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Ars Pharmaceutica es una revista científica multidisciplinar en el ámbito de las Ciencias Farmacéuticas, abarcando su sentido más amplio. Destaca por su enfoque en áreas como Atención Farmacéutica, Tecnología y Química Farmacéutica, Farmacología y Farmacovigilancia, siendo pionera en España en estas disciplinas. Desde su fundación en 1960, la revista ha sido editada de manera ininterrumpida por la Facultad de Farmacia de la Universidad de Granada.

A lo largo de su trayectoria, Ars Pharmaceutica ha evolucionado para adaptarse a las tendencias editoriales de las revistas científicas, pasando de su publicación en formato impreso a convertirse en una revista electrónica de libre acceso. Esta transformación ha permitido una mayor accesibilidad para investigadores de todo el mundo, lo que se refleja en el incremento de visitas a su página web y en el creciente interés por publicar trabajos en ella. Además, la aceptación de manuscritos tanto en español como en inglés ha contribuido significativamente al aumento de originales recibidos durante la última década.

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La revista cuenta con diversas categorías de trabajos publicables y utiliza el sistema Open Journal Systems (OJS), un programa de código abierto diseñado para gestionar y publicar revistas académicas en línea, lo que facilita y agiliza el envío de originales. Además, se han destinado recursos humanos y económicos para garantizar que la revista se ofrezca en formato electrónico y de manera gratuita a toda la comunidad científica y profesional interesada.

Ars Pharmaceutica se alinea con los principios de las revistas de acceso abierto (**Open Access Journal**) y, desde 2018, publica sus artículos bajo los términos de la licencia **Creative Commons 4.0 Internacional (CC BY-NC-SA 4.0)**. Asimismo, la revista no cobra tasas ni por el envío de manuscritos ni por la publicación de los artículos.

Desde 2012, esta revista ha sido el órgano de expresión de la **"Cátedra María José Faus Dáder de Atención Farmacéutica"**. En 2024, se incorporaron el **Aula de Farmacovigilancia** y el **Aula de Promoción de la Salud y Educación Sanitaria**, todas ellas con sede en la Facultad de Farmacia de la Universidad de Granada.

En el año 2024, la revista ha recibido la renovación del **Sello de Calidad Editorial** otorgado por la FECYT. Además, continúa figurando en el nuevo índice de impacto **JCI (Journal Citation Indicator)**, lo que la sitúa entre las 357 revistas más destacadas del mundo en el campo de la Farmacología y la Farmacia incluidas en los JCR de la Web of Science.

Por todo ello, invitamos a los autores a enviar sus contribuciones a las distintas secciones de la revista, consolidando así su compromiso con la excelencia científica y académica.

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Editorial

Inteligencia híbrida aplicada a los servicios profesionales farmacéuticos asistenciales: potenciando la inteligencia humana con las tecnologías avanzadas

Hybrid Intelligence Applied to Professional Pharmaceutical Healthcare Services: Enhancing Human Intelligence with Advanced Technologies

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Atención farmacéutica y servicios profesionales farmacéuticos asistenciales

La atención farmacéutica (AF) es una tecnología en salud orientada a contribuir al logro de los mejores resultados en salud posibles y a mejorar la calidad de vida de las personas, mediante la estructuración, implementación y prestación de servicios profesionales farmacéuticos asistenciales (SPFA). En esencia, la AF contribuye a la utilización efectiva, segura y económica de los medicamentos, al igual que a la promoción de la salud y a la prevención la enfermedad. Para ello, la AF se soporta en la prestación de servicios profesionales por el farmacéutico (con o sin medicamentos), con el foco del bienestar y calidad de vida del paciente y con el objetivo de ***conseguir el máximo beneficio posible en términos de salud.*** Por tanto, la dispensación, el seguimiento farmacoterapéutico (SFT), la revisión de la medicación, la educación para la salud y los tamizajes, son ejemplos de SPFA[1].

En este escenario y de forma más específica, los SPFA corresponden a procesos/actividades desempeñados por un farmacéutico, que emplea sus competencias profesionales en la prevención de la enfermedad y la mejora de la salud, desempeñando un papel activo en la optimización del proceso de uso y de los resultados de los tratamientos en salud[1]. Desde una perspectiva práctica, los SPFA, en el contexto de la filosofía de la AF, son la vía para contribuir a solucionar uno de los retos existentes en la sociedad: la utilización adecuada de los medicamentos y, con ello, la reducción de la morbimortalidad y costos evitables atribuidos a estos recursos terapéuticos.

En este sentido, por ejemplo, existe evidencia del efecto del SFT en la contribución al logro de los objetivos terapéuticos en ciertos grupos de pacientes, entre ellos con enfermedad cardiovascular[2] o con trastorno afectivo bipolar-I[3]. En el caso de Colombia, se ha evidenciado que el SFT disminuye los reingresos hospitalarios en pacientes con trastorno afectivo bipolar-I de forma costo-efectiva[4].

Inteligencia híbrida: articulación del pensamiento automatizado inteligente con la inteligencia humana

Este momento histórico se caracteriza por la incursión de los desarrollos tecnológicos, en especial del aprendizaje automatizado y de la inteligencia artificial (IA), en la forma como se diseñan, ejecutan y evalúan los resultados de los procesos y actividades en diferentes campos, incluyendo el desarrollo de medicamentos[5] y la farmacia clínica[6]. Lo anterior invita a todas las profesiones a reflexionar sobre el camino que deben seguir para integrar de manera efectiva las tecnologías avanzadas en su ejercicio profesional. En particular, es fundamental establecer cómo el pensamiento automatizado inteligente puede complementarse con la inteligencia humana, sin sustituirla. Esta cuestión cobra especial relevancia en disciplinas orientadas al bienestar y la calidad de vida de las personas, como las ciencias de la salud. En este ámbito, los profesionales deben aprovechar sus habilidades humanas –como el juicio clínico, la empatía y la toma de decisiones éticas– mientras potencian sus capacidades cognitivas mediante el uso de la tecnología. De esta manera, la potenciación entre ambos tipos de inteligencia permitirá desarrollar intervenciones más precisas, personalizadas y efectivas en el cumplimiento de sus propósitos.

La IA puede contribuir a mejorar de forma notoria la gestión de la medicación, la atención al paciente y los resultados en salud alcanzados, optimizando la prestación de los SPFA[7]. Por ejemplo, mediante el uso de algoritmos de IA y aprendizaje automático (automatizado), los farmacéuticos pueden analizar un gran volumen de datos de pacientes, incluidos registros médicos, resultados de laboratorio y perfiles de medicamentos, lo que favorece los resultados de los SPFA, debido a una mayor efectividad de[8]:

- El seguimiento y evaluación de la efectividad y seguridad de la farmacoterapia.
- La estructuración de recomendaciones/intervenciones informadas y adaptadas a las características particulares del paciente.

• La identificación de interacciones medicamentosas clínicamente relevantes y otros actores asociados a la variabilidad farmacológica.

Por su parte, en el contexto de los avances tecnológicos emerge el concepto de "Fármaco-inteligencia", como resultado de la integración/articulación de la IA, el aprendizaje automatizado y tecnologías avanzadas similares, en la práctica farmacéutica, incluyendo la prestación de los SPFA, con el objetivo de mejorar la atención, la seguridad y el logro de mejores resultados en salud del paciente[8].

Con este referente, en un marco más amplio, se ha establecido el concepto de FarmacIA[7] o, en su versión ajustada a la esencia de la quinta revolución industrial, el de Farmacia 5.0[9]. De forma global, la industria 4.0 proporciona el contexto y soporte para el desarrollo de la Farmacia 5.0, destacando que, la industria 5.0, se diferencia de la 4.0, en establecer la necesidad de la interacción/articulación esencial de los sistemas automatizados inteligentes con la inteligencia humana.

La Farmacia 5.0 se puede asimilar a un sistema de AF con las siguientes características: a) centrado en el paciente; b) estructurado y ejecutado por equipos interprofesionales; c) utilización de los principios y conceptos de la industria 5.0 y tecnologías avanzadas: Big data, analítica de datos, IA, automatización, farmacogenómica, monitores portátiles e impresión 3D de medicamentos y dispositivos médicos; y d) finalidad de favorecer la optimización de la farmacoterapia y de aumentar el desarrollo y la implementación de tratamientos personalizados[9].

Propósitos del sistema farmacia 5.0[9]:

• Maximizar la farmacoseguridad, la calidad y efectividad de la dispensación, la adherencia terapéutica, los resultados terapéuticos, las intervenciones claves, la colaboración intra equipo de atención y el cumplimiento normativo.

• Minimizar los errores en la cadena terapéutica (disponibilidad, prescripción, transcripción, dispensación, administración/uso y evaluación de resultados alcanzados).

• Reducir la entrada de datos redundantes, las interrupciones no planificadas en la atención, las tareas sin valor agregado y las intervenciones menos efectivas.

Componentes relevantes del sistema farmacia 5.0[9].

• Funciones claves "5C": Sistemas ciber-físicos, conexión de datos, conversión de información de datos, conocimiento y configuración.

• Fundamentos tecnológicos "4T": Tecnologías (1) de plataforma, (2) de datos, (3) analíticas y (4) operaciones.

• Objetivos de atención al paciente ("3S"): (1) Seguridad de la medicación (farmacoseguridad) inteligente, (2) farmacia inteligente y (3) adherencia y resultados de la medicación inteligentes (resultado de la articulación de las 5C y 4T, para brindar SPFA personalizados y efectivos a quien la necesita).

• Paciente: Centro y esencia del sistema.

Desde esta perspectiva, la inteligencia híbrida juega un papel clave al integrar la capacidad analítica de la inteligencia artificial con los juicios individuales del profesional farmacéutico. Mientras que las tecnologías avanzadas fortalecen los procesos cognitivos mediante el procesamiento de grandes volúmenes de información o la identificación de patrones que mejoren la toma de decisiones clínicas, la inteligencia humana sigue siendo necesaria para la interacción amable con los pacientes y la colaboración con otros profesionales. Aspectos como la interpretación ética ante un caso clínico, la empatía en la comunicación con el paciente y la sensibilidad ante la particularidad de cada caso son dimensiones en las que la inteligencia humana debe seguir siendo preponderante. Es decir, la tecnología no reemplaza la inteligencia humana, sino que la amplifica en el plano lógico-cognitivo, manteniendo intactas las competencias humanas esenciales para una atención integral y humanizada.

En este sentido, y considerando que los procesos y actividades realizadas por los seres humanos siempre han estado mediadas por las dimensiones múltiples de la inteligencia (han sido inteligentes), sería conveniente adicionar, a los ***procesos y actividades***, el término I***nteligencia híbrida*** y, con ello, sugerir

su atributo de ***"ultraInteligentes"***. Propiedad resultante de la articulación efectiva del pensamiento automatizado inteligente con la inteligencia humana, siempre con el foco del bienestar y calidad de vida de las personas. Por tanto, ¿Será posible hablar de SPFA-híbridos, con el atributo de ser ultraInteligentes? Reto claro, con un camino con recorridos aún por dibujar.

Bibliografía

1. Foro de Atención Farmacéutica-Farmacia Comunitaria (Foro AF-FC), panel de expertos. Guía práctica para los Servicios Profesionales Farmacéuticos Asistenciales desde la Farmacia Comunitaria. Madrid: Consejo General de Colegios Oficiales de Farmacéuticos; 2024. Disponible en: https://www.farmaceuticos.com/wp-content/uploads/2021/02/2021-guia-practica-spfa.pdf.

2. Amariles P, Sabater-Hernández D, García-Jiménez E, et al. Effectiveness of Dader Method for pharmaceutical care on control of blood pressure and total cholesterol in outpatients with cardiovascular disease or cardiovascular risk: EMDADER-CV randomized controlled trial. J Manag Care Pharm. 2012;18(4):311-23. doi: 10.18553/jmcp.2012.18.4.311.

3. Salazar-Ospina A, Amariles P, Hincapié-García JA, et al. Effectiveness of the Dader Method for Pharmaceutical Care on Patients with Bipolar I Disorder: Results from the EMDADER-TAB Study. J Manag Care Spec Pharm. 2017;23(1):74-84. doi: 10.18553/jmcp.2017.23.1.74

4. Monsalve-David M, Hincapié-García JA, Salazar-Ospina A, et al. Coste-efectividad del seguimiento farmacoterapéutico en pacientes con trastorno afectivo bipolar-I: ensayo clínico aleatorizado EMDADER-TAB. PharmacoEcon Span Res Artic. 2017;14:31-38. doi: 10.1007/s40277-017-0068-z.

5. Niazi SK, Mariam Z. Artificial intelligence in drug development: reshaping the therapeutic landscape. Ther Adv Drug Saf. 2025;16:20420986251321704. doi: 10.1177/20420986251321704.

6. Ranchon F, Chanoine S, Lambert-Lacroix S, Bosson JL, Moreau-Gaudry A, Bedouch P. Development of artificial intelligence powered apps and tools for clinical pharmacy services: A systematic review. Int J Med Inform. 2023;172:104983. doi: 10.1016/j.ijmedinf.2022.104983.

7. González-Pérez Y, et al. Approaching artificial intelligence to Hospital Pharmacy. Farm Hosp. 2024;48 Suppl 1:S35-S44. doi: 10.1016/j.farma.2024.02.007.

8. Chalasani SH, Syed J, Ramesh M, et al. Artificial intelligence in the field of pharmacy practice: A literature review. Explor Res Clin Soc Pharm. 2023;12:100346. doi: 10.1016/j.rcsop.2023.100346.

9. Lin AC, Lee J, Gabriel MK, et al. The Pharmacy 5.0 framework: A new paradigm to accelerate innovation for large-scale personalized pharmacy care. Am J Health Syst Pharm. 2024;81:e141-e147. doi: 10.1093/ajhp/zxad212.

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Artículos originales

Solubility Enhancement of Brexpiprazole for Schizophrenia using HPβ Cyclodextrin Ternary Complexation

Mejora de la solubilidad del brexpiprazol para la esquizofrenia mediante complejación ternaria de ciclodextrina HPβ

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The authors declare no conflict of interest.

Resumen

Introducción: El presente estudio se centra en la mejora de la solubilidad y la velocidad de disolución del brexpiprazol con hidroxipropil β-ciclodextrina y ácido succínico como solubilizador para la esquizofrenia.

Método: Brexpiprazol se obtuvo como muestra de regalo de CTX Life sciences, Sachin, Surat. La hidroxipropil beta ciclodextrina fue donada por Good Health Pvt. Limitado. Ltd. Sachin, Surat. Los complejos binarios y ternarios se prepararon utilizando tres métodos diferentes y se caracterizaron mediante espectroscopia infrarroja por transformada de Fourier, calorimetría diferencial de barrido, difracción de rayos X y estudios de disolución in vitro.

Resultados: Se observó que entre las diferentes proporciones de fármaco y polímero utilizadas, la proporción 1:5 de fármaco y polímero para el complejo binario resultó ser la optimizada y el método de evaporación del disolvente dio los mejores resultados. Luego se preparó el complejo ternario usando esta proporción en diferentes concentraciones de ácido succínico (0,25, 0,5 y 1 % p/p) y la concentración óptima de ácido succínico como solubilizador fue del 1 %. Se encontró que la liberación del fármaco era máxima del 92% en comparación con el complejo binario. Los resultados de la espectroscopia infrarroja por transformada de Fourier, la calorimetría diferencial de barrido y la difracción de rayos X confirmaron la formación de un complejo estable.

Conclusiones: Se concluyó que el complejo ternario tuvo una liberación máxima del fármaco del 92 % al final de 60 minutos, lo que resultó en una mejora de la solubilidad y la velocidad de disolución.

Palabras clave: Esquizofrenia; Brexpiprazol; Complejo ternario; solubilidad de fase; tasa de disolución

Abstract

Introduction: The present study focuses on the solubility and dissolution rate enhancement of Brexpiprazole with Hydroxy Propyl β-cyclodextrin and succinic acid as a solubiliser for schizophrenia.

Materials & Method: Brexpiprazole was obtained as a gift sample from CTX Life sciences, Sachin, Surat. Hydroxypropyl beta cyclodextrin was gifted by Good Health Pvt. Ltd Sachin, Surat. Binary and ternary complexes were prepared using three different methods and characterized using the fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray diffraction and invitro dissolution studies.

Results: It was observed that among different ratios of drug and polymer used, 1:5 ratio of drug and polymer for binary complex was found to be the optimized and solvent evaporation method gave the best results. The ternary complex was then prepared using this ratio in different concentration of succinic acid (0.25, 0.5 and 1 % w/w) and optimum concentration of succinic acid as a solubiliser was of 1 %. The drug release was found to be maximum 92 % as compared to binary complex. The fourier transform infrared spectroscopy, differential scanning calorimetry and X-ray diffraction results confirmed the formation of stable complex.

Conclusions: It was concluded that ternary complex had maximum drug release of 92% at the end of 60 minutes resulting in solubility and dissolution rate enhancement.

Keywords: Schizophrenia; Brexpiprazole; Ternary complex; Phase solubility; Dissolution rate

Highlights

Solubility enhancement of Biopharmaceutical Classification System (BCS) class II drugs is a need in order to have the maximum drug dissolution and quick onset of action for diseases like schizophrenia where the patients require quick onset of action and faster delivery of drug to brain.

Various polymers and techniques can be used as solubility enhancing agents among which cyclodextrin complexation is one of the highly recommended techniques. Using cyclodextrins in combination with solubilizers like succinic acid are more effective as they give a synergistic effect to solubility enhancement.

The result obtained can be used for practically water insoluble drug's solubility enhancement and can be used to enhance the dissolution rate which can be used to make fast dissolving dosage forms.

Introduction

People with schizophrenia experience a serious mental disease in which they interpret reality in an unusual way. Childhood schizophrenia is an uncommon but dangerous mental disorder. Children with the disease show poor emotional regulation and reasoning skills, which causes them to lose interest in reality. Delusions include things like hearing or seeing things that aren't there, hallucinations, and false beliefs[1]. Delusions, or irrational, incorrect beliefs, or hallucinations, or misleading sensations involving any sensory modality, are the most typical characteristics of psychotic conditions[1]. The development of new neurotherapeutics is limited by the challenges associated with translocating molecules across the blood-brain barrier (BBB) and the low water solubility of most of the new medication candidates. Because non-target regions, particularly those outside the central nervous system, are exposed to larger drug concentrations, poor solubility increases systemic exposure. This raises the possibility of adverse outcomes[2-3]. Solubility can be enhanced by using various techniques like co-solvency, complexation, hydrotropy, cryogenic method, liquisolid compact, high pressure homogenization, manipulation of solid state, micellar solubilisation, microemulsion & self-emulsifying system, micronization, nano crystallization, nanosuspension, neutralization, cosolvency, particle size reduction, pH adjustment, solid dispersion, solubilisation, precipitation, salt formation, solvent deposition, sonocrystallization, spray drying, supercritical fluid process[4-6].Cyclodextrins (CDs) have received increasing attention in pharmaceutical field because of their ability to enhance solubility, stability, and bioavailability through the formation of inclusion complexes[7-10]. However, it is imperative that pharmaceutical dosage form should contain appropriate and approved concentration of CDs, because use of excess amounts of CDs may lead to potential toxicity and other related side effects, which may impede its use in drug development. Hence, optimization of complexation process and possibility to explore multicomponent systems to gauge the cyclodextrin concentration within permissible limits and further improve efficiency in terms of dissolution and bioavailability were mandated. In this context, the influence of additional (ternary) components in enhancing cyclodextrin solubilization of poorly water-soluble drug has received research focus and momentum over the years[11].

The current study attempts to improve brexpiprazole's solubility and dissolution. Atypical antipsychotics like brexpiprazole are prescribed to treat schizophrenia and depression in addition to depression. It's practically water-insoluble nature leads to an extended half-life of 91 hours and a protein binding percentage of >99%. Brexpiprazole was found to be 0.0063 mg/ml soluble in water at pH 7.0. Drug dissolving rate is slowed down by low water solubility. In this investigation, hydroxypropyl β cyclodextrin and a solubilizer succinic acid were used to produce a ternary complex, which improved the solubility and dissolution of brexpiprazole[12,13].

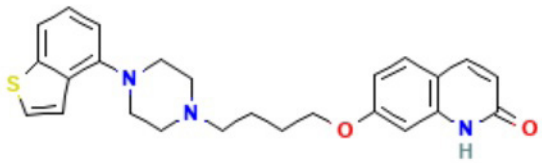

Figure 1. Structure of brexpiprazole

Material and Methods

Materials

Brexpiprazole was obtained as a gift sample from CTX Lifesciences Pvt. Ltd. located in Sachin, Surat, Gujarat, India. A gift sample of hydroxypropyl β cyclodextrin was also provided from Good Health Pvt. Ltd. in Sachin, Surat, Gujarat, India. All other ingredients were of analytical grade.

Methods

Phase solubility studies

Phase solubility studies were conducted to examine the drug's solubility in HPβCD solutions at several concentrations (4–20 mM/L). A saturated solution was generated in a vial by gradually adding excess medication to each of the various solutions. After being screwed shut and sealed, the vials were agitated at room temperature for a full day. The samples were taken out and filtered using a 0.22 µm nylon filter after a 24-hour period. After that, these saturated systems were examined using a UV spectrophotometer set at 214.5 nm[14,15].

Buffer solubility studies of the brexpiprazole:

Solutions of various pH values were generated in order to determine the drug's saturation solubility at various pH values (pH 2, 3, 5, 6, 8, and 9). The drug was gradually added to each vial of buffer solution until a saturated solution was achieved. Each buffer solution was taken in its own vial. After that, all of the solutions were left to agitate for a full day. The samples were taken out and filtered using a 0.22 µm nylon filter after a 24-hour period. After the filtrate was diluted, a UV spectrophotometer was used to measure the absorbance[14,15].

Preparation of Binary Complex of Drug and Hydroxy propyl beta cyclodextrin

The inclusion complex of drug and HPβCD was prepared by three different methods including physical mixture, kneading method and solvent evaporation method by taking three different ratios of drug: HPβCD 1:1, 1:3 & 1:5[16-20].

Physical mixture

The drug powder that had been milled was combined with HP-β-CD in several drug to CD ratios, such as 1:1, 1:3, and 1:5, to create the physical combinations. After going through sieve mesh #60, the mixes were kept in a desiccator until more testing was completed.

Kneading method

With precision, various ratios of Brexpiprazole: HP-β-CD were weighed. In order to get a slurry-like consistency, a tiny amount of ethanol: water (1:1 v/v) was added to the mortar along with HP-β-CD. After that, the medication was gradually added to the slurry, and trituration was carried out for an additional forty-five minutes. After a 24-hour drying period at 45°C, the slurry was grinded, put through a no. 60# filter, and kept in desiccators until needed again.

Solvent Evaporation Method

After dissolving the medication in 20–25 ml of ethanol, the mixture was stirred magnetically until a transparent solution was created. The necessary quantity of cyclodextrin was added to the clear solution to make it clear, and the mixture was heated intermittently to produce the dry residue. After being scraped from the beaker, the dried residue was ground into a fine powder and put through a sieve with a 60# mesh.

Preparation of ternary complex and its characterization

According to the research, the solvent evaporation approach yielded an 80 % drug release in salivary medium at the conclusion of a 60-minute duration. One to five was the ideal ratio. Thus, the ternary

complex was prepared using this optimized ratio (1:5) and solvent evaporation method. The medication was dissolved in the least amount of solvent possible and stirred with a magnetic stirrer until a transparent solution formed. To create a clear solution, cyclodextrin was combined with the necessary amount of succinic acid as solubiliser at varied concentrations (0.25 %, 0.5 %, and 1 % w/w) separately. After that, the mixture were heated sporadically on the magnetic stirrer until the solvent had completely evaporated. After being scraped from the beaker, the dried residue was ground into a fine powder and put through a 60# sieve[19,21-22].

In vitro dissolution studies

Through the dissolution of the complexes formed in water and salivary buffer, the inclusion complex was optimized. Using the United States Pharmacopoeia (USP) dissolution tester Lab India Disso Smart 8S, in vitro dissolution tests were carried out under sink conditions for medication cyclodextrin complexes. 900 mL of distilled water kept at 37.0±0.5 °C and rotating at 100 rpm made up the dissolving media. Weighing precisely, 10 mg of the drug's equivalent of powder complex was placed into each vessel. At predetermined intervals of 15, 30, 45, and 60 minutes, 5 mL samples were manually removed and filtered using a 0.22 µm nylon filter. Using a UV spectrophotometer, the drug concentration in the sample was determined.

Fourier transform infrared spectroscopy

Infrared spectra were recorded on FTIR (Nexus FTIR, J Thermo Nicolet, and USA). In order to create the sample disk, 1–2 mg of a solid sample was grinded in the mortar and was quickly triturated with 0.10–0.20 g of potassium bromide for infrared spectrophotometry. Care was taken to prevent moisture absorption. The pellet was then placed in the sample holder of the FTIR spectrometer. And the IR spectrum was recorded in the desired range (usually 4000–400 cm^{-1}).

Differential scanning calorimetry

The differential scanning calorimetry measurement was performed using a DSC. A sample of 3 – 10 mg was weighed in aluminium pans and crimped. The samples were heated over a temperature range of 0 – 350 °C at a constant rate of 10 °C/min under nitrogen flow of 20 mL/min. An empty aluminium pan was used as a reference.

X-Ray Diffraction (XRD) studies

This study was performed at PW1710 X-ray diffractometer with Cu as anode material and graphite monochromator, operated at a voltage of 35 kV, current 40 mA in the 2θ angle range of 10-70 °C with scan time of 0.5 s. The X-ray diffractogram showed narrow and broad peaks reflecting the nature of drugs, excipients and complexes.

Results

Results & Discussion

Phase solubility determination:

Phase solubility tests show that when the molar concentration of hydroxy propyl beta cyclodextrin increases, the drug's concentration climbs linearly. The stability constant's value was ascertained by applying the Higuchi & Conors method. Based on the Kc value, it was also thought that the medication and polymer showed a 1:1 stoichiometric ratio. With a stoichiometric ratio of 1:1 between the drug and polymer, and a better complex formation, the values of Kc were found to be 176 and 133 cm^{-1} in water and buffer, respectively. An A_L-type graph was also anticipated because the medication concentration increased linearly with the HPβCD concentration. (Table 1).

Table 1. Phase solubility of drug in water and buffer

Medium	mM/L conc.	R^2	Kc (M^{-1})	Graph type
Water	4-20mM/L	0.98	133	A_L type
Buffer	4-20mM/L	0.97	176	A_L type

pH solubility studies of brexpiprazole

pH solubility studies are critical in evaluating how a drug's solubility changes across different pH levels, which reflect the physiological conditions of the human body, particularly in the gastrointestinal tract. These studies are especially important for weakly acidic or basic drugs, as their ionization state—and therefore solubility—can vary significantly with pH. The ionization of a compound affects its dissolution and absorption in the body, impacting its bioavailability. Understanding solubility at various pH levels can guide the design of formulations to improve drug solubility and absorption. pH-dependent solubility profiles can assist in predicting drug absorption and identifying potential challenges in drug delivery systems, aiding in the development of more effective pharmaceutical formulations. It was found that the drug has maximum solubility at acidic pH 4. Amongst different pH range it could be predicted that as it was maximum soluble at 4 pH it would be weakly basic in nature[23] (Table 2).

Table 2. pH solubility profile of Brexpiprazole

S. No.	pH	Conc of drug (mg/ml)	Drug conc(mg/ml)
1	2	0.0063	0.0063±0.0001
2	3	0.0208	0.0208±0.0001
3	4	0.0346	0.0345±0.0001
4	5	0.0297	0.0297±0.0003
5	6	0.0064	0.0064±0.0001
6	8	0.0060	0.0060±0.0001
7	9	0.0055	0.0055±0.0001

In vitro drug release

Using a USP type II equipment dissolution device, the drug dissolving tests were conducted in vitro at 37 ±0.5 °C. Three distinct approaches were used to conduct drug dissolving tests in water and salivary buffer pH 6.8 for various drugs and the HPβCD binary complex. The solvent evaporation method was discovered to be the optimal approach, and the optimized ratio was found to be 1:5. The highest drug release in the binary complex was discovered to be 86.10 % after 60 minutes. In order to create the ternary complex, this optimized batch of complex was utilized together with varying concentrations of succinic acid as a solubilizer (0.25, 0.5, and 1 % w/w). At the end of the 60-minute period, 92 % of the ternary complex's medication was released. Therefore, it can be inferred that the addition of a co-solubiliser, succinic acid at a concentration of 1 % w/w resulted in maximal drug release. Additionally, hydroxypropyl beta cyclodextrin and succinic acid demonstrated a synergistic effect that improved drug release and sped up the beginning of action. (Table 3 and 4).

Table 3. Drug release profile of binary complex

Method	Drug:HPBCD Ratios	Maximum percent cumulative drug release
Physical mixture	1:1	20.56±1.78
	1:3	34.33±2.0
	1:5	35.50±2.45
Kneading method	1:1	39.24±1.54
	1:3	56.56±1.45
	1:5	61.60±2.04
Solvent evaporation method	1:1	53.14±1.65
	1:3	67.09±2.31
	1:5	86.10±2.11

Table 4. Drug release profile of ternary complex

Time (min)	%Drug Release		
	Ternary Complex 0.25 %(w/w)	Ternary Complex 0.5 % (w/w)	Ternary Complex TC 1 %(w/w)
0	0	0	0
15	54.90±1.11	59.48±2.01	57.00±1.44
30	55.28±1.43	61.50±1.67	59.55±1.23
45	57.75±1.78	62.10±2.61	89.63±1.99
60	63.68±1.23	70.13±2.22	91.95±1.56

FTIR Studies

Standard FTIR of the drug shows characteristic peaks of -C-O stretching at 1219.06 cm^{-1}, -C=O ketone at 1653.73, CH alkane stretch at 2815.89 and secondary NH amine group at 3429.90 cm^{-1}(Illustrated in figure 1). In case of binary inclusion complex the physical mixture did not show any change in shift in peaks as compared to standard drug indicating no complex formation. Comparatively, in case of inclusion complex the characteristic peaks shifted to 1250, 1600, 2900 and 3300-3400 cm^{-1} of the respective functional groups in the pure drug. This shift in peaks and also the decrease in peak sharpness or broadening of peak indicate the formation of complex. When a ternary complex is prepared using succinic acid it was found that the characteristic drug peaks of -C-O stretching at 1219.06 cm^{-1}, -C=O ketone at 1653.73, CH alkane stretch at 2815.89 and secondary NH amine group at 3429.90 cm^{-1} shifted to 1153, 1650.17, 2901.06 and 3047.77 cm^{-1} of the respective functional groups. These shifts of peaks confirmed the formation of ternary complex. The FTIR graph of binary and ternary complex is illustrated in figure 2 and 3.

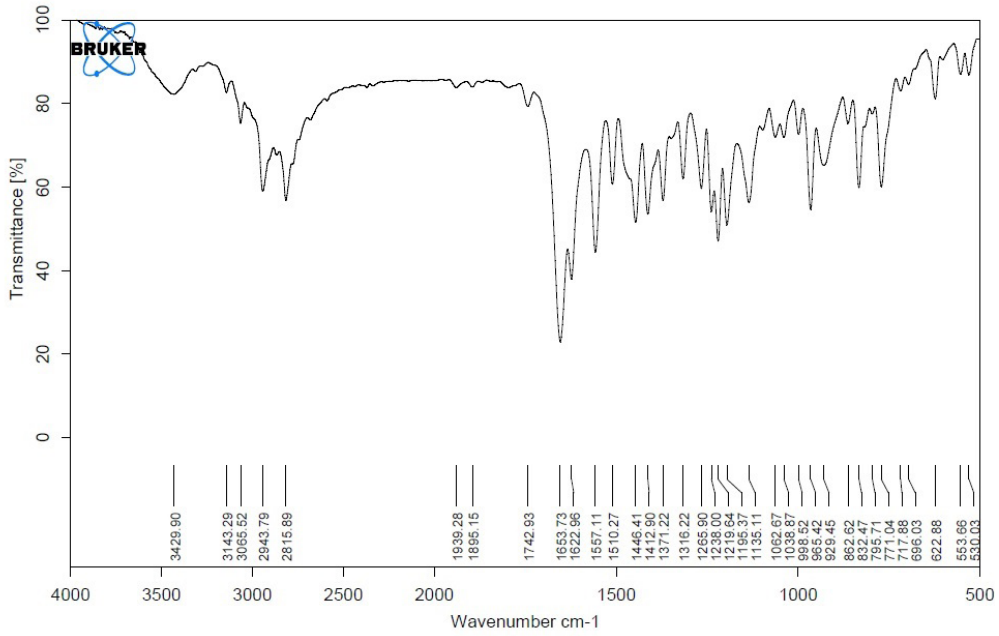

Figure 1. FTIR of Brexpiprazole

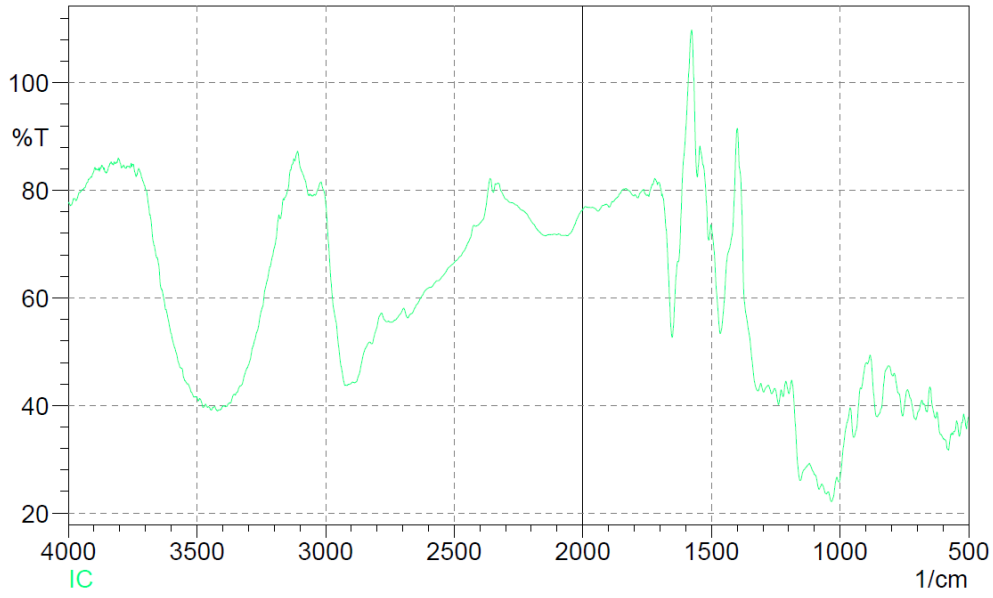

Figure 2. FTIR of binary complex

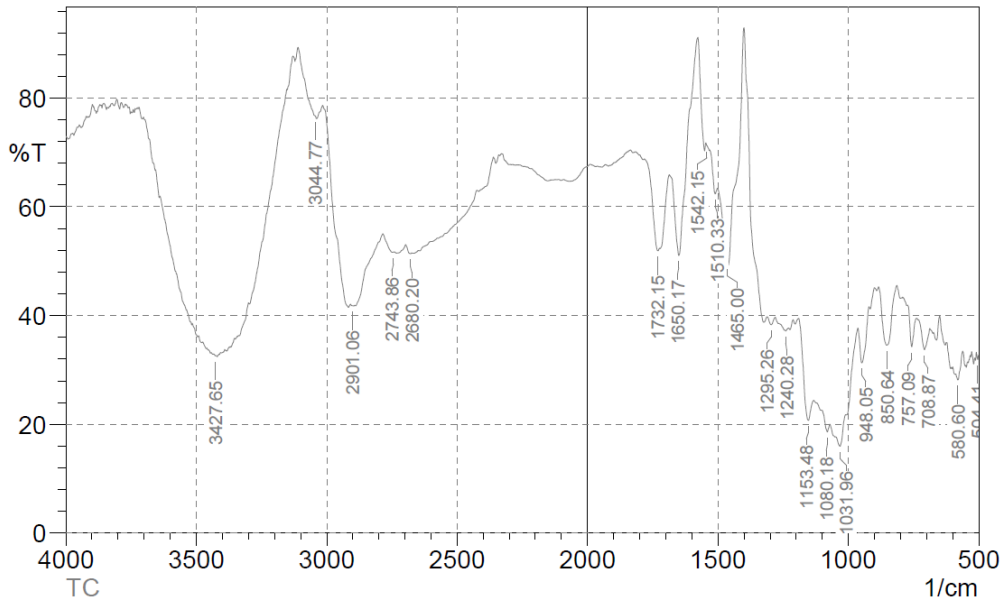

Figure 3. FTIR of ternary complex

Differential Scanning Calorimeter

Differential Scanning Calorimetry (DSC) is a thermal analysis technique used to measure the heat flow associated with material transitions as a function of temperature or time. This method provides critical insights into the thermal properties of substances, such as melting point, crystallization, glass transition, and thermal stability, which are crucial for material characterization in fields like pharmaceuticals, polymers, and food science. DSC is particularly valuable in the pharmaceutical industry for studying drug-excipient compatibility, polymorphism, and the solid-state stability of active pharmaceutical ingredients (APIs). DSC can effectively identify phase transitions in drug substances, which helps optimize formulation processes by ensuring the stability and efficacy of the final product. To create the medication, solubilizer, and cyclodextrin complex, the solvent evaporation method was employed. The medication cyclodextrin and solubilizer complex's DSC data verified the complex's formation. The pure drug DSC exhibited a prominent endothermic peak in the binary complex, close to its melting point of 184.4 ^{0}C. The physical mixing of the binary complex likewise showed no alterations in minor peak as compared to pure drug. However, at 86.68 °C, the complicated endothermic peak was observed. Along with the peak›s decrease in length, the peak was also seen to be broad in nature. This suggested the formation of an inclusion complex. When the DSC of the ternary complex was compared to the standard drug, the ternary complex›s endothermic peak was observed at 87.39 ^{0}C, nearly parallel to the base line, suggesting the formation of a more stable complex. Additionally, the ternary complex was significantly more stable than the binary complex since it had a greater drop in peak area and enthalpy value[24]. The DSC curve of pure drug is shown in (figure 4) while the overlay graph of DSC of binary and ternary complex is shown in (figure 5).

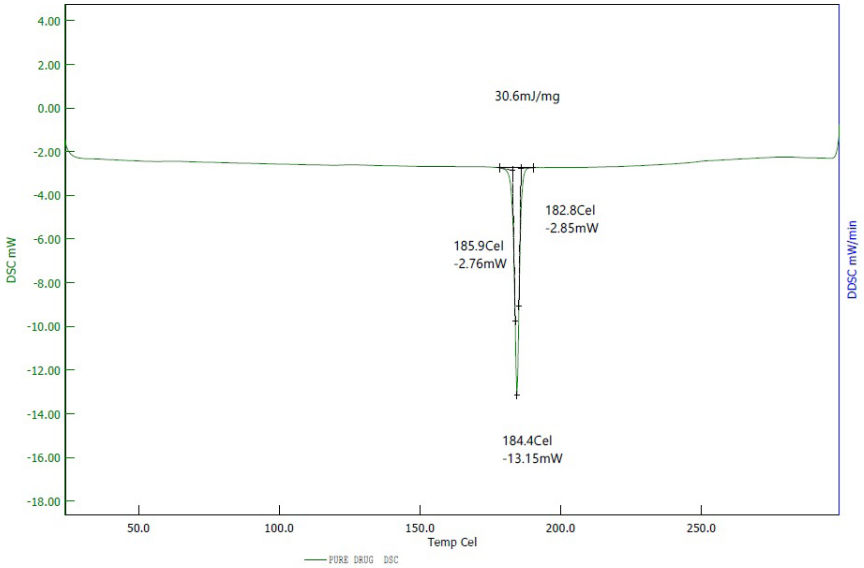

Figure 4. DSC of Brexpiprazole

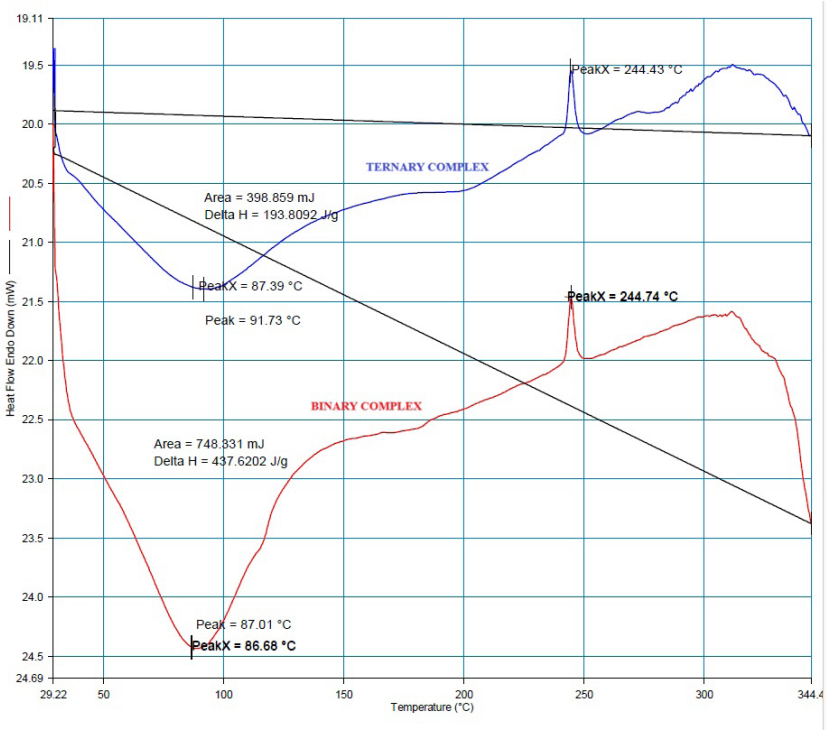

Figure 5. DSC curve of binary and ternary complex

XRD Studies

The ternary complex, binary complex, and pure drug were all studied using XRD. The pure drug's diffractograms showed a number of strong peaks, indicating that they were crystalline in nature. On the other hand, the diffractograms of the binary complexes showed a notable reduction in peak intensity (amorphization), and the ternary complex showed an even greater reduction in peak intensity when compared to the binary complex. This suggested that the drug's crystalline to amorphous state shifted, increasing its solubility and dissolution. X-ray Diffraction (XRD) studies are widely employed to analyze the crystalline structure of materials, providing essential information about the phase composition, crystallinity, and molecular arrangement within a product. This technique is particularly important in the pharmaceutical field, where it is used to differentiate between polymorphic forms of a drug, assess the degree of crystallinity, and ensure batch-to-batch consistency of solid-state products. XRD can also be used to detect amorphous content, which is critical for understanding solubility and bioavailability. XRD analysis plays a vital role in pharmaceutical development by helping identify different solid forms of a drug substance, which can significantly impact its therapeutic performance and stability. The results of XRD of pure drug, binary complex and ternary complex are illustrated in an overlay form in (figure 6).

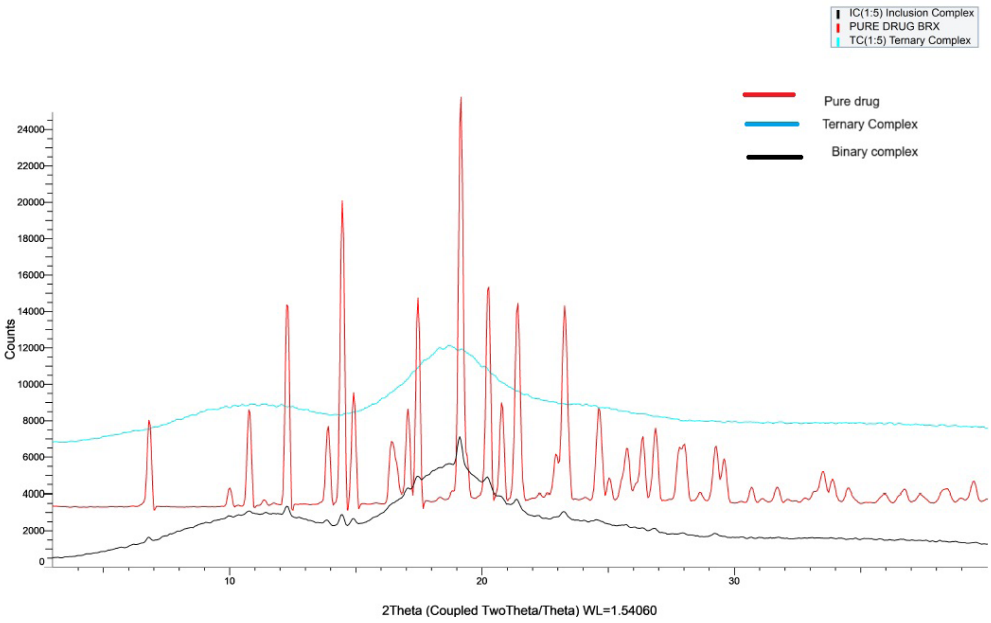

Figure 6. XRD graph of pure drug, binary and ternary complex

Conclusion

The studies mentioned above indicated that the ternary complex enhanced the solubility of brexpiprazole, resulting in a higher rate of dissolution and quicker onset of action. One of the preferred complexation methods was presumed to be solvent evaporation, and succinic acid was thought to be a promising solubilizer for developing a ternary complex.

References

1. Read J, van Os J, Morrison AP, Ross CA. Childhood trauma, psychosis and schizophrenia. A literature review with theoretical and clinical implications. Acta Psychiatr Scand. 2005;112(5):330–50. doi: 10.1111/j.1600-0447.2005.00634.x.

2. Upadhyay RK. Drug delivery systems, CNS protection, and the blood brain barrier. Biomed Res Int. 2014; 2014:869269. doi: 10.1155/2014/869269.

3. Deshmukh AS, Tiwari KJ, Mahajan VR. Solubility Enhancement Techniques for Poorly Water-Soluble Drugs. Int J Pharm Sci Nanotechnol. 2017;10(3):3701–8. Doi:10.37285/ijpsn.2017.10.3.1

4. Turunen E. Cyclodextrins in Fast- Dissolving Drug Formulations for Intraoral Administration. Publications of the University of Eastern Finland. Dissertations in Health Sciences 229; 2014.

5. Manne ASN, Hegde AR, Raut SY, Rao RR, Kulkarni VI, Mutalik S. Hot liquid extrusion assisted drug-cyclodextrin complexation: a novel continuous manufacturing method for solubility and bioavailability enhancement of drugs. Drug Deliv Transl Res. 2021;11(3):1273-1287. doi: 10.1007/s13346-020-00854-w.

6. Chaudhary A, Nagaich U, Gulati N, Sharma VK, Khosa RL. Enhancement of solubilization and bioavailability of poorly soluble drugs by physical and chemical modifications: A recent review. J Adv Pharm Educ Res. 2012;2(1):32–67.

7. Chaudhary VB, Patel J.K. Cyclodextrin inclusion complex to enhance solubility of poorly water soluble drugs. A Review. Int J Pharm Sci Res. 2013;4(1): 68-76. Doi: 10.13040/IJPSR.0975-8232

8. Kumar SK, Raju PY, M Sushma. Dissolution Enhancement of Poorly Soluble Drugs by Using Complexation Technique-A Review. J. Pharm. Sci. & Res. 2013; 5(5): 120 – 124.

9. Sharma GS, Srikanth MV, Sunil SA, Rao NS, Murthy KR. Dissolution rate enhancement of poorly soluble gliclazide by complexation with hydroxy propyl β cyclodextrin. Res J Pharm Biol Chem Sci. 2011;2(3):814-23.

10. Basavaraj S, Sihorkar V, Shantha Kumar TR, Sundaramurthi P, Srinivas NR, Venkatesh P, et al. Bioavailability enhancement of poorly water soluble and weakly acidic new chemical entity with 2-hydroxy propyl-β-cyclodextrin: Selection of meglumine, a polyhydroxy base, as a novel ternary component. Pharm Dev Technol. 2006;11(4):443–51. doi:10.1080/10837450600770577.

11. Loftsson T, Duchêne D. Cyclodextrins and their pharmaceutical applications. Int J Pharm. 2007;329(1–2):1–11. doi: 10.1016/j.ijpharm.2006.10.044.

12. Hope J, Castle D, Keks NA. Brexpiprazole: a new leaf on the partial dopamine agonist branch. Australas Psychiatry. 2018;26(1):92-94. doi: 10.1177/1039856217732473.

13. Eaves S, Rey JA. Brexpiprazole (Rexulti): A new monotherapy for schizophrenia and adjunctive therapy for major depressive disorder. P T. 2016;41(7):418-422.

14. Loh Gok, Tan YTF, Peh KK. Enhancement of norfloxacin solubility via inclusion complexation with β-cyclodextrin and its derivative hydroxypropyl-β-cyclodextrin. Asian J Pharm Sci. 2016 Aug 1;11(4):536–46. Doi: 10.1016/j.ajps.2016.02.009

15. Jagdale SK, Dehghan MH, Paul NS. Enhancement of dissolution of fenofibrate using complexation with hydroxy propyl β-cyclodextrin. Turk J Pharm Sci. 2019 Mar 1;16(1):48–53. doi: 10.4274/tjps.60490.

16. Pathak SM, Musmade P, Dengle S, Karthik A, Bhat K, Udupa N. Enhanced oral absorption of saquinavir with Methyl-Beta-Cyclodextrin-Preparation and in vitro and in vivo evaluation. Eur J Pharm Sci. 2010;41(3-4):440-451. doi: 10.1016/j.ejps.2010.07.013.

17. Kaushik, S., Verma, R., Purohit, D. et al. (2020). Development of binary and ternary complex of cefuroxime axetil with cyclodextrin for improving pharmaceutical characteristics. International Journal of Applied Pharmaceutics, 12(6),107–117. Doi: 10.22159/ijap.2020v12i6.38927

18. Patel SG, Rajput SJ. Enhancement of oral bioavailability of cilostazol by forming its inclusion complexes. AAPS PharmSci Tech. 2009;10(2):660–9. Doi: 10.1208/s12249-009-9249-7

19. Jafar, Mohammed & Salahuddin, Mohammed & Kayed, M.T. & Ahmed, N. & Al-Eid, H.A. & Al-Qarross, A.H. Solid State Analysis and In-Vitro Dissolution Behavior of MeloxicamHydroxy Propyl Beta Cyclodextrin-Ethanolamines Ternary Complexes. International Journal of Pharmaceutical Quality Assurance. 2018 Mar 30;9(01). Doi: 10.25258/ijpqa.v9i01.11919

20. Patel HM, Suhagia BN, Shah SA, Rathod IS, Parmar VK. Preparation and characterization of etoricoxib-β-cyclodextrin complexes prepared by the kneading method. Acta Pharmaceutica. 2007;57(3):351–9. doi: 10.2478/v10007-007-0028-2.

21. Chowdary, K & Naresh, A. A Factorial study on the effects of β Cyclodextrin, PVP K30 and SLS on the solubility and dissolution rate of efavirenz. Int J App Bio Pharm Technol. 2010;2: 228-34.

22. Crestani De Miranda J, Elyan T, Martins A, Veiga F, Gomes Ferraz H. Cyclodextrins and ternary complexes: technology to improve solubility of poorly soluble drugs. Braz. J. Pharm. Sci.2011; 47 (4). Doi:10.1590/S1984-82502011000400003

23. Avdeef A. Absorption and Drug Development: Solubility, Permeability, and Charge State. Hoboken, NJ: John Wiley & Sons; 2003.

24. He R, Craig DQ. An investigation into the thermal behaviour of an amorphous drug using low frequency dielectric spectroscopy and modulated temperature differential scanning calorimetry. J Pharm Pharmacol. 2001;53(1):41-48. doi:10.1211/0022357011775172.

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Artículos originales

Formulation, Development, and Evaluation of Flubiprofen Sustained Release Tablets Using a Quality-by-Design Approach

Formulación, desarrollo y evaluación de comprimidos de liberación sostenida de flubiprofeno mediante un enfoque de calidad por diseño

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Resumen

Introducción: El flurbiprofeno actúa bloqueando las enzimas ciclooxigenasa (COX) del organismo para que no realicen su función normal. El estudio produjo comprimidos de flubiprofeno de larga duración mediante compresión directa con polímeros carbopol, HPMC K100M y HPMC K4M. El flubiprofeno es un antiinflamatorio no esteroide.

Método: La investigación formula y desarrolla comprimidos de liberación sostenida de flubiprofeno por compresión directa con polímeros carbopol, HPMC K100M, y HPMC K4M. La mayor concentración de flubiprofeno en etanol, según las pruebas de preformulación, fue de 215 nm.

Resultados: Se examinó el grosor, el diámetro, el contenido de medicamento y la friabilidad de la tableta comprimible. Todos los exámenes fueron buenos. El índice de compresibilidad, la densidad aparente, el ángulo de reposo y la densidad roscada mostraron buenos resultados para la mezcla de comprimidos. Se realizaron pruebas de liberación in vitro utilizando un dispositivo USP tipo II a 50 RPM, HCl 0,1 N en el medio de disolución durante dos horas, y tampón fosfato pH 6,8 durante seis horas a 37 +0,5°C. Un espectrofotómetro UV-visible con un ajuste de 215 nm evaluó la liberación del fármaco en diferentes periodos. Esta investigación sobre la formulación indicó una liberación del 99,25% del fármaco a partir de F2.

Conclusiones: Una de las ventajas de la forma farmacéutica de liberación sostenida es que permite administrar un medicamento de forma gradual durante un periodo de tiempo prolongado para mantener constante el nivel de concentración en sangre. Esto puede mejorar el cumplimiento del paciente y aumentar la producción de fármacos.

Palabras clave: Flubiprofeno, Liberación sostenida, Disolución, Polímero, DOE, Calidad por diseño.

Abstract

Introduction: Flurbiprofen acts by blocking the cyclooxygenase (COX) enzymes in your body from carrying out their normal function. The study made flubiprofen tablets that last a long time by directly compressing them with carbopol, HPMC K100M, and HPMC K4M polymers. An anti-inflammatory non-steroid is flubiprofen.

Method: The research formulates and develops sustained-release flubiprofen tablets by direct compression with carbopol, HPMC K100M, and HPMC K4M polymers. The highest flubiprofen concentration in ethanol, according to pre-formulation tests, was 215 nm.

Results: The compressibility tablet was tested for thickness, diameter, medication content, and friability. Every exam was good. The compressibility index, bulk density, angle of repose, and tapped density showed good results for the tablet mix. In-vitro release tests were performed utilizing a USP device type II at 50 RPM, 0.1 N HCl in the dissolving media for two hours, and phosphate buffer pH 6.8 for six hours at 37 +0.5°C. A UV-visible spectrophotometer with a 215 nm setting assessed drug release at different periods. This formulation research indicated 99.25 % drug release from F2.

Conclusions: One benefit of the sustained release dosage form is that it allows a medication to be administered gradually over an extended period in order to keep the blood level of concentration constant. This may improve patient compliance and increase drug output.

Keywords: Flubiprofen, Sustained release, Dissolution, Polymer, DOE, Quality by Design

Highlights

In this study, the flurbiprofen tablet was formulated by using the direct compression method which includes carbopol, HPMC K100M, and HPMC K4M polymers.

SR formulations were developed to improve drug function by lengthening half-lives, decreasing frequency of administration, minimizing side effects, lowering dose, and delivering the medication in the shortest time using the least amount through the most effective route.

This study aims to maximize yield percentage and flow quality while reducing moisture. The factorial design used statistics to find the best formulation parameters and looked at how the spray dryer's process parameters affected the co-excipient's moisture content, percentage yield, and compressibility index. It also looked at the effects of these parameters on each other and on a quadratic scale. Quadratic and linear response surfaces were examined utilizing Design Expert's 3-factor, 3-level design.

The study found that in vitro dissolution was successful. Batch F2 had the highest drug release rate of 99.25%, according to the formulation. So batch F2 is optimized.

Introduction

Painful disorders like migraines, sprains and strains, menstruation pain, and arthritis can all be treated with flurbiprofen. It is also recommended to reduce discomfort following surgery[1].

The way that flurbiprofen functions is by preventing your body's cyclo-oxygenase (COX) enzymes from doing their job. These enzymes aid in the body's production of prostaglandins, another type of molecule. At the locations of damage or injury, some prostaglandins are created, which results in pain and inflammation. Pain and inflammation are reduced because fewer prostaglandins are generated when COX enzymes are blocked[2].

Additionally, flurbiprofen is sold as throat lozenges and eye drops. Two different medication pamphlets named Flurbiprofen eye drops and Flurbiprofen lozenges have more information about these[3].

Most non-steroidal anti-inflammatory drugs (NSAIDs) block cyclooxygenase in a non-selective manner, thereby inhibiting the enzymes COX-1 and COX-2. Flubiprofen is part of a class of medications known as propionic acid derivatives. This prescription is a suitable candidate for a controlled or sustained-release medicine because it requires three to six daily doses and has a plasmatic half-life of one to two hours, potentially limiting drug release in the upper GI tract. The kind and amount of polymer utilized in the preparations has a big impact on how quickly the medication releases from the dosage form[4].

We developed a sustained-release formulation specifically for patients who required reasonably consistent blood levels over an extended period, thereby eliminating the need for multiple dosage schedules[5].The sustained release drug delivery system (SRDDS) seeks to minimize side effects while releasing medication at a predetermined rate. The main goal of developing SR formulations was to improve the way drugs functioned by lengthening their half-lives, decreasing the frequency of administration, minimizing side effects, lowering the required dose, and delivering the medication in the shortest amount of time using the least amount through the most effective route[6-8]. Therefore, the current work aims to develop flubiprofen tablets that gradually release medication through a variety of hydrophilic polymers. We employ the hydrophilic polymers of HPMC K100M, HPMC K4M, and Carbopol 940 for tablet formulation, and add relevant additives using the direct compression process[9].

Methods

Materials[10-15]

Flubiprofen, magnesium stearate, and talc are procured from a research lab chem industry in Mumbai, and HPMC K 100 m, HPMC K 4M, carbopol, avicel, and lactose are the other excipients; they were collected from the Modern Industry C-74, MIDC Malegaon, India.

Pre-formulation study

Characterization of drug

The organoleptic properties of flubiprofen: The organoleptic characteristics of the Flubiprofen drug sample, such as color and odor.

Melting point determination: The capillary technique was used to determine the drug's melting point.

Solubility determination: Different solvents, including water, alcohol (ethanol/ ethyl alcohol), ether, acetone, and chloroform, were used to test the drug's solubility.

UV spectra (λ max): Flubiprofen 100 mg was weighed, transferred into a 100 ml volumetric flask, and then mixed with alcohol to make 100 ml. It contained 10 mg/ml of the normal stock solution of flubiprofen. Dilutions were made from this solution, and max was then determined.

Standard curve of flubiprofen: The flubiprofen is dissolved in ethanol and then prepared the 5 dilutions 10 µg/ml, upto 50 µg/ml) and calculate the absorbance with the help of UV.

Formulation table

Formulation table of sustained release tablet of flubiprofen represents in table 1.

Table 1. Formulation table of sustained release tablet of flubiprofen

Sr. No	Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
1	Flubiprofen	200	200	200	200	200	200	200	200
2	HPMC K100M	10	15	15	10	15	10	10	15
3	Carbopol	15	20	15	20	15	20	15	20
4	HPMC K4M	20	20	20	25	25	20	25	25
5	Avicel	15	15	15	15	15	15	15	15
6	Magnesium Stearate	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
7	Talc	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
8	Lactose	QS	QS	QS	QS	QS	QS	QS	QS

Sr. No: Serial Number, QS: Quantity sufficient

Total weight of each tablet =320 mg

Method of preparation

The Various batch formulations of tablets (F1-F8) were made using the direct compression technique. Flubiprofen, a pure medication, and the polymers, HPMC K4M and K100M Carbapol, were each passed through #40 sieves separately before being thoroughly combined in a mortar and pestle for ten minutes. After going through #40 sieves, lactose and Avicel were added to this mixture and vigorously mixed for five minutes. After going through #60 sieves, there was adequate talc and magnesium stearate to lubricate this powder blend. Next, using 8 mm circular punches to hardness 4-5 kg/cm^3, the necessary amount of powder was weighed and manually fed into the single punch rotary machine to manufacture tablets weighing 320 mg[16,17].

Experimental design

The experimental design was used to optimize the processing parameters. Developing and improving medication delivery systems is frequently accomplished through the application of response surface methodology (RSM). Using a range of experimental designs, polynomial equations are generated, and the response is mapped throughout the experimental domain to determine the optimal formulation or formulations, all in accordance with the design of experiments (DOE) principle. The procedure is substantially more cost-effective and efficient than the conventional methods of producing dose types since it requires the least amount of effort and experimentation. The current study has chosen to maximize yield percentage and flow property while lowering moisture content as its objective function. The formulation parameters were statistically optimized using the factorial design, which also allowed for the evaluation of the primary, interaction, and quadratic impacts of the spray dryer's process parameters on the co-excipient's moisture content, yield percentage, and compressibility index. Using Design Expert, the quadratic and linear response surfaces were investigated using a 3-factor, 3-level design. Based on the Design Expert software's analysis of variance (ANOVA) feature, the polynomials' statistical validity was determined. A significant threshold of p & it; 0.05 was used. Formulation and Development of Flubiprofen Sustained Release Tablet: The mathematical model that fitted the data the best was chosen through comparison of various statistical parameters, such as the predicted residual sum of squares (PRESS), the multiple correlation coefficient (R^2), the adjusted multiple correlation coefficient (adjusted R^2), and the coefficient of variation (CV)[18-20].

When comparing the selected model to the other models being considered, PRESS—a measure of how well the model matches the data—should be small. The 2-D contour plots and 3-D response surface graphs were also generated by the Design Expert® application. To illustrate how the elements, interact to affect answers, these graphs are helpful. The experimental design was employed to maximize the processing parameters[21].

Levels selection of parameters of flubiprofen sustained released tablet:

The trial batch served as the foundation for choosing the level of independent elements. Table 2 shows the translation of the coded level in actual units. Table 3 shows Factorial design was used in the experimental design to optimise the processing parameters of the flubiprofen sustained-release tablet[22].

Dependent factors (response)

- Friability
- Dissolution

Table 2. Translation of the coded level in actual units

Sr. No.	Coded Level	Independent Factor		
		HPMC K4M (X1)	HPMC K100M (X2)	Carbopol (X3)
1	Lower Level (-1)	10	15	20
2	Higher Level (+1)	15	20	25

Table 3. Factorial design was used in the experimental design to optimise the processing parameters of the flubiprofen sustained-release tablet.

Sr. No.	Run	Friability (Factor 1)	Dissolution (Factor 2)
1	1	0.518	74.42
2	2	0.515	99.25
3	3	0.520	99.11
4	4	0.520	92.18
5	5	0.518	97.02
6	6	0.512	82.31
7	7	0.520	92.08
8	8	0.526	79.58

Evaluation Parameters of Flubiprofen Tablet

Pre-formulation evaluation methods[23-26]

Determine the bulk density

Flubiprofen granules' bulk density was calculated by measuring the volume of the packing after a weighed quantity of granules was added.

Formula-

$$Bulk\ density = \frac{Weight\ of\ the\ powder}{Volume\ of\ the\ packing}$$

Tapped density

The tapping method was used to determine the tapped density. Once the beginning volume was noted, a predefined quantity of granules was placed within a measuring cylinder. Next, tapping the cylinder was done until the granules' volume stopped changing. The final volume of the tapped packing was then recorded.

Formula -

$$\boldsymbol{Tapped\ density = \frac{weight\ of\ the\ powder}{volume\ of\ the\ tapped\ package}}$$

Carr's index

The compressibility index of the granules was calculated using Carr's index. The proportion Carr's index can be computed using the process below.

Formula -

$$Carr's\ index\ (\%) = \frac{TD - BD}{TD} \times 100$$

Haunser's ratio

The Hausner ratio is the ratio of the bulk density to the tapped density. The granule flow is judged as unsatisfactory if the Hausner ratio is greater than 1.25, while flow properties are regarded as excellent if it is less than 1.25.

Formula-

$$Haunsers\ ratio = \frac{Tapped\ density}{bulk\ density}$$

Angle of repose

The angle of repose is calculated as the arctangent of the ratio between the height (h) and radius (r) of a conical powder pile. It can be achieved within the space between the horizontal plane and the surface of the powder heap that is not supported by anything. The fixed funnel is positioned with its tip set at a vertical distance h above the graph paper, which is laid out on an even and level surface. The powder is progressively added to the funnel until the conical heap's peak is just barely touching the funnel's tip.

Formula -

$$Angle\ of\ repose = tan - 1\frac{h}{r}$$

Where,

r = Radius of the base of the pile

h = Height of the pile

θ= The angle of repose

Post-compression evaluation methods[27-30]

Hardness

The strength of the pill is indicated by its hardness. By calculating the force required to break the tablet, you may test it. The force is measured in kg. Calculate the hardness of 5 tablets from the formulation.

Percent friability

Tablet strength is gauged by friability. In this test, many tablets are dropped to a distance of 6 inches during each rotation of a plastic chamber rotating at a speed of 25 rpm, subjecting the tablets to the combined effects of shock and abrasion. In Roche friability, a sample of pre-weighed tablets was put, and the device was then turned on and off 100 times. The tablets were afterward cleaned and reweighed. Generally speaking, a weight decreases of less than 1 % is acceptable. The formula used to compute percent friability (% F),

Formula -

$$Percent\ friability = \frac{Initial\ Weight - Final\ weight}{Initial\ weight} \times 100$$

Dimension (thickness and diameter)

The thickness and diameter of the tablets determined the uniformity of tablet size. The diameter and thickness of the tablets were measured with a Vernier caliper. For each type of formulation, five tablets were used to calculate the average values.

Disintegration

To find each formulation's disintegration time, we used six Tablets. The pH 6.8 phosphate buffer solution, which served as the disintegration medium, was carefully kept at 37±0.5°C. Using a media capacity of 900 ml, the average disintegration time of six tablets was determined.

In-vitro dissolution studies

The dissolve experiments for the flubiprofen SR tablets were carried out using the USP dissolving testing apparatus II, namely the paddle type. For the first two hours of the in-vitro dissolving experiment, an acidic solution (0.1 N HCl) was used. After that, the mixture was spun at 50 revolutions per minute and 37°C while the 6.8-pH phosphate buffer in 900 mg was utilised as the dissolving medium. Every six hours, 5 mg of the material were taken out of the dissolving apparatus. An equivalent volume of medium was utilised in place of the samples. The absorbance of these solutions was measured with a UV spectrophotometer, and the result was 215 nm. The drug concentration released at various time intervals was calculated using the traditional graph. To analyze the drug release pattern, plotting the cumulative proportion of medication release against time was done. The rates of drug release were established[31,32].

Results

Preformulation study

Organoleptic properties of flubiprofen: The sample of flubiprofen was studied for organoleptic such as color is White, odour is slight and appearance is crystalline power.

Melting point determination result: Flubiprofen melting point was measured by using the capillary technique. According to IP, the melting point of flubiprofen was found to be between 74 °C to 76 °C.

Solubility determination: The solubility of flubiprofen was checked in different solvents and it is soluble in ethanol and is insoluble or partially soluble in Water.

UV spectrum (λ max):

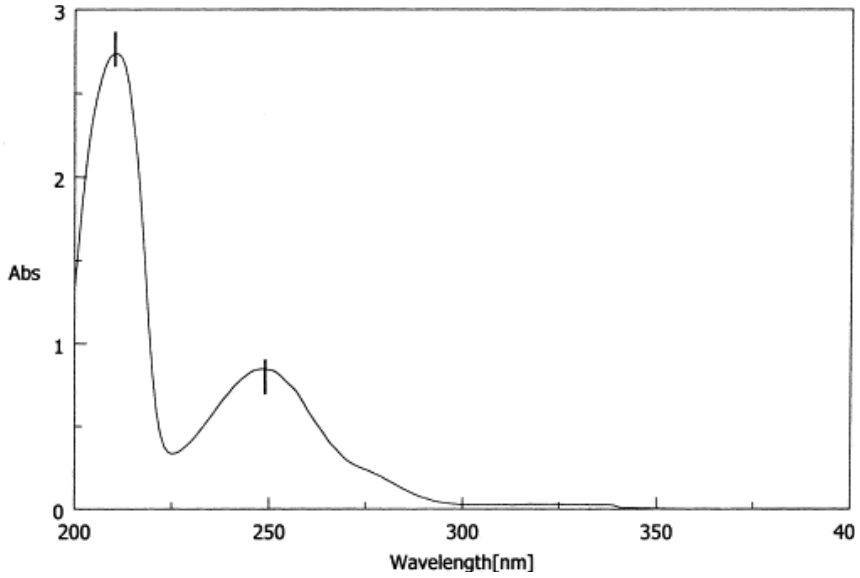

Figure 1. UV visible Spectrum of flubiprofen in ethanol

Standard curve of Flubiprofen

Table 4. Concentration and absorbance of flubiprofen drug

Sr. No.	Concentration (ppm)	Absorbance
1	10	0.177
2	20	0.261
3	30	0.295
4	40	0.393
5	50	0.505

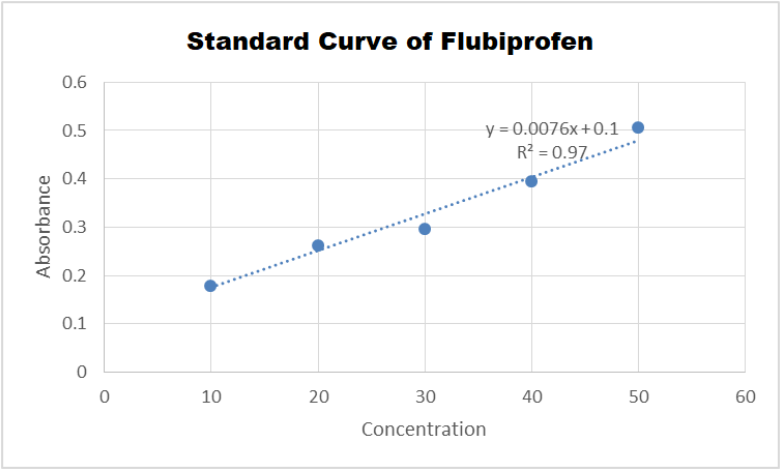

Figure 2. Calibration curve of flubiprofen

The flubiprofen was scanned and the wavelength (max) was found to be 215 nm in ethanol using a UV spectrophotometer. It was found that flubiprofen shows absorbance in the UV range of 200 to 350 nm. The equation of the regression line was obtained Y= 0.0079x + 0.0898 and the regression value R^2= 0.97. Figure 1 shows UV visible spectrum of flubiprofen in etanol and figure 2 shows calibration curve of flubiprofen.

Evaluation parameters

Pre-compression parameters

Table 5. Pre-compression Evaluation parameters results

Sr. No	Batch	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose (θ)	Haunser s Ratio	CI (%)
1	F1	0.40	0.48	32.61	1.20	16.66
2	F2	0.40	0.47	32.61	1.17	14.89
3	F3	0.39	0.49	32.52	1.25	20.40
4	F4	0.38	0.52	33.06	1.36	26.92
5	F5	0.40	0.50	31.59	1.25	20.00
6	F6	0.41	0.52	33.22	1.26	21.15
7	F7	0.43	0.47	33.42	1.09	8.51
8	F8	0.41	0.52	32.29	1.21	21.15

CI: Compressibility Index

A number of characteristics, including as tapped density, bulk density, Haunser's ratio, Carr's compressibility index, and angle of repose, were evaluated for formulating flubiprofen and other excipients. flubiprofen and the other excipients were also subjected to measurements and analyses of these parameters. The bulk density ranged from 0.38 to 0.43 g/cm^3, and the tapped density ranged from 0.47 to 0.52 g/cm^3. It was found that both values fell within the required range. These two density measurements were used to calculate Carr's compressibility index. Hausner's ratio and the compressibility index were used to find that all powder mixes had flow characteristics that ranged from good to acceptable.

The compressibility index ranged from 8.57 % to 26.92 %, and the Hausner's ratio varied from 1.09 to 1.36. The angle of repose provided the best explanation for the flow characteristic of all powder combinations. It was found that the angle of repose ranged from 32.29° to 33.42°. All powder blends exhibited well to acceptable flow characteristics, according to the angle of repose test (Table 5).

Post-compression evaluation parameters result

Table 6. Evaluation parameters result of post compression

Sr. No	Formulation n number	Hardness (kg/cm^2)	Thickness (mm)	Diameter	Friability (%)	Weight Variation
1	F1	4.00	5.776	8.00	0.518	315
2	F2	4.00	5.846	8.00	0.515	320
3	F3	5.00	5.798	8.00	0.520	317
4	F4	4.00	5.822	8.00	0.520	319
5	F5	5.00	5.832	8.00	0.518	320
6	F6	4.00	5.832	8.00	0.512	322
7	F7	5.00	5.776	8.00	0.520	321
8	F8	4.00	5.940	8.00	0.526	320

The table displays the post-compression parameters for all formulas. The thickness ranged from 5.766 mm to 5.846 mm, suggesting a consistent thickness across all samples. All formulations had a diameter of 8mm and there was no statistically significant difference seen. The hardness of all formulations falls within the range of 4.00 to 5.00 kg/cm^2. The friability, which measures the tendency of the tablet to crumble or break, was found to be less than 1%, showing that the tablets have good integrity (Table 6).

In-vitro dissolution studies result

Table 7. In vitro dissolution studies of different batches.

Sr. No	Buffer Medium (pH)	Time	F1	F2	F3	F4	F5	F6	F7	F8
1	0.1 N HCl	00	00	00	00	00	00	00	00	00
2		1	10.22	4.01	3.08	5.06	3.87	3.47	9.432	14.86
3		2	20.84	20.17	27.57	31.95	15.27	9.45	16.23	33.47
4	Phosphate buffer 6.8 pH	3	28.32	52.29	38.70	55.00	18.51	18.34	38.14	53.96
5		4	35.05	66.49	49.48	65.58	33.52	18.92	52.52	58.04
6		5	46.40	81.79	65.95	75.84	62.13	31.50	67.00	59.78
7		6	56.78	86.38	88.41	84.75	79.4	50.37	81.80	63.76
8		7	63.46	87.46	94.09	90.46	90.42	63.83	90.89	71.73
9		8	74.42	99.25	99.11	92.78	97.02	82.31	92.08	79.58

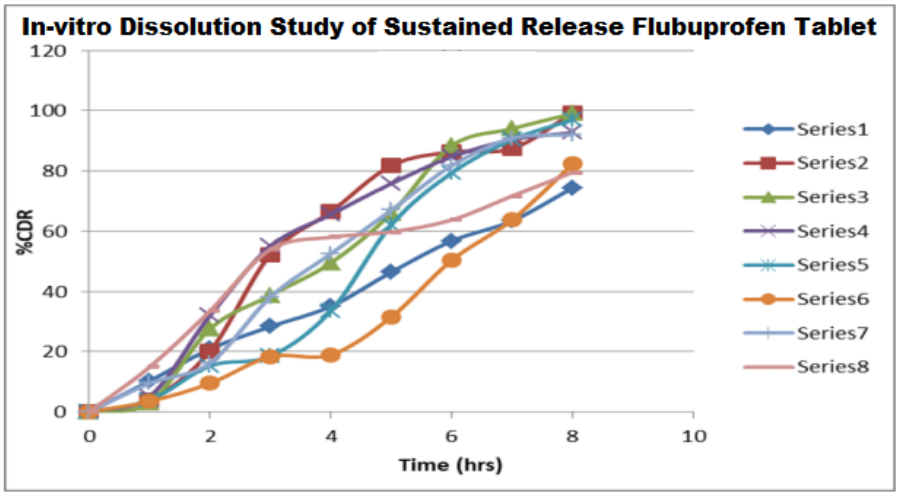

Figure 3. In-vitro dissolution study of flubiprofen sustained release tablets

The USP type II dissolution test apparatus paddle type was used to investigate the dissolution of the manufactured formulation using 900 ml of phosphate buffer solution (PH 6.8). The medication release data expressed as a percentage in a table. The F2 formulation in this formulation research demonstrates 99.25% drug release (Table 7 and Figure 3).

Discussion

We examined the Flubiprofen sample's white color, mild flavor, and crystalline power look. Flubiprofen melts at 74–76°C, according to IP. It dissolves in ethanol, whereas water does not. We used a UV spectrophotometer to scan Flubprofen in ethanol and discovered the maximum wavelength at 215 nm. We found that flubiprofen absorbs 200–350 nm UV light. When synthesizing flubiprofen and other excipients, we considered tap density, bulk density, Haunser's ratio, Carr's compressibility index, and angle of repose. We tested and studied flubiprofen and other excitants. The bulk density was 0.38–0.43 g/cm^3, while the tapped density was 0.47–0.52. Both values met the criteria. Using these two densities, we estimated Carr's compressibility index. According to Hausner's ratio and compressibility index, all powder blends had a good to acceptable flow. The compressibility index was 8.57 %–26.92 %, and Hausner's ratio was 1.09–1.36. Angle of Repose provided the best explanation for the angle of flow in all powder combinations. The reposing angle was 32.29°–33.42°. The angle of repose tests showed that all powder blends had a good flow.

The thickness ranged from 5.766 to 5.846 mm, indicating consistency between samples. All formulations were 8 mm in diameter, and there was no statistical difference. All formulations are 4.00–5.00 kg/cm^2 hard. The tablets' friability was less than 1 %, indicating good integrity. We tested the formulation's solubility with 900 ml of phosphate buffer solution (PH 6.8) and the USP type II paddle-type dissolution test device. A table presents the data on the percentage of drug release. This investigation shows that the F2 formulation releases 99.25 % of the medication.

Conclusion

In this study, the sustained release tablets of flubiprofen were formulated and developed, for the formulation development Quality by design was used. The direct compression methods were used to

formulate sustained-release flubiprofen tablets, the goal of the current work study was established. Controlled release formulations are designed for achieving plasma drug concentrations for a longer duration by controlling the rate, time, and site of the drug release. Utilizing a suitable polymer, such as HPMC K100M, HPMC K4M, and Carbopol, the formulation was prepared. The final product was tested for friability, hardness, diameter, thickness, and in-vitro release after being crushed into tablets. The dissolution experiments were conducted using acidic solutions (0.01 N HCl for the initial two hours, followed by six hours in the pH of the phosphate buffer is 6.8). The study concluded that the investigation of in vitro dissolution was carried out successfully. According to the formulation result, batch F2 exhibited the greatest drug release rate of 99.25 %. Therefore, batch F2 is considered the optimized batch

References

1. Jadhav KR, Ambade KW, Jadhav SL, Gambhire MN, Kurmi SD, Kadam VJ. Formulation and evaluation of flurbiprofen microemulsion. Curr Drug Deliv. 2008; 5(1):32-41. Doi: 10.2174/156720108783331032

2. Kumar S. Proniosomal gel of flurbiprofen: formulation and evaluation. J drug Deliv Therap. 2012;2(1). Doi: 10.22270/jddt.v2i1.31

3. Mettu SR, Veerareddy PR. Formulation, evaluation and pharmacokinetics of flurbiprofen fast dissolving tablets. Brit J Pharm Res. 2013;3(4):617-31.

4. Swain RP, Kumari TR, Panda SA. Formulation development and evaluation of sustained release ibuprofen tablets with acrylic polymers (eudragit) and HPMC. Int J Pharm Pharmaceut Sci. 2016;8(2):131-5.

5. Radhika PR, Pal TK, Sivakumar T. Formulation and Evaluation of Sustained Release Matrix Tablets of Glipizide: Sustained release matrix tablets of glipizide. Iran J Pharm Sci. 2009; 5(4):205-14. Doi: 10.22037/ijps.v5.41224

6. Bhowmik D, Yadav R, Jayakar B, Kumar KS. Formulation and evaluation the oral tablets ibuprofen. The Pharma Innovation. 2012;1(9, Part A):32. Doi: NA

7. Ratnaparkhi MP, Gupta Jyoti P. Sustained release oral drug delivery system-an overview. Terminol. 2013;3(4):10-22270.

8. Bakre LG, Akinyele E, Bamiro O, Adeleye O, Kunle O. Formulation and evaluation of sustained release ibuprofen matrix tablets using starch from maize genotypes as polymer. Acta Marisiensis-Seria Medica. 2021;67(2):122-6. Doi: 10.2478/amma-2021-0018

9. Sharma V, Sharma S, Khokra SL, Sahu RK, Jangde R, Singh J. Formulation, development and evaluation of Pregabalin sustained release matrix tablets. Der Pharmacia Lettre. 2011;3(5):326-3.

10. Dabbagh MA, Beitmashal L. Sustained release formulation and in-vitro evaluation of ibuprofen-hpmc matrix tablets. Jundishapur J Nat Pharm Prod. 2006 Dec 20;1(1):1-7.

11. Joshi RS, Nangare AK, Sanap DS, Sase SM. Development and Validation of UV-Spectrophotometric Method for Estimation of Metformin Hydrochloride and Pioglitazone in Tablet Dosage Form. J Drug Deliv and Therap. 2019 Aug 30;9(4-A):381-4. Doi: 10.22270/jddt.v9i4-A.3499

12. Lopes CM, Manuel Sousa Lobo J, Costa P, Pinto JF. Directly compressed mini matrix tablets containing ibuprofen: preparation and evaluation of sustained release. Drug Dev Ind Pharm. 2006;32(1):95-106. Doi: 10.1080/03639040500388482

13. Sahilhusen IJ, Mukesh RP, Alpesh DP. Sustained release drug delivery systems: A patent overview. Aperito J Drug Desig a Pharmacol. 2014 Oct;1(1):1-4.

14. Alhalmi A, Altowairi M, Saeed O, Alzubaidi N, Almoiliqy M, Abdulmalik W. Sustained release matrix system: an overview. World J Pharm Pharm Sci. 2018; 7(6):1470-86.

15. Almeida H, Amaral MH, Lobão P. Prolonged-release solid dispersions of ibuprofen. J Appl Pharm Sci. 2012; 2(6):67-70. DOI: 10.7324/JAPS.2012.2603

16. Bhowmik D, Yadav R, Jayakar B, Kumar KS. Formulation and evaluation the oral tablets ibuprofen. The Pharma Innovation. 2012 Nov 1;1(9, Part A):32.

17. Uddin M, Halder S, Kumar U, Rouf AS. Design and evaluation of once daily losartan potassium sustained release matrix tablet. Int J Pharm Sci Res. 2014;5(2):519. Doi: 10.13040/IJPSR.0975-8232.5(2).519-25

18. Guerra-Ponce WL, Gracia-Vásquez SL, González-Barranco P, Camacho-Mora IA, Gracia-Vásquez YA, Orozco-Beltrán E, Felton LA. In vitro evaluation of sustained released matrix tablets containing ibuprofen: a model poorly water-soluble drug. Braz J Pharm Sci. 2016; 52: 751-9. Doi: 10.1590/S1984-82502016000400020

19. Hapse SA, Kadaskar PT, Shirsath AS. Difference spectrophotometric estimation and validation of ibuprofen from bulk and tablet dosage form. Der Pharmacia Lettre. 2011;3(6):18-23.

20. Patil SJ, Surana KR, Mahajan SK. Quantification of active phytoconstituents in ethanolic extract of Mentha piperita by modern analytical tools. Res Jr Agril Sci. 2024; 15(3): 615-21.

21. Patil SJ, Surana KR, Mahajan SK. In vitro antimicrobial and antifungal activity of Mentha piperita active phytoconstituents. Res Jr Agril Sci. 2023; 14(6): 1875-7.

22. Sonawane VN, Suryawanshi KG, Wagh KH, Sonawane SL, Sonar AD, Sakle SJ, Sonawane DD. A Comparative Study of Dissolution Profiles on Various Brands of Diclofenac Sodium Prolonged Release Tablet Formulation. Prog Chem Biochem Res. 2023; 6(4):341-55. Doi: 10.48309/pcbr.2023.410170.1271

23. Patel N, Madan P, Lin S. Development and evaluation of controlled release ibuprofen matrix tablets by direct compression technique. Pharm Develop Tech. 2011;16(1):1-1.Doi: 10.3109/10837450903460467

24. Lakio S, Tajarobi P, Wikström H, Fransson M, Arnehed J, Ervasti T, Simonaho SP, Ketolainen J, Folestad S, Abrahmsén-Alami S. Achieving a robust drug release from extended release tablets using an integrated continuous mixing and direct compression line. Int J Pharma. 2016; 511(1):659-68. Doi: 10.1016/j.ijpharm.2016.07.052

25. Wahab A, Khan GM, Akhlaq M, Khan NR, Hussain A, Zeb A, Rehman A, Shah KU. Pre-formulation investigation and in vitro evaluation of directly compressed ibuprofen-ethocel oral controlled release matrix tablets: A kinetic approach. Afr J Pharmacy Pharmacol. 2011;5(19):2118-27. Doi: 10.5897/AJPP11.128

26. Modi A, Pandey A, Singh V, Bonde CG, Jain D, Shinde S. Formulation and evaluation of fast dissolving tablets of diclofenac sodium using different superdisintegrants by direct compression method. Pharmacia. 2012; 1(3):95-101.

27. Guerra-Ponce WL, Gracia-Vásquez SL, González-Barranco P, Camacho-Mora IA, Gracia-Vásquez YA, Orozco-Beltrán E, Felton LA. In vitro evaluation of sustained released matrix tablets containing ibuprofen: a model poorly water-soluble drug. Braz J Pharm Sci. 2016; 52:751-9. Doi: 10.1590/S1984-82502016000400020

28. Gil-Chávez J, Padhi SS, Leopold CS, Smirnova I. Application of Aquasolv Lignin in ibuprofen-loaded pharmaceutical formulations obtained via direct compression and wet granulation. Int J Biol Macromol. 2021; 174:229-39. Doi: 10.1016/j.ijbiomac.2021.01.064

29. Ervasti T, Simonaho SP, Ketolainen J, Forsberg P, Fransson M, Wikström H, Folestad S, Lakio S, Tajarobi P, Abrahmsén-Alami S. Continuous manufacturing of extended release tablets via powder mixing and direct compression. Int J Pharma. 2015; 495(1):290-301.Doi: 10.1016/j.ijpharm.2015.08.077

30. Wiranidchapong C, Ruangpayungsak N, Suwattanasuk P, Shuwisitkul D, Tanvichien S. Plasticizing effect of ibuprofen induced an alteration of drug released from Kollidon SR matrices produced by direct compression. Drug Dev Ind Pharm 2015;41(6):1037-46. Doi: 10.3109/03639045.2014.925917

31. Wei Q, Yang F, Luan L. Preparation and in vitro/in vivo evaluation of a ketoprofen orally disintegrating/sustained release tablet. Drug Dev Ind Pharm. 2013; 39(6):928-34. Doi: 10.3109/03639045.2012.664146

32. Shittu AO, Njinga SN, Joshua A. Formulation and Evaluation of Fast Disintegrating Oral Tablets of Ibuprofen using Mannitol, PEG 4000, Cellactose and Sodium Starch Glycolate via Direct Compression: https://doi. org/10.51412/psnnjp. 2022.32. Niger J Pharm. 2022; 56(2). Doi: 10.51412/psnnjp.2022.32

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Artículos originales

Evaluation of the Probiotic Potential of *Bacillus velezensis SNR14-4* Strain from Nile Tilapia Gills Using Genomic and In Vitro Approach

Evaluación del potencial probiótico de la cepa *Bacillus velezensis SNR14-4* de branquias de tilapia del Nilo mediante un enfoque genómico e in vitro

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Conflict of interest

The authors declare no conflict of interest.

Resumen

Introducción: La presente investigación evaluó una nueva cepa *de Bacillus velezensis SNR14-4*, aislada de las branquias de tilapia del Nilo con la intención de considerarla como un contendiente probiótico prometedor.

Métodos: Inicialmente, se llevó a cabo un análisis extenso del genoma del aislado particular empleando herramientas bioinformáticas para anticipar sus características y potenciales atributos probióticos. El genoma total de SNR14-4, reconocido como *B. velezensis* mediante ARNr 16S y secuenciación del genoma completo y análisis filogenético, está compuesto por un cromosoma circular singular con un tamaño de genoma de 4,1 Mb, una longitud total de 4.183.910 pb y una longitud media de 4183910 pb. contenido de guanina-citosina (GC) del 46,52 %. Se adquirieron conocimientos valiosos utilizando AntiSMASH para detectar grupos de genes biosintéticos de metabolitos secundarios, y se logró la anotación de genes funcionales relevantes para los rasgos probióticos utilizando RASTtk y PROKKA. La ausencia de elementos de virulencia, determinada mediante análisis genómico, facilitó una exploración in vitro específica.

Resultados: SNR14-4 mostró características probióticas notables y demostró eficacia antimicrobiana contra patógenos comunes de los peces. El análisis HR-LCMS QTOF del extracto microbiano reveló varios compuestos antimicrobianos potentes sintetizados por la cepa.

Conclusiones: *B. velezensis* SNR14-4 se muestra prometedor como candidato a probiótico, ya sea como punto de fuente único o como parte de consorcios de probióticos formados por cepas similares.

Palabras clave: Probióticos; Acuicultura; Antimicrobiano; secuencia del genoma

Abstract

Introduction: The current investigation assessed a novel strain of *Bacillus velezensis SNR14-4*, isolated from the gills of Nile tilapia intending to consider it as a promising probiotic contender.

Methods: Initially, an extensive analysis of the genome of the particular isolate was carried out employing bioinformatics tools to anticipate its characteristics and potential probiotic attributes. The total genome of *SNR14-4*, recognized as *B. velezensis* via 16S rRNA and whole-genome sequencing and phylogenetic analysis, is composed of a singular circular chromosome with a genome size of 4.1 Mb, a total length of 4183910 bp, and an average guanine-cytosine (GC) content of 46.52 %. Valuable insights were acquired utilizing AntiSMASH to detect secondary metabolite biosynthetic gene clusters, and functional gene annotation relevant to probiotic traits was accomplished by utilizing RASTtk and PROKKA. The absence of virulence elements, ascertained via genomic analysis, facilitated a targeted *in vitro* exploration.

Results: *SNR14-4* displayed notable probiotic characteristics and exhibited antimicrobial efficacy against common fish pathogens. HR-LCMS QTOF analysis of the microbial extract unveiled several potent antimicrobial compounds synthesized by the strain.

Conclusions: *B. velezensis SNR14-4* showcases promise as a probiotic candidate, either as a single point of source or as a part of probiotic consortia made of similar strains.

Keywords: Fish immunity; Probiotics; Aquaculture; Antimicrobial; Genome sequence

Highlights

A novel strain of *Bacillus velezensis* SNR14-4 was identified from Nile tilapia which showed no virulence factors. SNR14-4 showed probiotic features and antimicrobial activity *in vitro* against prevalent fish pathogens and also shown to produce many bioactive components. Genetic analysis of SNR14-4 showed that the stain could be a potential probiotic candidate that might play a valuable role if it is to be incorporated in fish/animal feed or combined with other candidates as a probiotic consortium.

As one of the top ten fishes consumed on a worldwide scale, Tilapia culture requires shielding from bacterial infections that can lead to economic losses for farmers. The most common bacterial pathogens infecting fish, especially tilapia, *Vibrio parahaemolyticus, Listeria monocytogenes,* and *Flavobacterium psychrophilum*, are identified and defended by the use of antibiotics when they lead to infection. Although antibiotics have made it easier to treat these pathogenic diseases in fish, the birth of new antibiotic-resistant mutants is a serious threat to tilapia aquaculture and worldwide fish production.

New microbes with potential antibiotic and probiotic characters are required due to the increased resistance of bacteria. This study provides the new strain with all these properties.

This strain has the potential probiotic properties that might play a valuable role if it is to be incorporated in fish/animal feed or combined with other candidates as a probiotic consortium.

Introduction

As one of the most common species of fish, *Oreochromis niloticu*s, commonly known as Nile tilapia, has contributed to more than 80 % of the edible fish population in the past few years[1]. As one of the top ten fishes consumed on a worldwide scale, Tilapia culture requires shielding from bacterial infections that can lead to economic losses for farmers[2]. The most common bacterial pathogens infecting fish, especially tilapia, *Vibrio parahaemolyticus, Listeria monocytogenes,* and *Flavobacterium psychrophilum*, are identified and defended by the use of antibiotics when they lead to infection[3,4]. Although antibiotics have made it easier to treat these pathogenic diseases in fish, the birth of new antibiotic-resistant mutants is a serious threat to tilapia aquaculture and worldwide fish production[5].

Probiotics have proven to be a good defense against the rising concern of using antibiotics leading to antibiotic-resistant mutants[6]. The current paper has been successful in revealing the probiotic features of a newly discovered strain of *B. velezensis*. Previously, the strain's candidacy in xenobiotic degradation was revealed using 'gene-before-lab' approach and it also addressed the existence of cryptic gene clusters[7]. The production of antimicrobial compounds essentially does not guarantee that these compounds are good candidates for use as probiotic supplements. *Bacillus spp*., a broad class of organisms, has long been known to produce many nontoxic compounds that can be effectively used in probiotics[8]. Numerous probiotic microbes have been used in aquaculture for many years[9], and *Bacillus* species are known for their large number of secondary metabolites that can act against fish pathogens by regulating water quality and the gut microbiota[10]. Mining natural sources such as water, soil, and air, for novel strains, could be strenuous and unpredictable due to the incompatibility of the isolates with fish *in vivo*.

Bacillus species, namely, those members of the operational group *Bacillus amyloliquefaciens*[11], with a keen focus on *B. velezensis* of this group, have proven to be a treasure house of secondary metabolites that can be applied to numerous industrial sectors[12]. Many researchers have previously explored the potential of *B. velezensis,* and their work has contributed to the discovery of many novel bioactive secondary metabolites that can be used as probiotics in animals, including fish[8, 13-16]. The majority of the probiotics used today are counterproductive, as most are originally obtained from non-fish candidates. The need for more effective, naturally adaptive probiotic candidates of fish origin has to have opted for prolonged and fully efficient immunity against fish pathogens. The proximity of antimicrobial-producing microbes in the mouth, gut, or gills can assure resistance against pathogens. As many pathogens can enter through the gills or mouth, it is necessary to protect fish of commercial value by supplementing feeds that contain probiotic isolates[17].

Methods

Characterization of the isolate

The sample collection, screening, isolation, and cultural and morphological characterization of the isolate were done previously[7]. Three healthy *Oreochromis niloticus* (Nile tilapia) were procured from the hatchery of Kerala University of Fisheries and Ocean Studies, located in Kochi, Kerala, India. The health status of the fish was ascertained to be optimal, devoid of any viral, bacterial, or fungal infections. Carefully, the gills of the fish were dissected and positioned onto sterile Petri dishes. A quantity of five grams of gill tissue was precisely measured, followed by rinsing with an equivalent amount of Phosphate-buffered saline (PBS) to cleanse the gills thoroughly. This rinsing procedure was repeated thrice to guarantee the elimination of all impurities and potential contaminants. Subsequently, the

gills were homogenized utilizing a sterile mortar and pestle in 3 ml of PBS. A milliliter of the homogenized specimen was then transferred to a sterile 10 ml screw cap tube along with 9 ml of distilled water to achieve a 10-1 dilution. The solution was subjected to further serial dilutions up to 10-4. Following this, 100 µl of the solution was inoculated onto nutrient agar plates and placed in an incubator at 37 °C for 24 hours. The predominant bacterial strain was subcultured multiple times to attain a pure culture, initially identified *via* microscopic examination and Gram staining.

Biochemical characteristics

The strain was tested for biochemical characteristics, and most of the protocols for the procedure have been adopted from previously conducted research on other strains of *Bacillus genus*[18,19,20,21]. The biochemical assays involved were the indole test, methyl red test, Voges–Proskauer test, citrate test, urease test, catalase test, hemolysis, starch hydrolysis, and spore formation. Each test was performed in triplicate.

DNA isolation and 16S rRNA sequencing

The DNA isolation was carried out using the protocol described by Green and Sambrook, 2107[22]. Briefly, cells were lysed using sodium dodecyl sulfate and proteinase K. The DNA was then extracted with phenol:chloroform, and precipitated with isopropanol. The 16S rRNA gene sequence analysis was carried out to accurately identify the species and the strain that have been isolated[23,24]. PCR amplification was performed using a PCR master mix (2X) from Emerald, with purified and spooled-out isolated DNA. PCR was initiated by denaturation at 94 °C for 5 mins, followed by 35 cycles of denaturation at 94 °C, annealing at 50 °C for 30 s, extension at 72 °C for 2 mins, and a final extension at 72 °C by holding the reaction mixture for 7 mins. The PCR products were analyzed by 1 % agarose gel electrophoresis. The product was subsequently sequenced using the universal bacterial primers forward primer 27F (AGAGTTTGATCCTG GCTCAG) and reverse primer 1492R (GGTTACCTTGTTACGACTT) through polymerase chain reaction (PCR). The 16S rRNA sequence homology was compared with 16S rRNA sequences of other *B. velezensis* strains available in the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). Similarity analysis was carried out to specifically identify the strain isolated. For further clarity, a phylogenetic tree was constructed using MEGA 11 software to establish an evolutionary relationship. The initially identified sequence was deposited in NCBI.

Gene-before-lab Approach: Whole-genome sequencing combined with de novo assembly

Whole-genome sequencing of the isolated strains was performed with the aid of Medgenome Labs Ltd., Bangalore, India. The quality of the raw data was assessed using the fastq-mcf tool (version – 1.04.8030), and a 2.2 GB file was generated. The sample was sequenced using NovaSeq after the removal of all human contaminants using the BWA-MEM tool (version 0.7.12). De novo assembly was performed using the SPAdes assembler (version. 3.11.1). ORF prediction followed by annotation of the WGS data was carried out using Prodigal software. Reference guide assembly was carried out, and consensus genome FASTA sequences were developed from the aligned bam files using the SAMtools and BCFtools versions. The identity of the isolate was evaluated *via* the genome-based taxonomic classification tool: Type Strain Genome Server (TYGS) run against nine other *Bacillus* members[25]. The tool was accessed through (https://tygs.dsmz.de/). Additionally, the strain identity was evaluated by using the Genome-to-Genome Distance Calculator (GGDC) software[26]. PubMLSt web service accessed through *https://pubmlst.org/species-id/* was used to compare multiple housekeeping genes that are conserved across related species[27]. Average Nucleotide Identity (ANI) was calculated using JSpeciesWS accessed *via https://jspecies.ribohost.com/jspeciesws/*[28].

Functional gene annotation, genome mining for Probiotic marker genes

The consensus genome FASTA file was also used for gene annotation and gene function prediction using several different web-based and online gene annotation pipelines, such as Prokka in Proksee (https://proksee.ca/), BAKTA (https://bakta.computational.bio/)[29], and DFAST (https://dfast.ddbj.nig.ac.jp/dfc/)[30]. The results were further cross-checked against the comprehensive database of the Bac-

terial and Viral Bioinformatics Resource Center (BV-BRC) (https://www.bv-brc.org/[31]. The Virulence Factor Database (VFDB) was used to analyze the presence of any virulence genes in the present isolate. The non-virulence properties were further evaluated by using Virulence Finder 2.0[32], AntiSMASH 7.0 was employed for the rapid and thorough genome-wide mining for secondary metabolite-synthesizing gene clusters and gene clusters encoding antibiotics[33]. The false prediction level was minimized by manually BLASTing the coding sequences of the biosynthetic gene clusters from AntiSMASH against Pfam[34].

Many *Bacillus* strains are known to be good probiotics and are used in feeds and food[35,36]. The probiotic marker genes were manually detected by citing previous studies on probiotic *Bacillus* spp., and the specific genes were searched in both BV-BRC/PATRIC, Prokka, and Protein Data Bank (PDB)[37,38,39]. The sequences were further confirmed using BLASTp against amino acid sequences in the NCBI database. Functional pathways were confirmed by the *Kyoto Encyclopedia of Genes and Genomes* (KEGG) database.

Antibiotic susceptibility and safety of the strain

The isolated strain was tested against several commercial antibiotics[40,41] purchased from HiMedia. *In silico* analysis was centered on the Resistance GeneIdentifier (RGI) (*https://card.mcmaster.ca/analyze/rgi*)[42]. ResFinder 4.5.0 was used to double-check the results of RGI[43,44]. ResFinder was accessed through http://genepi.food.dtu.dk/resfinder. The culture (24 hours) was swabbed using sterile cotton swabs on Mueller–Hinton agar plates with proper dilution for uniform growth. The antibiotic discs were carefully placed gently over the swabbed MHA plates and incubated overnight at 37 °C. The results were read by measuring the zone produced, in mm. Pathogen Finder 1.1 was used to evaluate the pathogenicity of the isolate towards the human host[45].

Bile tolerance, acid tolerance, and high-temperature resistance of the isolate

Genomic analysis data on stress-tolerant genes of probiotic relevance generated from PATRIC, PROKKA, and NCBI BLAST was used as a guiding principle for the *in vitro* evaluation. The evaluation of bile tolerance was carried out using a 24-h-old culture of an isolate that was inoculated in Luria Bertani broth supplemented with 0.30 % (w/v) bile salts at 30 °C and incubated for 0, 1, 2, 3, or 4 h[41,46]. After each hour of incubation, the viable cell count was calculated by streaking the incubated samples onto NA plates. Acid tolerance was determined by inoculating the isolate SNR14-4 into Luria Bertani broth with the pH adjusted to 2, 3, 4, or 5[36].

Antimicrobial activity

Based on AntiSMASH evaluation, antibacterial activity was evaluated by checking the activities against *L. monocytogenes, Fl. psychrophilum, V. parahaemolyticus.* The pathogenic strains were all previously isolated from infected fish hosts[8,16,47]. The antibacterial activity was evaluated initially by the well diffusion method using a cell-free supernatant of the *Bacillus* isolate SNR14-4. Minimal activity was observed against *Flavobacterium* and *V. parahaemolyticus,* and therefore, the crude cell extract was subjected to solvent extraction. The inoculum concentration was set to 1 x 10^{12} CFU/ml. The cell-free extract of SNR14-4 was further subjected to solvent extraction using different solvents, such as hexane, petroleum ether, and ethyl acetate. 200 ml of CFE were mixed with 200 ml of each solvent in a separating funnel and kept overnight for mass transfer of antimicrobial compounds. The respective fractions were then separated and evaporated using a rotary evaporator. The evaporated residue was dissolved in 0.5 % DMSO. The activity of each fraction was evaluated by the well diffusion method. Fifty microliters of the SNR14-4 sample were added to one well, 50 µl of 0.5 % DMSO was used as a control, and streptomycin was used as a positive control. To increase the reliability of the results obtained, nutrient media without the inoculate was subjected to the same treatment as carried out with the inoculated media. Solvent extraction of the uninoculated media was carried out using hexane, petroleum ether, and ethyl acetate. The uninoculated media was fractioned and evaporated and used as the negative control. The antimicrobial activity was seen as clear zones, whose diameter was measured in mm. The antibacterial activity was evaluated in triplicate.

Autoaggregation test

The autoaggregation test was carried out using previous reports on similar strains with some modifications[37]. A 24-hour-old culture of SNR14-4 in 10 ml of Luria media was incubated at 30°C, and the culture was centrifuged at 5000 × g for 15 mins. The pellet was washed with PBS, and after two washes, the pellet was resuspended in PBS at pH 7.2. The absorbance was measured at 620 nm after 1, 2, 3, 4, and 5 h of *incubation. The autoaggregation of B. velezensis* SNR14-4 was calculated by the following formula: autoaggregation % = (1-[A_t/A_0]) × 100, where A_t denotes the absorbance at time t = 0, 1, 2, 3 and 4 h and A_0 denotes the absorbance at time = 0 h.

Hydrophobicity assay

Hydrophobicity was measured by measuring the adhesion of the strains to organic solvents. For the determination of cell surface hydrophobicity, a protocol previously carried out for probiotic strains with slight modifications[48]. One ml of 24-hour culture was mixed with 100 µl of xylene. The mixture was vortexed for 1 min. After the phases were separated, the aqueous part was removed, the optical density was measured at 480 nm, and the hydrophobicity was calculated using the following equation: Hydrophobicity % = (H_0 – H_t)/H_0 × 100 %, where H is the optical density at 630 nm, H_0 is the initial optical density and H_1 is the final optical density.

Hemolytic activity

The activity of the isolated Bacillus strain was determined on agar plates supplemented with 5% human blood. The isolate was streaked onto agar plates from a 24-hour-old culture. Hemolysis activity was recorded as the extent of the hemolytic zone. Green zones represent α-hemolysis, and the plates were incubated at 37°C for 24-36 hours, clear zones represent β-hemolysis, and no zones represent no hemolysis.

HR-LCMS/MS–Q-TOF analysis

The ethyl acetate fraction that showed prominent activity was analyzed to detect the active component that exhibited antimicrobial activity using HR-LCMS-QTOF (Agilent Technologies, USA). Mass Hunter (Agilent) software was used for data acquisition. A ZORBAX Eclipse Plus – C18 (150 × 2.1 mm, 5 microns, Agilent) column was used with the following gradient solvent system (A: water; B: methanol 95:5), and sample profiling was carried out with a flow rate of 0.3 ml/min.

In silico ADME and toxicity analysis

The *in silico* prediction of the toxicity and ADME parameters, pharmacokinetic properties, druglike nature, and the medicinal chemistry friendliness of the molecules identified through HRLC-MS Qtof was evaluated using Swiss ADME software (http://www.swissadme.ch/) and protox-3.0 (https://tox.charite.de/protox3/) software.

Statistical analysis

All the tests were conducted in triplicate. The mean values of the observations taken from each replicate were used for statistical analysis. The mean values were statistically analyzed by the Web Agri Stat Package 2.0 (ICAR Goa), and the mean differences were compared at 5 % significance.

Results

Identification of the organism

A total of three isolates were recovered from the homogenized gill fraction. Only one of the three isolates showed appreciable and prominent growth. Hence, this strain was chosen as the primary focus of the present study. After streaking on nutrient agar, the isolated strains formed single colonies that were white, opaque, wrinkled, and dry. The edges were irregular, and the colony as a whole was flat and

rough in appearance with a lobate margin. When picked with an inoculating loop, the colony was sticky and dense. Microscopic observation revealed that the strain was a gram-positive rod, small in cell size, and showed positive results for endospore formation, exhibiting the ability to sporulate. Observations of cell morphology and culture characteristics revealed that the strain was a *Bacillus* species. The isolate showed positive results for methyl red, Voges-Proskauer, urease, starch hydrolysis, and catalase. The citrate utilization test, indole test, and oxidase test were negative. The experiment was carried out in triplicate and all generated the same result[7].

Molecular identification of the strain as *B. velezensis* SNR14-4

The 16S rRNA sequencing results were compared with other 16S rRNA sequences available in NCBI using BLAST. The isolated strain showed 99% similarity to *B. velezensis*. The phylogenetic tree constructed using MEGA 11 software (Fig 1) shows the clade distance score, which confirmed that the organism was a novel strain of *B. velezensis*.

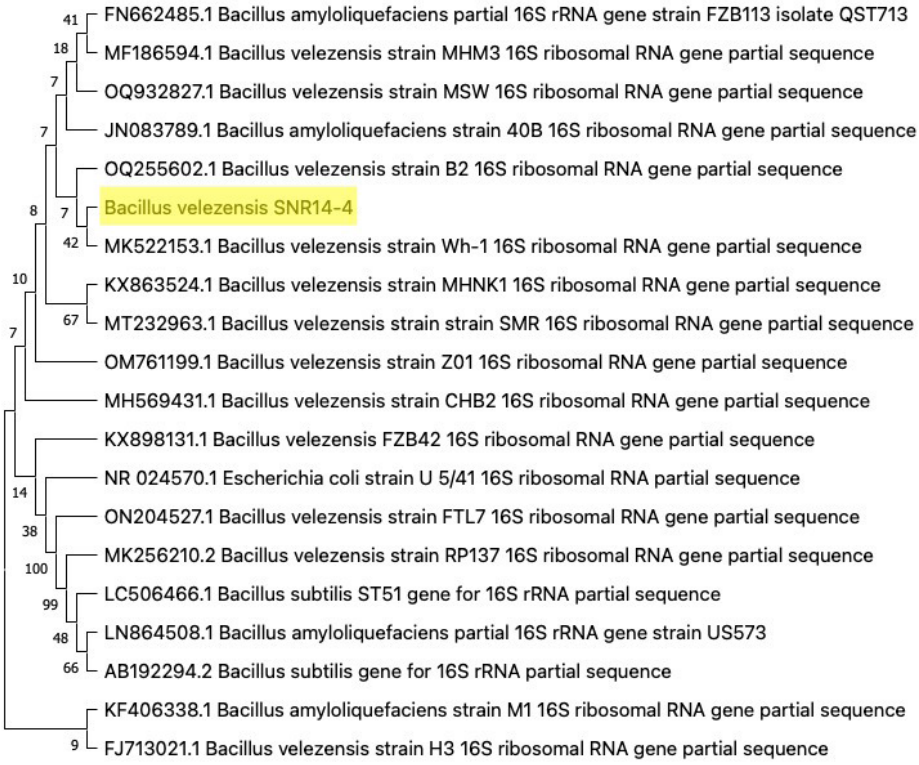


Figure 1. Phylogeny of the isolated strain. Phylogenetic tree constructed by the neighbor-joining method using MEGA 11 software, showing the relatedness of *B. velezensis* SNR14-4 to other *B.* species (Madhavan et al., 2024).

A detailed phylogentic analysis was done on the strain previously while exploring the genomic potential of the strain by the same authors and the obtained 16S rRNA sequence was submitted to GenBank under the designation *B. velezensis* SNR14-4 (accession number: OR226766)[7].

Insights into the complete genome assembly of *B. velezensis* SNR14-4

De novo assembly was completed using the SPAdes assembler (v3.11.1), and the assembly was executed with a k-mer size of 55 using the de-Bruijn graph method, which revealed that the number of scaffolds (>=0_bp) was 343, the N50 was 166965 bp, the N75 was 117388 bp, the L50 was 8 bp, and the L75 was 16 bp. The assembly created a 4.1 Mb file with a single circular chromosome and 1 contig with a genome length of 41,83,910 bp, with an average guanine-cytosine (GC) content of 46.52 %, which is consistent with the GC ratio of other *B. velezensis* variants[7]. The sample was aligned to the reference genome using NCBI BLAST, and the closest match was with *B. velezensis strain SGAir0473,* with an alignment percentage of 93.64 %. The strain was proposed as a novel. The sequence was then named *B. velezensis* strain *SNR14-4* and deposited in the SRA-NCBI database with Bioproject accession ID: PRJNA994302 (https://www.ncbi.nlm.nih.gov/sra/PRJNA994302). 16S rRNA sequencing analysis showed that the strain was *B, velezensis*. 16S rRNA sequencing analysis showed that the strain was *B. velezensis*. The strain's identity was confirmed using additional tools such as PubMLST, TYGS, JSpeciesWS and GGDC. TYGC analysis of the isolate against nine members of *Bacillus* genus showed that *B. velezensis* with NRRL B-41580 showed more similarity. GGDC data generated based on TYGC evaluation by comparing with several sample strains also showed that the strain is *B. velezensis* (Table 1).

Table 1. Genome-to-Genome Distance Calculation of different reference genome against Sample

Query genome	Reference genome	DDH	Model C. I	Distance	Prob.DDH>=70 %
Formula 1 (HSP length/total length)					
B. velezensis SNR14-4	*B. velezensis* F3A	87.20	83.6 - 90.1	0.0959	96.12
B. velezensis SNR14-4	*B. velezensis* ATR2	90.8	87.7 - 93.2	0.0765	97.56
B. velezensis SNR14-4	*B. velezensis* FZB42	85.6	81.9 - 88.6	0.1040	95.31
B. velezensis SNR14-4	*B. velezensis* CYS06	86.1	82.4 - 89.1	0.1017	95.16
B. velezensis SNR14-4	*B. velezensis* B8	87.6	84.1 - 90.4	0.0224	91.2
B. velezensis SNR14-4	*B. velezensis* R-71003	83.7	79.9 - 86.9	0.1135	94.15
B. velezensis SNR14-4	*B. velezensis* LF 01	86	82.3 - 89	0.1022	95.51
B. velezensis SNR14-4	*B. velezensis* CPA1-1	87.6	84 - 90.4	0.0939	96.3
Formula 2 (identities/HSP length)					
B. velezensis SNR14-4	*B. velezensis* F3A	81	78.1 - 83.6	0.0222	91.33
B. velezensis SNR14-4	*B. velezensis* ATR2	90.5	88.3 - 92.4	0.0115	95.97
B. velezensis SNR14-4	*B. velezensis* FZB42	85	82.3 - 87.4	0.0176	93.72
B. velezensis SNR14-4	*B. velezensis* CYS06	80.8	77.9 - 83.4	0.0224	91.2
B. velezensis SNR14-4	*B. velezensis* B8	83.4	80.6 - 85.9	0.0194	92.87
B. velezensis SNR14-4	*B. velezensis* R-71003	83.6	80.8 - 86.1	0.0192	92.98
B. velezensis SNR14-4	*B. velezensis* LF 01	80.3	77.4 - 82.9	0.023	90.82
B. velezensis SNR14-4	*B. velezensis* CPA1-1	83.4	80.6 - 85.9	0.0194	92.86
Formula 3 (identities/total length)					
B. velezensis SNR14-4	*B. velezensis* F3A	89	86.1 -91.3	0.1160	99.27
B. velezensis SNR14-4	*B. velezensis* ATR2	93.2	90.9 - 95	0.0871	99.73
B. velezensis SNR14-4	*B. velezensis* FZB42	88.4	85.5 - 90.8	0.1198	99.16
B. velezensis SNR14-4	*B. velezensis* CYS06	88.1	85.1 - 90.	0.1218	99.11
B. velezensis SNR14-4	*B. velezensis* B8	89.7	86.9 - 91.9	0.1114	99.38
B. velezensis SNR14-4	*B. velezensis* R-71003	86.7	83.6 - 89.2	0.1305	98.79
B. velezensis SNR14-4	*B. velezensis* LF 01	87.9	84.9 - 90.4	0.1228	99.07
B. velezensis SNR14-4	*B. velezensis* CPA1-1	89.7	86.9 - 91.9	0.1115	99.37

DDH- DNA-DNA Hybridization: HSP - High-Scoring Segment Pair

The process of calculating distances involves two steps: (i) comparing two genomes using the BLAST program to retrieve HSPs/MUMs, and (ii) utilizing three different formulas to estimate the distance from

the set of HSPs/MUMs. The distances are converted to DDH-analogous values. The likelihoods that DDH is > = 70% and > = 79% are reported using logistic regression (a particular kind of GLM). The primary purpose of GGDC is to determine the species' in silico relatedness.

PubMLST, and JSpeciesWS analysis also further confirmed the identity of the species as *B. velezensis*. The comparative G+C percentage content of SNR 14-4 with other *velenzensis* species showed slight difference (Table 2).

Table 2. Percentage of G+C content difference of reference to *B. velezensis* SNR14-4

Query genome	Reference genome	Difference in % G+C
B. velezensis SNR14-4	*B. velezensis* F3A	0.02
B. velezensis SNR14-4	*B. velezensis* ATR2	0.18
B. velezensis SNR14-4	*B. velezensis* FZB42	0.05
B. velezensis SNR14-4	*B. velezensis CYS06*	0.07
B. velezensis SNR14-4	*B. velezensis B8*	0.02
B. velezensis SNR14-4	*B. velezensis* R-71003	0.39
B. velezensis SNR14-4	*B. velezensis* LF 01	0.04
B. velezensis SNR14-4	*B. velezensis* CPA1-1	0.03

>1 within a single species; ≤1 between distinct species; (G+C : Guanine + Cytosine)

Gene annotation and functional gene prediction show the absence of virulence factors

The RASTtk bioinformatic tool in BV-BRC produced more detailed analytical data on the genome of the isolates, and the resulting contig data showed a total of 4163 CDSs, 205 repeat regions, 84 tRNAs, and 27 rRNAs. The data were cross-reviewed with other gene annotation pipelines, such as DFAST and BAKTA[7]. VFDB analysis through PATRIC showed that no potent virulence factors were present in the isolated strain which was confirmed through Virulence Finder2.0 that showed no hits. The absence of virulence factors hints at the safety of the strain for use as a probiotic. Moreover, there were 50 drug targets and 197 transporters in the overall genome of the isolate.

Genome mining revealed several probiotic markers in *B. velezensis* SNR14-4

Genome analysis using AntiSMASH revealed the presence of different antibiotic-, antifungal-, and bacteriocin-encoding gene clusters in SNR14-4. The results are summarized in Table 3.

Table 3. AntiSMASH analysis of SNR14-4 for secondary metabolites and biosynthesis-related gene clusters.

Region	Type	Nucleotide length		Probable similarity to known cluster	Similarity (%)
		From	To		
Region 1	NRPS, transAT-PKS, betalactone	108,443	242,587	Fengycin (NRP)	92
Region 2	Terpene	271,311	291,438	-	-
Region 3	T3PKS	373,617	413,965	-	-
Region 4	transAT-PKS	542,626	634,999	Diffricidin (Polyketide)	100
Region 5	NRP-metallophore, NRPS, RIPP-like	1,275,489	1,327,282	Bacillibactin (NRP)	100
Region 6	NRPS	1,607,518	1,673,902	-	-
Region 7	Other	1,868,903	1,910,321	Bacilysin (Other)	100
Region 8	NRPS	2,522,641	2,587,693	Surfactin (NRP:Lipo-peptide8)	100

Region 9	PKS-like	3,157,783	3,199,027	butirosin A/butirosin saccharide	7
Region 10	Terpene	3,281,765	3,302,505	-	-
Region 11	TransAT-PKS	3,606,557	3,694,385	Macrolactin H (Polyketide)	100
Region 12	TransAT-PKS, T3PKS, NRPS	3,914,441	4,017,072	Bacillaene (Polyketide+NRP)	100

NRPS: nonribosomal peptide synthetase; T3PKS: Type III polyketide synthases; AT-PKS: Transacyltransferase polyketide synthases; RiPP: Ribosomally synthesized and posttranslationally modified peptides.

The presence of difficidin, fengycin, bacillaene, bacilysin, bacillibactin, and macrolactin H biosynthesis-related gene clusters may indicate the probiotic characteristics of the strain. The genes that impart probiotic characteristics are summarized in Table 4.

Table 4. Marker genes of SNR14-4 exhibiting probiotic properties generated by genomic mining using PATRIC, NCBI and Prokka

Probiotic Gene Clusters and Functions	Coverage percentage	Similarity percentage
Antimicrobial Production Genes		
Surfactin (srfAA)	99	97
Bacillomycin (bmyD)	100	97.48
Fengycin (fenA)	100	98.61
Iturin (ItuD (ituD), ItuA (ituA), ItuB (ituB), and ItuC (ituC))	84	97.14
Difficin (dfnF)	100	97.99
Macrolactin (mlnI)	100	98.35
Bacillibactin(dhbE)	95	67.92
Bacilysin (bacA)	100	99.72
Bacillaene (baeI)	100	99.6
Bacillaene (baeS)	99	97.84
Stress Resistance Genes		
Sigma factor B(sigB)	100	99.13
GroEL/ES	79	78.01
Adhesion and Biofilm Formation		
Biofilm matrix protein (tasA)	100	99.24
Immune System Modulation		
Spo0A	98	68.01

The genes were curated from previously reported works on *Bacillus,* and the genes were manually searched in the annotated assembly using PATRIC, NCBI, and Prokka[18,19]. All results align with previously reported research on probiotic strains. The presence of these genes is beneficial for targeting and confirming the probiotic characteristics and safety of the novel *B. velezensis* strain. The manual search for probiotic genes and BLAST analysis of the genome showed that the isolate's genome contained genes that encode for Iturin, an antifungal lipopeptide that can disturb the plasma membrane of fungal pathogens mediated by oxidative stress as well as by interfering with glycolysis, gluconeogenesis and the tricarboxylic acid cycle[20]. AntiSMASH analysis does not reveal these antimicrobial genes. These genes could be inactive or non-functional but their presence in the genome could address the existence of cryptic genes. They may be activated under the right circumstances.

SNR14-4 shows antibiotic susceptibility

The isolates were susceptible to the following antibiotics: clarithromycin (CLR), streptomycin (S), chloramphenicol (C), tetracycline (TE), kanamycin (K), vancomycin (VA), doxycycline hydrochloride (DO), erythromycin (E), levofloxacin (LE), and ampicillin (A/S). Clarithromycin and ampicillin had the greatest effects on the growth of the isolates. Comparatively, the isolate showed less sensitivity to vancomycin and tetracycline. The diameter of each disc formed against the isolated strain is summarized in Table 5.

Table 5. Antibiotic susceptibility of the isolate of SNR14-4

Antibiotic used	Zone Diameter
Clarithromycin	37 ± 0.2 mm
Streptomycin	34 ± 0.2 mm
Chloramphenicol	30 ± 0.1 mm
Vancomycin	28 ± 0.1 mm
Doxycycline hydrochloride	30 ± 0.1 mm
Tetracycline	28 ± 0.1 mm
Kanamycin	29 ± 0.1 mm
Erythromycin	30 ± 0.1 mm
Levofloxacin	35 ± 0.1 mm
Ampicillin	37 ± 0.1 mm

The values are the means ± SDs of three independent estimations

The genome was evaluated for antibiotic-resistant genes (ARGs) using ResFinder and CARD RGI, which showed the absence of significant virulence factors. Even though RGI reported the presence of clbA, tet(45), and Bcl-resistant genes the *in vitro* analysis showed no resistance, hence these genes could either be cryptic or non-functional. Pathogen Finder showed that the isolate is not harmful to humans.

SNR14-4 is tolerant to pH, and bile

Strain *SNR14-4* showed appreciable resistance to low pH values of 2 and 3, making the isolate valuable for being incorporated into fish feed to survive acidic conditions within the gut of the fish (Fig 2)[49,50].

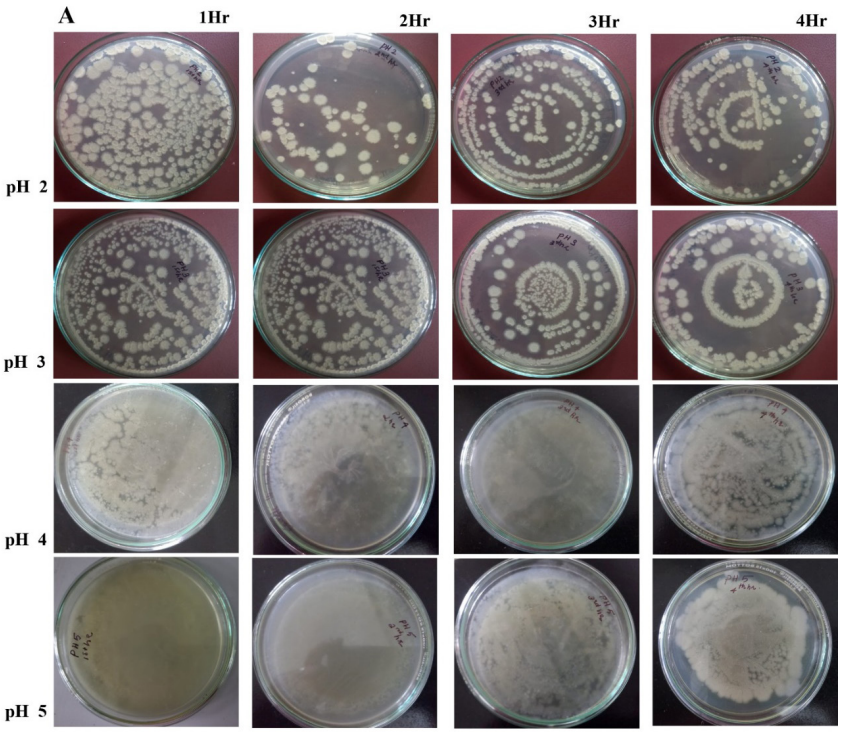


Figure 2. Acid tolerance of SNR14-4 in the pH range of 2-5. A: Growth pattern of SNR14-4 at different pH values: pH 2-, pH 3, pH 4, and pH 5 tolerant colonies from the first to fourth hours of incubation to fourth hours of incubation, M-P: pH 5-tolerant colonies from the first to fourth hours of incubation. The experiment was conducted in triplicate.

A bile tolerance assay was carried out using varying concentrations of bile salt in Luria Bertani, with a maximum concentration of 0.3 %. The maximum exposure time was 4 hours, and all plates incubated showed more than 50 % survivability. The experiment was carried out in triplicate (Fig 3).

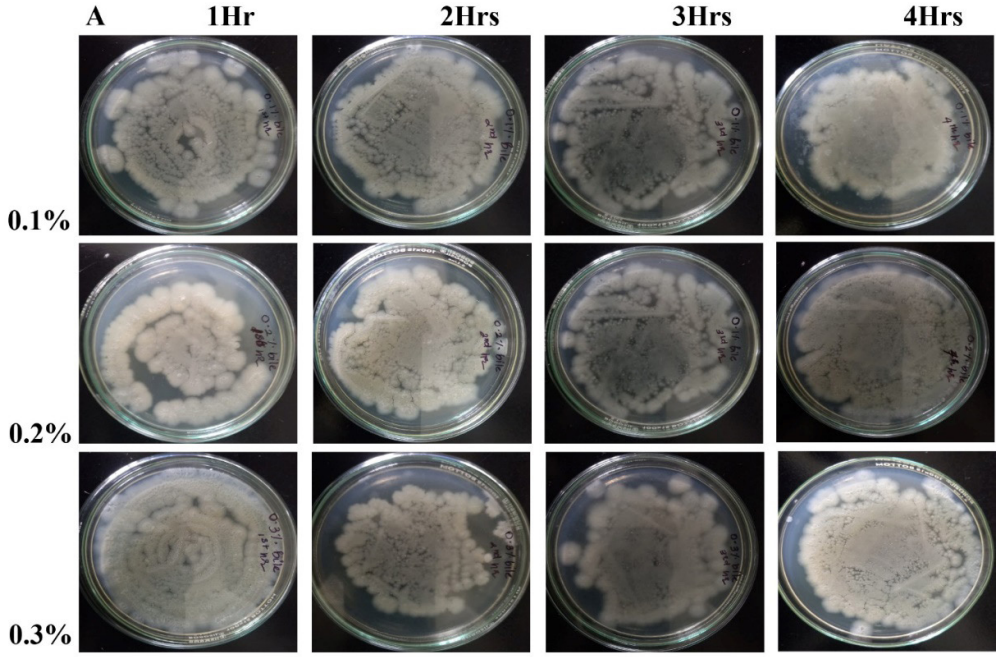


Figure 3. Bile tolerance of SNR14-4 to varying concentrations of 0.1-0.3 % bile for incubation periods of 1 to 4 hrs. The experiment was conducted in triplicate.

Tolerance to acid and bile has proven to be advantageous to the isolate, as it can effectively colonize the guts of the fish under *in vivo* conditions. The results were found to be consistent with the analysis of PATRIC and NCBI.

SNR14-4 shows promising in vitro antimicrobial activity

The isolated strain could produce antimicrobial activity against the prominent pathogenic *Flavobacterium* spp., *Listeria, and Vibrio spp*. Among the different solvent fractions, the ethyl acetate fraction exhibited antimicrobial activity. The control of DMSO (0.5 %) showed no clear zone, and the positive control of streptomycin was used to compare the diameter of the clear zone produced by the isolate. The diameters, summarized in Table 6, show that the isolate has good potential for use as a substitute for synthetic or commonly available antibiotics, with the greatest activity against *V. parahaemolyticus* (18 mm zone) and the least activity against *Flavobacterium* (14 mm zone).

Table 6. Antibacterial activity of the isolate of SNR14-4

Pathogen	Control (mm)	EA_f (mm)
Flavobacterium spp.	33 ± 0.1 mm	15 ± 0.1 mm
Vibrio parahaemolyticus	26 ± 0.1 mm	18 ± 0.1 mm
Listeria monocytogenes	19 ± 0.1 mm	17 ± 0.1 mm

EA_f: Ethyl acetate Fraction; The values are the means ± SDs of three independent estimations.

The uninoculated media that was treated as same as the inoculated media showed no activity. The analysis of antibiotic isolation and purification from the ethyl acetate fraction is the scope of further research. The results generated were parallel to those predicted by the gene-before-lab approach.

SNR14-4 showed promising probiotic characteristics; autoaggregation, hydrophobicity, and hemolytic activity

The autoaggregation assay of the isolated strain SNR14-4 revealed 85.7 % autoaggregation at the first hour (T_1), 84.2 % at the second hour, and 85.3 %, 85.5 %, and 85.9 % at 3 h, 4 h and 5 h, respectively. The hemolytic assay carried out using 5% human blood showed no hemolytic zones, as observed for alpha hemolysis. Strains with alpha and gamma hemolytic activity were previously used as probiotic strains (data not shown). A strain isolated from fish gills has proven to be a good candidate for use as a probiotic because of its α-hemolysis property[51]

Preliminary phytochemical analysis and HR-LCMS–QT reveals several bioactive compounds from SNR14-4

Preliminary phytochemical analysis revealed the presence of several components in the ethyl acetate fraction of *viz.* Coumarins, terpenoids, flavonoids, polyphenolic compounds, etc. Although the total ion chromatogram of the HRLC MS-QTOF analysis of ethyl acetate showed several peaks, a few compounds were identified. The identified compounds were saponins (cyclopassifloic acid C), alkaloids (guanvoline and dihydroaspidospermatine), biosurfactants (surfactin), aliphatic nitro compounds (meriserotoxin) and aspergillic acid (Table 7).

Table 7. Biologically active compounds identified using HRLCMS-QTOF

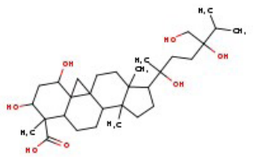
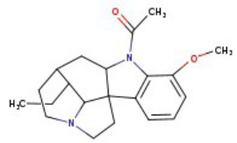
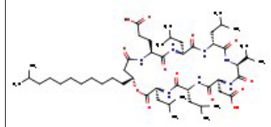
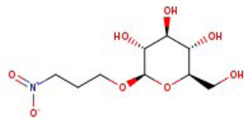
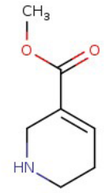
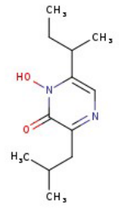
Si No	RT	Mode Compound	Mass	Formula
1.	13.803 -ve	Cyclopassifloic acid C	536.3691	Ca1 H2O7
2.	23.463 -ve	14,19-Dihydroaspidospermatine	340.2129	Ca1 H28 N2 O2
3.	24.972 -ve	Surfactin	1035.6881	Cs3 H93 N7 O13
4.	1.629 +ve	Miserotoxin	267.0954	Co H17 N O8
5.	3.338 +ve	Guvacoline	141.0799	Cr H11 N O2
6.	7.727 +ve	Aspergillic acid	224.1527	Cu2 H20 N2 O2

RT; Retention time

Compounds showed no toxicity with acceptable ADME properties

The compounds produced by SNR14-4 was found to be non-toxic without any mutagenicity. 14,19-Dihydroaspidospermatine and miserotoxin were predicted to be insoluble. Except cyclopassifloic acid C all other compounds satisfied the Lipinski rule for a potential drug (Table 8).

Table 8. *In silico* ADME and toxicity properties of the compounds produced by SNR14-4

Compound	Structure	Lipophilicity Log $P_{o/w}$	Water Solubility Log S (SILICOS-IT)	GI absorption	BBB permeant	Druglikeness (Lipinski)	Mutagenicity; Predicted Toxicity class; LD_{50}
Cyclopassifloic acid C		3.66	Soluble	Low	No	No; 2 violations: MW>500, NHorOH>5	No;6; 9800mg/kg
14,19-Dihydroaspido-spermatine		2.88	Moderately soluble	High	Yes	Yes; 0 violation	No; 4; 325mg/kg
Surfactin		4.83	Insoluble	Low	No	No; 3 violations: MW>500, NorO>10, NHorOH>5	No;4; 1190mg/kg
Miserotoxin		-2.25	Insoluble	Low	No	Yes; 0 violation	No; 6; 23000mg/kg
Guvacoline		0.56	Soluble	High	No	Yes; 0 violation	No;4; 750mg/kg
Aspergillic acid		2.14	Soluble	High	Yes	Yes; 0 violation	No; 4; 600mg/kg

ADME : Absorption, Distribution, Metabolism, and Excretion; GI : Gastrointestinal ; BBB : Brain-blood barrier

Discussion

With decades of research and scientists stating *Bacillus spp*. as the most useful and diverse hub of bioactive secondary metabolites, the present study added a new member to the list. *B. velezensis SNR14-4* was isolated from Tilapia gills, and relevant assays that define this bacterium as a probiotic were carried out. The present study aimed to introduce a novel and targeted approach for unlocking various characteristics of an organism through a "gene-before-lab" approach. When the approach was employed to analyze the probiotic characteristics of *B. velezensis SNR14-4*, the in vitro analysis results were consistent with the bioinformatics predictions. The gene-to-lab approach was beneficial for reducing the *in vitro* analysis time, and 100% of the probiotic test results were confirmed. The strain was culturally, morphologically, and biochemically identified as a member of *Bacillus* There is a strong genetic similarity of 99% with members of *B. amyloliquefaciens* and *B. velezensis.*[52,53]. Several disputes have been claimed over the identity of specific members of the *Operational Group Bacillus amyloliquefaciens* (OGBa) (which requires techniques such as 16S rRNA sequencing, phylogenetic tree construction, genome to genome calculation and DNA-DNA hybridization value[54]. All these techniques pointed out that the current strain shared most similarity with *B. velezensis.*[55] Given the close identity morphological and biochemical characterization gives limited information about the strain, but interestingly the strain was urease-positive and amylase-positive. As most *B. velezensis* strains are urease-negative, the urease-positive nature denotes the strain similarity to *B. velezensis* isolated from plant rhizospheres as well as from other soil sources[56].

Although many *B. velezensis* strains have been isolated, a complete evaluation of the entire gene pool has rarely been reported[57,58]. This is the first genome-guided study in the *B. velezensis* strain to explore the probiotic features. Genome mining and bioinformatics-guided analysis of the whole genome of the isolate revealed the presence of probiotic markers, and these findings were consistent with previously reported work in Nile Tilapia[51,59]. The genetic markers were observed to be functional through *in vitro* evaluation, and their products were confirmed through HR-LCMS analysis. Genome-guided evaluation of strains can also be considered a primary step in building a consortium as a strategic laboratory, and *in vivo* protocols can be designed over time. *Bacillus velezensis* TS5[60], *Bacillus velezensis* R-71003[49], *Bacillus velezensis* LF01[61], *Bacillus velezensis* CYS06[62], *Bacillus velezensis* CPA1-1[63] are some of the strains that has been extensively studied for its probiotic effects, some on fish, with a focus on the genetic information. Considering the undeniable genetic similarity, it can be argued that the current strain can also harbour the same capability. This hypothesis has been proven with the progress of the evaluation.

The work carried out previously on the *Bacillus* species was anchored around the usefulness of the Virulence Finder tool in evaluating the safety of isolates[60,64], The result obtained using this tool showed that the present strain is safe and free from virulent genetic factors and is not pathogenic to humans, indicating that if fish supplemented with the strain as probiotic feed probably poses no threat to humans. The sporulation ability explains the stress tolerance of the isolate at higher temperatures[8], bile tolerance and acid tolerance were vital to determine the ability of the isolate to survive in the gastrointestinal tract of Nile tilapia as well as other commercially cultured fish species.

Given the history of using *Bacillus* species as potentially useful probiotics[65], the present strain also showed hydrophobicity and autoaggregation properties[66], which, from the viewpoint of a probiotic candidate, are essential, as binding to the mucus epithelium may facilitate the proliferation of probiotic bacteria and lead to better immunity[67].The research has covered all necessary *in vitro* assays that are required to determine the safety of the strain for incorporation into fish, especially into tilapia, as the strain has no pathogenic traits. Being α-hemolytic as it produces no clear zones but does show a greenish-brown discoloration, results in incomplete hemolysis. The addition of alpha- and gamma-hemolytic strains to fish is safe, as shown in previous research, hence the addition of the present strain along with *in vivo*-proven probiotic strain could amplify the probiotic effect. The autoaggregation values were calculated using previously published works on *Bacillus* as well as probiotics[67,68]. The higher values of autoaggregation show that the strain has better potential to colonize the fish gut and stimulate immune responses, as well as establish better immunity. Hydrophobicity is an important prop-

erty of probiotic bacteria, and the current isolate has shown 78 % hydrophobicity[69]. Hydrophobicity is predicted based on the MATH formula, which corresponds to van der Waals forces and electrostatic interactions between microbes and organic solvents and indirectly reflects the efficiency of binding or adhering to the gut tissue of the fish[67].

A potential fish probiotic candidate should show good activity against fish-pathogen by producing antibiotics. The evaluation of the present strain in comparison to other similar strains proposes to be a substitute for normally available antibiotics and boost aquaculture as never before if incorporated with feed or as a part of a consortium[70-73]. The *in silico* analysis has guided the research to be time-efficient and resource-saving. It also addresses the presence of cryptic genes present in the genome. With further study of the genome characters, it is possible to configure an *in vitro* condition that can activate the cryptic gene clusters and possibly generate novel bioactive compounds.

The ethyl acetate fraction actively contains a large number of strong antibacterial and fungicidal chemicals, according to the HR-LCMS-QTOF analytical results. These substances could be the cause of the inhibitory effects on fish pathogenicity. The chemicals of interest identified are alkaloids, dihydroaspidospermatine, which inhibits TNF-α[74,75], guvacolinean, which has some cytotoxic activity[76], cyclopassifloic acid C, which belongs to the class saponins[77], miserotoxin, a nitro compound[78] with proven antibacterial activity, and aspergillic acid[79], which is most widely used as an antibiotic and an antifungal agent. Surfactin, an extremely potent cyclic lipopeptide biosurfactant made by several *Bacillus* strains, shows haemolytic, antiviral, antibacterial, and anticancer effects, particularly on Ehrlich ascites, breast and colon cancers, leukemia and hepatoma[80,81]. The presence of these compounds in the active fraction explains the effect of the novel strain against the selected pathogens. These fungicidal compounds, as well as biocontrol agents against plant fungi, also illustrate the immense potential of *SNR14-4* against fungal pathogens in tilapia (data unpublished). Through a comprehensive approach starting from 'gene-before-lab', *in vitro* analysis, and HR-LCMS analysis, *B. velezensis SNR14-4* can be considered a safe, useful, and effective member of the *Bacillus* genus for use as a probiotic candidate. The extent of further exploration of the industrial potential of this novel strain remains the scope of future research.

References

1. Fitzsimmons K M, Gonzalez-Alanis P, Martinez-Garcia R. Why is tilapia becoming the most important food fish on the planet? In Proceedings of the 9th International Symposium on tilapia in Aquaculture, Shanghai Ocean University, Shanghai, China. 2011. 22-24 April 2011 8–16.

2. Prabu E, Rajagopalsamy CBT, Ahilan B, Jeevagan IJMA, Renuhadevi M, Tilapia – An Excellent Candidate Species for World Aquaculture: A Review. Annu. Res. Rev. Biol. 2019: 1–14. doi:10.9734/arrb/2019/v31i330052.

3. Santos R A, Oliva-Teles A, Pousão-Ferreira P, Jerusik R, Saavedra M J, Enes P, Serra C R. Isolation and characterization of fish-gut Bacillus spp. as surce of natural antimicrobial compounds to fight aquaculture bacterial diseases. Marine Biotechnology., 2021;23: pp.276-293. doi: 10.1007/s10126-021-10022-x.

4. Hesami S, Parkman J, MacInnes JI, Gray JT, Gyles CL, Lumsden John S, Antimicrobial Susceptibility of *Flavobacterium psychrophilum* Isolates from Ontario. J. Aquat. Anim. Health. 2010; 22: 39–49. doi: 10.1577/H09-008.1.

5. FAO, *Tilapia lake virus disease strategy manual*, 2021. FAO.

6. Wan MLY, Forsythe SJ, El-Nezami H, Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. Crit. Rev. Food Sci. Nutr. 2019;59:3320–3333. doi: 10.1080/10408398.2018.1490885.

7. Madhavan S, Dinesh N, Muhammed R N, John D, Hariharan S, Nevin K. G. Bioinformatic insights into the xenobiotic degradation potential gene clusters of fish-associated novel *Bacillus velezensis* SNR14-4. Env Exp Biol, 2024; 22(2):79–86. Doi:10.22364/eeb.22.08.

8. Thankappan B, Ramesh D, Ramkumar S, Natarajaseenivasan K, Anbarasu K. Characterization of Bacillus spp. From the Gastrointestinal Tract of Labeo rohita—Toward to Identify Novel Probiotics Against Fish Pathogens. Appl. Biochem. Biotechnol., 2015;175: 340–353. doi: 10.1007/s12010-014-1270-y.

9. Banerjee G, Ray AK. The advancement of probiotics research and its application in fish farming industries. Res. Vet. Sci. 2017; 115:66–77. doi: 10.1016/j.rvsc.2017.01.016.

10. Elsabagh M, Mohamed R, Moustafa EM, Hamza A, Farrag F, Decamp O, Dawood MAO, Eltholth M. Assessing the impact of *Bacillus* strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*. Aquac. Nutr. 2018; 24: 613–1622. doi.org/10.1111/anu.12797.

11. Fan B, Blom J, Klenk HP., Borriss, R., 2017. Bacillus amyloliquefaciens, Bacillus velezensis, and *Bacillus siamensis* Form an "Operational Group B. amyloliquefaciens" within the *B. subtilis* Species Complex. *Front. Microbiol*., 2017, Vol. 8. doi: 10.3389/fmicb.2017.00022.

12. Rabbee M, Ali, MD, Choi J, Hwang B, Jeong S, Baek K, Bacillus velezensis: A Valuable Member of Bioactive Molecules within Plant Microbiomes. Molecules. 2019; 24: 1046. doi.org/10.3390/separations10110552.

13. Khalid F, Khalid A, Fu Y, Hu Q, Zheng Y, Khan S, Wang Z. Potential of Bacillus velezensis as a probiotic in animal feed: a review. J. Microbiol. 2021; 59: 627–633. doi: 10.1007/s12275-021-1161-1.

14. Rehman NU, Abed RMM, Hussain H, Khan HY, Khan A, Khan AL. Ali, M. Al-Nasri, A. Al-Harrasi, K. Al-Rawahi, A.N., Wadood, A., Al-Rawahi, A., Al-Harrasi, A., Anti-proliferative potential of cyclotetrapeptides from Bacillus velezensis RA5401 and their molecular docking on G-Protein-Coupled Receptors. Microb. Pathog. 2018; 123: 419–425. doi: 10.1016/j.micpath.2018.07.043.

15. Ye M, Tang X, Yang R, Zhang H, Li F, Tao F, Li Fei, Wang Z. characteristics and Application of a Novel Species of *Bacillus* : *Bacillus velezensis*. ACS Chem. Biol. 2018; 13: 500–505. doi: 10.1021/acschembio.7b00874.

16. Yi Y, Zhang Z, Zhao F, Liu H, Yu L, Zha J, Wang G. Probiotic potential of Bacillus velezensis JW: Antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on Carassius auratus. Fish Shellfish Immunol. 2018;. 78: 322–330. doi: 10.1016/j.fsi.2018.04.055.

17. Zhang DX, Kang YH, Zhan S, Zhao ZL, Jin SN, Chen C, Zhang L, Shen JY, Wang CF, Wang GQ, Shan XF, Qian AD. Effect of Bacillus velezensis on Aeromonas veronii-Induced Intestinal Mucosal Barrier Function Damage and Inflammation in Crucian Carp (Carassius auratus). Front. Microbiol. 2019; 10: 2663. doi: 10.3389/fmicb.2019.02663.

18. Zhou S, Xia Y, Zhu C, Chu W. Isolation of Marine Bacillus sp. with Antagonistic and Organic-Substances-Degrading Activities and Its Potential Application as a Fish Probiotic. Mar. Drugs. 2018; 196. doi: 10.3390/md16060196

19. Giri SS, Sukumaran V, Dangi NK, Characteristics of Bacterial Isolates from the Gut of Freshwater Fish, Labeo rohita that May be Useful as Potential Probiotic Bacteria. Probiotics Antimicrob. Proteins. 2012; 4: 238–242. doi: 10.1007/s12602-012-9119-6.

20. Xiong Q, Liu D, Zhang H, Dong X, Zhang G, Liu Y, Zhang R. Quorum sensing signal autoinducer-2 promotes root colonization of Bacillus velezensis SQR9 by affecting biofilm formation and motility. Appl. Microbiol. Biotechnol. 2020; 104: 7177–7185. doi: 10.1007/s00253-020-10713-w.

21. An J, Zhu W, Liu Y, Zhang X, Sun L, Hong P, Wang Y, Xu C, Xu D, Liu H. Purification and characterization of a novel bacteriocin CAMT2 produced by Bacillus amyloliquefaciens isolated from marine fish Epinephelus areolatus. Food Cont. 2015; 51: 278–282. doi:10.1016/j.foodcont.2014.11.038.

22. Green MR, Sambrook J. Isolating DNA from Gram-Negative Bacteria. Cold Spring Harb Protoc. 2017; 3: 2017. doi: 10.1101/pdb.prot093369.

23. Ray AK, Roy T, Mondal S, RingÃ E. Identification of gut-associated amylase, cellulase and protease-producing bacteria in three species of Indian major carps. Aquac. Res. 2009; 41(10): 1462-1469. doi:10.1111/j.1365-2109.2009.02437.x

24. Reda RM, Selim KM, El-Sayed HM, El-Hady MA. *In Vitro* Selection and Identification of Potential Probiotics Isolated from the Gastrointestinal Tract of Nile Tilapia, Oreochromis niloticus. Probiotics Antimicrob. Proteins. 2018; 10: 692–703. doi: 10.1007/s12602-017-9314-6.

25. Meier-Kolthoff JP, Gö. ker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun. 2019; 10(1): 2182. doi: 10.1038/s41467-019-10210-3.

26. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res. 2022; 50(D1):D801-D807. doi: 10.1093/nar/gkab902

27. Jolley, K.A., Bray, J.E., Maiden, M.C.J. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res*. 2018;24:124. doi: 10.12688/wellcomeopenres.14826.1.

28. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics. 2016; 32(6): 929-931. doi: 10.1093/bioinformatics/btv681.

29. Schwengers O, Jelonek L, Dieckmann MA, Beyvers S, Blom J, Goesmann. A.Bakta: Rapid and Standardized Annotation of Bacterial Genomes via Alignment-Free Sequence Identification Microbial Genome. 2021; 7/11: 000685. doi: 10.1099/mgen.0.000685

30. Tanizawa Y, Fujisawa T, Nakamura Y, DFAST: A Flexible Prokaryotic Genome Annotation Pipeline for Faster Genome Publication', ed. by John Hancock Bioinformatics. 2018; 34/6: 1037–1039. doi: 10.1093/bioinformatics/btx713.

31. Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, Davis JJ, Dempsey DM, Dickerman A, Dietrich EM, Kenyon RW, Kuscuoglu M, Lefkowitz EJ, Lu J, Machi D, Macken CT. et al. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): A Resource Combining PATRIC, IRD and ViPR Nuc Acids Res. 2023; 51/D1: 2023, D678–689. doi: 10.1093/nar/gkac1003.

32. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol. 2014;52(5):1501-1510. doi: 10.1128/JCM.03617-13.

33. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, Fetter A, Terlouw BR, Metcalf WW. EJN, van Wezel GP, Medema MH, Weber T. antiSMASH 7.0: New and Improved Predictions for Detection, Regulation, Chemical Structures and Visualization Nucleic Acids Res. 2023; 51/W1: W46–50. doi: 10.1093/nar/gkad344.

34. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, Tosatto SCE, Paladin L, Raj S, Richardson LJ, Finn RD., Bateman A. Pfam: The protein families database in 2021. Nucleic Acids Res. 2021; 49(D1):D412-D419. doi: 10.1093/nar/gkaa913.

35. Meidong R, Khotchanalekha K, Doolgindachbaporn S, Nagasawa T., Nakao M, Sakai K, Tongpim S, Evaluation of probiotic Bacillus aerius B81e isolated from healthy hybrid catfish on growth, disease resistance and innate immunity of Pla-mong Pangasius bocourti. Fish Shellfish Immunol. 2018; 73:1-10. doi: 10.1016/j.fsi.2017.11.032.

36. Kuebutornye FKA, Lu Y, Abarike ED, Wang Z, Li Y, Sakyi ME, *In vitro* Assessment of the Probiotic Characteristics of Three Bacillus Species from the Gut of Nile Tilapia, Oreochromis niloticus. Prob Antimicrob. Prot. 2020; 12: 412–424. doi: 10.1007/s12602-019-09562-5.

37. Sam-On M F S, Mustafa S, Hashim AM, Yusof MT, Zulkifly S, Malek A Z A, Asrore M S M. Mining the genome of Bacillus velezensis FS26 for probiotic markers and secondary metabolites with antimicrobial properties against aquaculture pathogens. Microb Pathog. 2023;1181:106161. doi: 10.1016/j.micpath.2023.106161.

38. Kapse NG, Engineer AS, Gowdaman V, Wagh S, Dhakephalkar PK, Functional Annotation of the Genome Unravels Probiotic Potential of Bacillus Coagulans HS243. Genomics. 2019; 111/4: 921–929. doi: 10.1016/j.ygeno.2018.05.022.

39. Seemann T, Prokka: Rapid Prokaryotic Genome Annotation Bioinformatics. 2014; 30/14: 2068–2069. doi: 10.1093/bioinformatics/btu153.

40. Charteris WP, Kelly PM, Morelli L, Collins JK. Gradient Diffusion Antibiotic Susceptibility Testing of Potentially Probiotic Lactobacilli. J. Food Prot. 2021;64: 2007–2014. doi: 10.4315/0362-028x-64.12.2007.

41. Zhou S, Xia Y, Zhu C, Chu W. Isolation of Marine Bacillus sp. with Antagonistic and Organic-Substances-Degrading Activities and Its Potential Application as a Fish Probiotic. Mar. Drugs. 2018; 16: 196. doi: 10.3390/md16060196

42. Baofeng J, Amogelang R. Raphenya, Brian Alcock, Nicholas Waglechner, Peiyao Guo, CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database, Nucleic Acids Research. 2017; 45: D566–D573. doi: 10.1093/nar/gkw1004.

43. Mahfouz N, Ferreira I, Beisken S, von Haeseler A, Posch AE. Large-scale assessment of antimicrobial resistance marker databases for genetic phenotype prediction: a systematic review. J Antimicrob Chemother 2020;75(11):3099-3108. doi: 10.1093/jac/dkaa257.

44. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother. 2020;75(12): 3491-3500. doi: 10.1093/jac/dkaa345.

45. Cosentino S, Voldby Larsen M, Møller Aarestrup F, Lund O. PathogenFinder--distinguishing friend from foe using bacterial whole genome sequence data. PLoS One. 2013; 8(10): e77302. doi: 10.1371/journal.pone.0077302.

46. Zhou J, Xie Y, Liao Y, Li X, Li Y, Li S, Ma X, Lei S, Lin F, Jiang W, He YQ. Characterization of a Bacillus velezensis strain isolated from Bolbostemmatis Rhizoma displaying strong antagonistic activities against a variety of rice pathogens. Front. Microbiol. 2022; 13: 983781. doi: 10.3389/fmicb.2022.983781.

47. Chu J, Wang Y, Zhao B, Zhang X, Liu K, Mao L, Kalamiyets E. Isolation and identification of new antibacterial compounds from Bacillus pumilus. Appl. Microbiol. Biotechnol. 2019; 103:8375–8381. doi: 10.1007/s00253-019-10083-y.

48. Balakrishna A, *In vitro* evaluation of adhesion and aggregation abilities of four potential probiotic strains isolated from guppy (Poecilia reticulata). Braz. Arch. Biol. Technol. 2013; 56: 793–800. doi. org/10.1590/S1516-89132013000500010.

49. Kang M, Su X, Yun L, Shen Y, Feng J, Yang G, Meng X, Zhang J, Chang X. Evaluation of probiotic characteristics and whole genome analysis of Bacillus velezensis R-71003 isolated from the intestine of common carp (Cyprinus carpio L.) for its use as a probiotic in aquaculture. Aquac. Rep. 2022; 25: 101254. doi.org/10.1016/j.aqrep.2022.101254.

50. Wu Z, Qi X, Qu S, Ling F, Wang G. Dietary supplementation of Bacillus velezensis B8 enhances immune response and resistance against Aeromonas veronii in grass carp. Fish Shellfish Immunol. 2021; 115: 14–21. doi: 10.1016/j.fsi.2021.05.012.

51. Amoah K, Dong X, Tan B, Zhang S, Kuebutornye FKA, Chi S, Yang Q, Liu H, Zhang H, Yang Y.. *In vitro* Assessment of the Safety and Potential Probiotic Characteristics of Three Bacillus Strains Isolated From the Intestine of Hybrid Grouper (Epinephelus fuscoguttatus♀ × Epinephelus lanceolatus♂). Front. Vet. Sci. 2021; 8: PP. 675962. doi: 10.3389/fvets.2021.675962.

52. Wang LT, Lee FL, Tai CJ, Kuo HP. Bacillus velezensis is a later heterotypic synonym of Bacillus amyloliquefaciens. Int. J. Syst. Evol. Microbiol. 2008; 58: 671–675. doi: 10.1099/ijs.0.65191-0.

53. Dunlap CA, Kim SJ, Kwon SW, Rooney AP.Bacillus velezensis is not a later heterotypic synonym of Bacillus amyloliquefaciens; Bacillus methylotrophicus, Bacillus amyloliquefaciens subsp. plantarum and 'Bacillus oryzicola' are later heterotypic synonyms of Bacillus velezensis based on phylogenomics. Int. J. Syst. Evol. Microbiol. 2016; 66: 1212–1217. doi: 10.1099/ijsem.0.000858.

54. Huynh T, Vörös M, Kedves O, Turbat A, Sipos G, Leitgeb B, Kredics L, Vágvölgyi C, Szekeres A. Discrimination between the Two Closely Related Species of the Operational Group *B. amyloliquefaciens* Based on Whole-Cell Fatty Acid Profiling. Microorganisms. 2022; 10(2):418. doi:10.3390/microorganisms10020418

55. Adeniji AA, Loots DT, Babalola OO. Bacillus velezensis: phylogeny, useful applications, and avenues for exploitation. Appl. Microbiol. Biotechnol. 2019; 103:3669–3682. doi: 10.1007/s00253-019-09710-5.

56. Wei, M., Zhang, M., Huang, G., Yuan, Y., Fu, C., Yu, L., Coculture with two Bacillus velezensis strains enhances the growth of Anoectochilus plants by promoting nutrient assimilation and regulating rhizosphere microbial community. Ind. Crops Prod., 2020, Vol. 154,pp 112697. doi:10.1016/j.indcrop.2020.11269.

57. Liu KF, Chiu CH, Shiu YL, Cheng W, Liu CH. Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, Litopenaeus vannamei larvae. Fish Shellfish Immunol. 2010; 28: 837–844. doi: 10.1016/j.fsi.2010.01.012.

58. Elshaghabee FMF, Rokana N, Gulhane RD, Sharma C, Panwar H. Bacillus As Potential Probiotics: Status, Concerns, and Future Perspectives. Front. Microbiol. 2017; 8:1490. doi: 10.3389/fmicb.2017.01490.

59. Shija VM, Amoah K, Cai J. Effect of bacillus probiotics on the immunological responses of Nile tilapia (Oreochromis niloticus): A Rev Fishes. 2023; 8(7):366. doi.org/10.3390/fishes8070366.

60. Chen B, Zhou Y, Duan L, Gong X, Liu X, Pan K, Zeng D, Ni X, Zeng Y. Complete genome analysis of *Bacillus velezensis* TS5 and its potential as a probiotic strain in mice. Front Microbiol. 2023;14:1322910. doi: 10.3389/fmicb.2023.1322910.

61. Zhang DF, Gao YX, Ke XL, Wang YJ. Genomic analysis of Bacillus velezensis LF01 strain and the biocontrol effect of its secondary metabolites. J Fish China. 2022; 46 (2): 196-206.

62. Liu L, Jiang D, Ren Y, Shi C, Wang Y, Yin J, Wang Q, Zhang D. The *Bacillus velezensis* CYS06 Strain Exhibits Promising Applications in Fighting Grass Carp Bacterial Diseases. *Fishes*. 2024; 9(1):7. doi:10.3390/fishes9010007.

63. Gao X, Chen A, Zhou Y, Qian Q, Qin L, Tang X, Jiang Q, Zhang X. Genomic characterization and probiotic potency of Bacillus velezensis CPA1-1 reveals its potential for aquaculture applications. Aquaculture. 2025; 596:741852. doi 10.1016/j.aquaculture.2024.741852

64. Bin Hafeez A, Pełka K, Worobo R, Szweda P. *In Silico* Safety Assessment of *Bacillus* Isolated from Polish Bee Pollen and Bee Bread as Novel Probiotic Candidates. Int J Mol Sci. 2024; 25(1):666. doi: 10.3390/ijms25010666.

65. Hai NV. Research findings from the use of probiotics in tilapia aquaculture: A review. Fish Shellfish Immunol. 2015: 45: 592–597. doi: 10.1016/j.fsi.2015.05.026.

66. Krausova G, Hyrslova I, Hynstova I. *In Vitro* Evaluation of Adhesion Capacity, Hydrophobicity, and AutoAggregation of Newly Isolated Potential Probiotic Strains. Fermentation. 2019; 5: 100. doi:10.3390/fermentation5040100.

67. Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Appl. Microbiol. Biotechnol. 2019; 103: 6463–6472. doi: 10.1007/s00253-019-09978-7.

68. Vinderola CG, Medici M, Perdigon G. Relationship between interaction sites in the gut, hydrophobicity, mucosal immunomodulating capacities and cell wall protein profiles in indigenous and exogenous bacteria. J. Appl. Microbiol. 2004; 96: 230–243. doi: 10.1046/j.1365-2672.2004.02158.x.

69. Hernandez-Hernandez O, Muthaiyan A, Moreno FJ, Montilla A, Sanz ML, Ricke SC. Effect of prebiotic carbohydrates on the growth and tolerance of Lactobacillus. Food Microbiol. 2012; 30: 355–361. doi: 10.1016/j.fm.2011.12.022.

70. Narimbi J, Mazumder D, Sammut J. Stable isotope analysis to quantify contributions of supplementary feed in Nile Tilapia *Oreochromis niloticus* (GIFT strain) aquaculture. Aquac. Res. 2018; 49: 1866–1874. doi:10.1111/are.13642.

71. Abarike, ED, Cai J, Lu Y, Yu H, Chen L, Jian J, Tang J, Jun L, Kuebutornye FKA. Effects of a commercial probiotic BS containing *Bacillus subtilis* and *Bacillus licheniformis* on growth, immune response and

disease resistance in Nile tilapia, Oreochromis niloticus. Fish Shellfish Immunol. 2018; 82: 229–238. doi: 10.1016/j.fsi.2018.08.037.

72. Liu G, Kong Y, Fan Y, Geng CE, Peng D, Sun Ming. Whole-genome sequencing of Bacillus velezensis LS69, a strain with a broad inhibitory spectrum against pathogenic bacteria. J Biotech. 2017; 249: 20–24. doi: 10.1016/j.jbiotec.2017.03.018.

73. Emam AM, Dunlap CA. Genomic and phenotypic characterization of Bacillus velezensis AMB-y1; a potential probiotic to control pathogens in aquaculture. Antonie Van Leeuwenhoek. 2020; 113: 2041–2052. doi: 10.1007/s10482-020-01476-5.

74. Prasanth D, Reddy LS, Dasari T, Bhavanam PR, Ahmad SF, Nalluri R, Pasala PK. LC/MS-Based Profiling of Hedyotis aspera Whole-Plant Methanolic Extract and Evaluation of Its Nephroprotective Potential against Gentamicin-Induced Nephrotoxicity in Rats Supported by In Silico Studies. Separations. 2023; 10(11):552.

75. Saleem H, Htar T T, Naidu R, Zengin G, Locatelli M, Tartaglia A, Ahemad N. Phytochemical composition and enzyme inhibition studies of Buxus papillosa CK Schneid. Processes. 2020;8(7):757. doi. org/10.3390/pr8070757.

76. Myers AL. Metabolism of the areca alkaloids–toxic and psychoactive constituents of the areca (betel) nut. Drug Metabol Rev. 2022;54(4):343-360. doi: 10.1080/03602532.2022.2075010.

77. Yoshikawa K, Katsuta S, Mizumori J, Arihara S, Four cycloartane triterpenoids and six related saponins from Passiflora edulis. J Nat Prod. 2000; 63(9):1229-1234. doi: 10.1021/np000126+.

78. Chandwani S, Dewala S, Chavan SM, Paul D, Kumar K, Amaresan N. Genomic, LC–MS, and FTIR Analysis of Plant Probiotic Potential of Bacillus albus for Managing Xanthomonas oryzae via Different Modes of Application in Rice (*Oryza sativa* L.). Prob Antimicrobial Prot. 2023; 18: 1-2. doi: 10.1007/s12602-023-10120-3.

79. Dutcher JD, Aspergillic Acid: An Antibiotic Substance Produced by Aspergillus Flavus: II. 1947.Bromination Reactions and Reduction With Sodium and Alcohol. J Biol Chem.1947;171(1): 341-53.

80. Seydlová G, Svobodová J. Review of surfactin chemical properties and the potential biomedical applications. Cent Eur J Med. 2008; 3: 123-33. doi.org/10.2478/s11536-008-0002-5.

81. Wu YS, Ngai S C, Goh B H, Chan K G, Lee LH, Chuah LH. Anticancer activities of surfactin and potential application of nanotechnology assisted surfactin delivery. Frontiers in Pharmacol. 2017; 8:761. doi: 10.3389/fphar.2017.00761

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Topical Ethosomal Formulation of Alpha Arbutin: Dermatokinetic Study and In-vitro Evaluation

Formulación etosomal tópica de alfa arbutina: estudio dermatocinético y evaluación in vitro

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Conflict of interest

The authors declare that there is no conflict of interest.

Resumen

Introducción: El melasma es un trastorno de la piel caracterizado por un aumento en la producción de melanina que genera molestias al paciente. La alfa-arbutina bloquea la biosíntesis de melanina epidérmica al inhibir la oxidación enzimática de la tirosina. La alfa-arbutina es hidrófila y penetra poco a través del estrato córneo. Los etosomas mejoran la permeabilidad de los fármacos a capas más profundas y prolongan la liberación. El objetivo principal de este estudio fue preparar un gel etosomal que contenga alfa-arbutina para mejorar la permeación a la piel.

Método: Se prepararon geles de etosomas de alfa arbutina mediante el método de frío utilizando lecitina de soja, etanol y propilenglicol (PG) y se evaluaron la difusión del fármaco in vitro, el tamaño de las vesículas, la eficiencia de atrapamiento y el estudio dermatocinético.

Resultados: Se encontró que la eficiencia de atrapamiento y la difusión del fármaco del gel etosomal preparado que contenía alfa arbutina era del 94,99 % y 106,63 %, respectivamente. El tamaño de la vesícula, el índice de polidispersidad y el potencial zeta de los etosomas formulados con 20 % p/p de etanol y 4 % p/p de lecitina de soja se registraron como 138,1 nm, 0,406 y -48 mV, respectivamente. El estudio de difusión in vitro ilustró la liberación ráfaga, con una liberación del fármaco del 97,56 ± 0,68 % a los 90 minutos. Al final de 8 horas, aproximadamente el 47,85 % del fármaco se había difundido desde el gel etosomal. El estudio dermatocinético demostró que el tiempo de retención del fármaco en la dermis y epidermis era significativamente mayor en el gel etosomal en comparación con la crema comercializada.

Conclusiones: La alfa arbutina se formuló con éxito como una suspensión etosomal y se convirtió en un gel. Gracias a la mayor concentración de etanol, se mejoró significativamente la penetración del fármaco en la dermis y la epidermis. Los estudios dermatocinéticos demostraron una mejor retención del fármaco en las capas de la dermis y la epidermis en comparación con la formulación comercializada.

Palabras clave: Alfa arbutina; Hiperpigmentación; etosomas; Permeación de la piel; Dermatocinética.

Abstract

Introduction: Melasma is a skin disorder characterized by increase in melanin production causes patient inconvenience. Alpha-arbutin blocks epidermal melanin biosynthesis by inhibiting enzymatic oxidation of Tyrosine. Alpha-arbutin is hydrophilic and poorly permeates through stratum corneaum. Ethosomes enhance permeability of drugs into deeper layers and extend the release. The main goal of this study was to prepare ethosomal gel containing alpha-arbutin to enhance permeation to skin.

Method: Ethosomes gel of alpha arbutin were prepared by cold method using soy lecithin, ethanol, and propylene glycol (PG) and evaluated for in vitro drug diffusion, vesicle size, entrapment efficiency and dermatokinetic study.

Results: The entrapment efficiency and drug diffusion of the prepared ethosomal gel containing alpha arbutin were found to be 94.99 % and 106.63 %, respectively. The vesicle size, polydispersity index, and zeta potential of the ethosomes formulated with 20 % w/w ethanol and 4 % w/w soy lecithin were recorded as 138.1 nm, 0.406, and -48 mV, respectively. The in vitro diffusion study illustrated burst release, with 97.56 ± 0.68 % drug released at 90 minutes. At the end of 8 hours, approximately 47.85% of the drug had diffused from the ethosomal gel. The dermatokinetic study demonstrated that the retention time of the drug in the dermis and epidermis was significantly higher in the ethosomal gel compared to the marketed cream.

Conclusions: Alpha arbutin was successfully formulated as an ethosomal suspension and converted into a gel. Due to the higher concentration of ethanol, drug permeation into the dermis and epidermis was significantly improved. Dermatokinetic studies demonstrated better retention of the drug in the dermis and epidermis layers compared to the marketed formulation.

Keywords: Alpha arbutin; Hyperpigmentation; Ethosomes; Skin permeation; Dermatokinetics.

Highlights

Skin pigmentation disorders are common issues linked to melanocytes. Alpha arbutin inhibits melanogenesis by blocking the tyrosinase enzyme. Its hydrophilic nature and log P value of -1.49 hinder its penetration into the stratum corneum, resulting in less than 1% reaching the melanocytes.

Alpha arbutin can be formulated as ethosomes. Ethanol acts as a permeation enhancer, and the nanosized particles further facilitate the drug's permeation into the dermis and epidermis, with better re-

tention compared to marketed products. Particle size and zeta potential values indicate the formation of a stable formulation.

The dermatokinetic study revealed better permeation and retention of alpha arbutin in the dermis and epidermis compared to the marketed formulation.

Introduction

Melanocyte-related disorders of skin pigmentation (hypopigmentaion and hyperpigmentation) are the most prevalent conditions. The majority of women have abnormal face pigmentation due to a combination of external and endogenous causes. Skin discoloration patients suffer from emotional and cognitive issues that affect their psychosocial health[1]. There are numerous causes of hyperpigmentation which negatively impacts an individual's quality of life. Addressing hyperpigmentation can be a challenging and discouraging process, particularly for women. The primary cause is the increase in melanin content in the dermis or epidermis[2].The process by which melanocytes produce melanin is known as melanogenesis. One important enzyme in the formation of melanin is tyrosinase. Tyrosine is converted to melanin by tyrosinase in a few steps. Melanin pigment's primary function is to absorb ultraviolet (UV) radiation (UVA and UVB), thereby protecting the skin from UV radiation. Hyperpigmentation is the result of an excess of melanin produced by UV exposure. Therefore, one of the main strategies for avoiding skin hyperpigmentation is melanin inhibition [3]. The most common treatment for hyperpigmentation is skin brightening [4]. The sources of skin-whitening chemicals are both synthetic and natural. The primary obstacle of whitening chemicals is its poor stability. As a result, encapsulating these whitening agents can enhance their concentration at specific places and physicochemical stability [5].

Hydroquinone inhibits the tyrosinase enzyme and halts the melanogenesis process, making it a depigmenting agent. However, it can lead to several negative effects, such as mutagenicity in African populations and an increased incidence of ochronosis [6,7].As such, the Food and Drug Administration (FDA) in the United States (US) and the European Union (EU) has prohibited the use of it in any over-the-counter preparation. The less cytotoxic derivatives of hydroquinone are utilized to achieve the whitening effects of the original drug. The derivative alpha arbutin is one of them. It is mostly made from hydroquinone through enzymatic synthesis. Alpha-arbutin is 4-hydroxyphenyl a-D-glucopyranoside structurally [8]. Beta-arbutin, or 4-hydroxyphenyl b-D-glucopyranoside, is another derivative [6]. When alpha-arbutin and its optical isomer, 4-hydroxyphenyl b-D-glucopyranoside, were tested for their ability to block tyrosinase, alpha-arbutin had a greater inhibitory action than beta-arbutin [8].Alpha-arbutin is used cosmetically, but it also has medicinal uses, including the treatment of urinary tract infections, anticancer activity, and antioxidant and anti-inflammatory qualities [6]. Bearberry extract naturally contains arbutin, but it can also be produced by glucosidation from hydroquinone [7].Alpha arbutin is hydrophilic and at 20 ± 5 °C, it has a water solubility of 151 g/L. The Scientific Committee on Consumer Safety (SCCS) recommends using up to 2% w/w and 0.5% w/w of alpha arbutin in face creams and body lotions, respectively [9]. Arbutin's high hydrophilicity (log P value of -1.49) and poor penetration through the stratum corneum restrict its use in topical preparations, despite the fact that it effectively inhibits tyrosinase to produce whitening effects. As a result, conventional skin formulations like lotions and creams fail to produce sufficient skin deposition [10].

These hurdles can be resolved by ethosomes, which effectively deliver drugs of all types (amphiphilic, lipophilic, and hydrophilic) to the epidermis as well as deeper into the skin by enhancing drug permeation[11,12]. Hence, in the present study, an attempt was made to develop an ethosomal suspension to encapsulate the drug in soy lecithin and incorporate it into a gel for easier application on the skin. Through this approach, drug penetration and retention in the deeper layers of the skin are improved.

Materials and Methods

Alpha arbutin was gifted by Barentz (Mumbai, India), Propylene glycol (PG) was obtained from Thomas Beaker (Mumbai, India), Soy Lecithin was purchased from HiMedia (Mumbai, India), Ethanol was ob-

tained from SD Fine Chemicals (Mumbai, India) and Sepineo P 600 was gifted by Blue Cross Laboratories (Nashik, India). All chemicals used were pharmaceutical grade.

Preparation of ethosomal suspension

Alpha arbutin ethosomal suspensions were prepared using the cold method, as described by Touitou et al. [14]. An ethanol solution with soy lecithin was made using a magnetic stirrer (REMI, Mumbai, India). PG was added to this ethanol-lipid mixture, referred to as the organic phase. Separately, a 2 % w/w drug solution was prepared in water, known as the aqueous phase. The system was maintained at 30 °C. The aqueous phase was added to the organic phase by means of syringe with continuous stirring at 700 rpm for 30 minutes [15]. The mixture was then sonicated using probe sonicator (Athena Technology, Electro lab, India) for 25-30 minutes to reduce particle size, then homiginized and refrigerated overnight to obtain stable ethosomes [16]. On next day, the mixture was centrifuged using cooling centrifugation at 10 ˚C, 8000 rpm for 10 minutes. Then, supernatant was collected as final ethosomal suspension. This suspension stored in refrigerator for further use. Various formulations with different lipid and ethanol concentrations were tested, as shown in Table 1. The optimal formulation was selected based on size analysis, zeta potential, polydispersity index (PDI), and % entrapment efficiency (% EE). The optimized ethosomal formulation was used to create a gel for further characterization.

Table 1: Formulation batches with varied concentration of ethanol and soylecithin

Formulation Batches	Drug %w/w	Ethanol %w/w	PG %w/w	Soy Lecithin %w/w	Water (q.s.) %w/w
F1	2	20	20	1	100
F2	2	20	20	2	100
F3	2	20	20	4	100
F4	2	30	20	1	100
F5	2	30	20	2	100
F6	2	30	20	4	100
F7	2	40	20	1	100
F8	2	40	20	2	100
F9	2	40	20	4	100

Incorporation of ethosomal suspension into gel

The gel base was prepared using Sepineo P 600, which offers several advantages: it works at room temperature, does not require high shear, and being non-thixotropic allows for flexibility in the processes and equipment used. The formulation was homogenized at 300 rpm with a measured amount of 5 % w/w Sepineo P 600 added until a clear gel was obtained. The formulation was then assessed for physical appearance, pH, and spreadability.

Characterization of ethosomes:

Vesicle size, PDI, zeta potential and entrapment efficiency

Using a Malvern Zetasizer, the dimensions, zeta potential, and PDI of each alpha arbutin ethosomal suspension were measured at 25 °C after diluting samples with distilled water. The % EE was determined by ultracentrifugation: suspensions were centrifuged at 8000 rpm for 10 minutes to separate the supernatant. This was then diluted to pH 5.6 with phosphate buffer and analyzed for absorbance

using a spectrophotometer. The medication amount in the supernatant was calculated with a linear equation, and %EE was determined using the provided formula [17].

$$\%EE = \frac{\text{Total amount of drug in sample} - \text{unentrapped drug in sample}}{\text{Total amount of drug in sample}} \times 100$$

In vitro drug diffusion of ethosomal suspensión

A Franz diffusion cell with a 90-m length was used for *in vitro* drug diffusion studies. Cellophane membrane, activated by immersion in pH 5.5 phosphate buffer for 1 hour, served as the diffusion membrane. The receptor medium, phosphate buffer at pH 5.6, filled the receptor compartment (1.76 cm^2 surface area). The membrane was placed between the donor and receptor compartments, with the temperature maintained at 37 ±2 °C. The donor compartment was covered with 1 ml of ethosomal suspension. To maintain sink conditions, 1 ml aliquots were taken from the receptor medium at 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min and replaced with fresh buffer. Drug diffusion was measured using a UV spectrophotometer, and permeation parameters were determined by plotting the total drug amount absorbed against time.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR analysis of alpha arbutin, soy lecithin, and the optimized formulation was conducted using an IR Affinity spectrometer (Shimadzu Co., Japan). The detection range was 400–4000 cm^{-1}. Spectra were produced showing the percentage transmittance of infrared light versus wavenumber, indicating the functional groups present.

pH measurement of ethosomal gel

The pH of the ethosomal gel was measured using a pH meter. Electrodes were immersed in the formulations, and the pH readings were displayed and recorded. Measurements were taken in triplicate, and the average pH was recorded.

Spreadability measurement

The gel base should spread easily without excessive drag or increased friction. Spredability was measured using an apparatus with a wooden board, a scale, and two glass slides with pans mounted on pulleys. The sample was sandwiched between the slides, compressed to a consistent thickness with 100 g of weight for five meters, and a 250 g weight was added to the pan. Spreadability was determined by the time (t) in seconds required to separate the slides, calculated using the following formula:

$$S = \frac{M \times l}{t}$$

Where, l = length of slide

Physical appearance of ethosomal gel

The prepare gel was further suspected to physical appearance such as color, texture and odour.

Comparative study by in vitro diffusion with marketed formulation

A Franz diffusion cell was used to compare the diffusion of ethosomal gel (alpha arbutin) with a cream formulation (alpha arbutin 2 % w/w). The donor compartment's cellophane membrane was coated with the formulations, and the receptor compartment was filled with phosphate buffer at pH 5.6. At 2, 3, 4, 5, 6, 7, and 8-hour intervals, 3 ml samples were withdrawn from the receptor compartment and replaced with an equal volume of receptor solution. The samples were then analyzed using a UV spectrophotometer.

Dermatokinetics study

For the dermatokinetic study, fresh goat skin was used, sourced from a nearby slaughterhouse with intact ear pinna skin. Hair was clipped, and subcutaneous fat and muscle debris were removed before cleaning the skin with phosphate buffer (pH 5.6). The dermis side of the skin was mounted in a Franz diffusion cell with the stratum corneum facing the donor compartment, where the gel was applied.

At designated intervals, the skin was removed from the diffusion cell, cleaned to remove any residual formulation, and then separated into epidermis and dermis. Both layers were cut into small pieces, macerated in 5 ml of ethanol for 24 hours, and sonicated for 2 hours to extract the drug. The samples were filtered through a 0.22 µm filter and analyzed using UV spectrophotometry. Results were assessed using a one-compartment model.

$$C_{skin} = \frac{K_p . C_{max}^{skin}}{(K_p - K_e)} (e^{-K_p t} - e^{-K_e t})$$

Where C_{skin} represents the concentration of drug in the skin at time t, K_p represents the dermal permeation constant, represents the maximum drug concentration achieved, and K_e represents elimination constant in skin [18,19].

Stability study

The stability study for ethosomal gel formulations was conducted over 12 weeks at two temperatures: refrigerated (4 ± 2 °C) and room temperature (30 ± 2 °C). The formulations were stored in borosilicate containers to prevent interactions. Physical changes, including color, odor, and appearance, were monitored and % CDR at initial time and after 12 weeks were determined.

Results

Vesicle size, PDI, and %EE:

Alpha arbutin ethosomal formulations exhibited entrapment efficiencies (EE) from 93.14 % to 94.99 %. The highest EE was achieved with F3, which had 20 % w/w ethanol and 4 % w/w soy lecithin (Table 2). Arbutin ethosomes ranged in size from 138.1 to 350.1 nm. The vesicle size was significantly influenced by the proportions of ethanol and soy lecithin. The smallest vesicles (138.1 ± 6.85 nm) were obtained with 20% w/w ethanol and 4 % w/w soy lecithin, while the largest vesicles were with 40 % w/w ethanol and 1 % w/w soy lecithin. Each formulation had a PDI ranging from 0. 383 to 0.498. Zeta potential, another key stability measure, reflects ethosome stability, with higher values indicating better stability. Ethanol imparts a negative charge to the vesicles, preventing aggregation. For batch F3, the zeta potential was -48.0 mV.

Table 2: % EE, vesicle size and PDI

Formulation Batches	% EE	Vesicle size (nm)	PDI
F1	93.14±0.03	289.4±10.65	0.498
F2	94.06±0.01	292.6±9.13	0.432
F3	94.99±1.52	138.1±6.85	0.406
F4	94.39±0.03	323.8±7.27	0.443
F5	94.56±0.03	138.6±8.12	0.476
F6	94.22±0.72	199.0±7.52	0.383
F7	94.36±0.05	350.1±12.63	0.406
F8	94.12±0.03	264±10.34	0.393
F9	93.58±0.84	240.2±9.52	0.420

In vitro drug diffusion of ethosomal suspension

The % CDR for each batch was evaluated up to 90 minutes, with batch F3 showing the highest % CDR at 106.63±4.23 % (Figure 1).

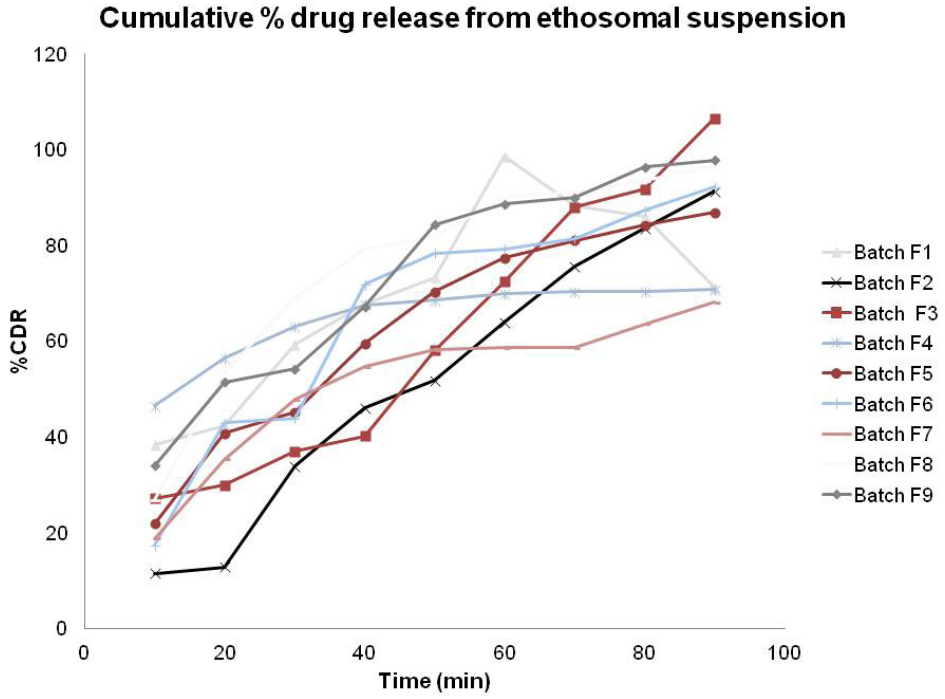

Figure 1: % CDR of ethosomal suspension at the end of 90 m.

FTIR analysis of drug and excipients:

The FTIR spectra of alpha arbutin, soy lecithin, and their mixture were analyzed in the 400-4000 cm^{-1} range (Figure 2). Key peaks for alpha arbutin included 3400-3600 cm^{-1} (OH), 1514.12 cm^{-1} (phenyl ring), 2924.09 cm^{-1} (C-H), and 1217.02 cm^{-1} (C-O). For soy lecithin, significant peaks were observed at 3529.15 cm^{-1} (OH), 2827.72 cm^{-1} (symmetric C-H), 1739.79 cm^{-1} (ester C=O stretch), and 721.38 cm^{-1} (C=C bending). In the mixture, major peaks included 3529.75 cm^{-1} (OH), 2924.09 cm^{-1} (C-H), 1739.79 cm^{-1} (C=O), 1514.12 cm^{-1} (phenyl ring), and 721.38 cm^{-1} (C=C).

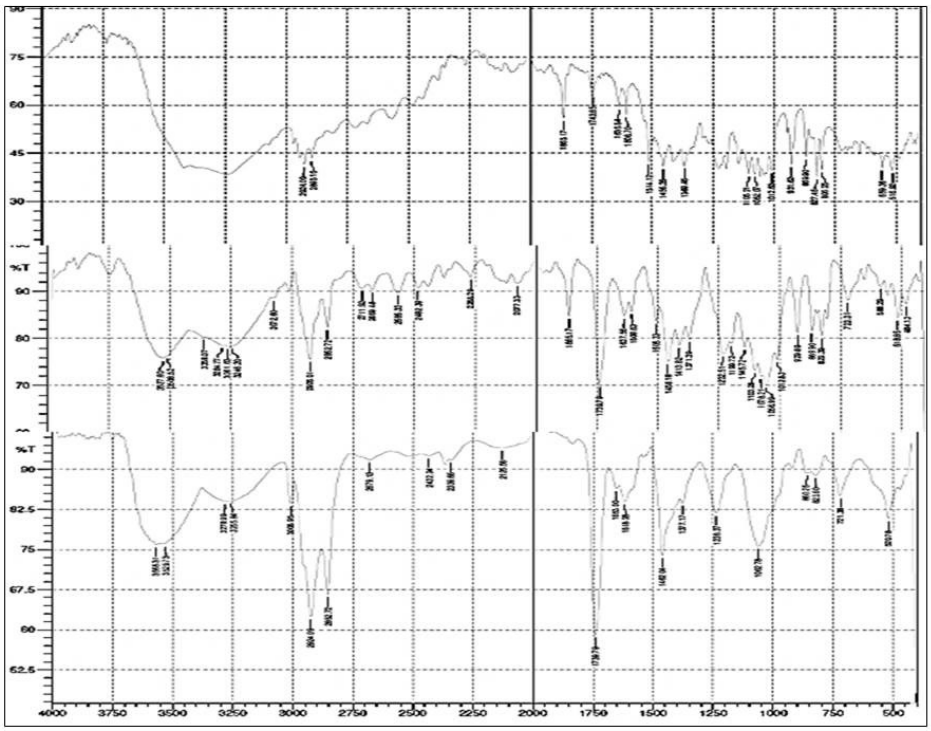

Figure 2: IR spectra of A. Alpha arbutin B. Soy lecithin and C. Alpha arbutin + Soy lecithin

FTIR of optimized ethosomal suspension:

FTIR spectrum of optimized formulation is shown in Figure 3. Key features include an -OH stretch at 3338.86 cm^{-1}, a C-H stretch at 2979 cm^{-1}, and an aromatic ring peak at 1508 cm^{-1}. Sharp peaks for $P\text{-}O_2$ and P-O-C shifted from 1062 cm^{-1} to 1041 cm^{-1}.

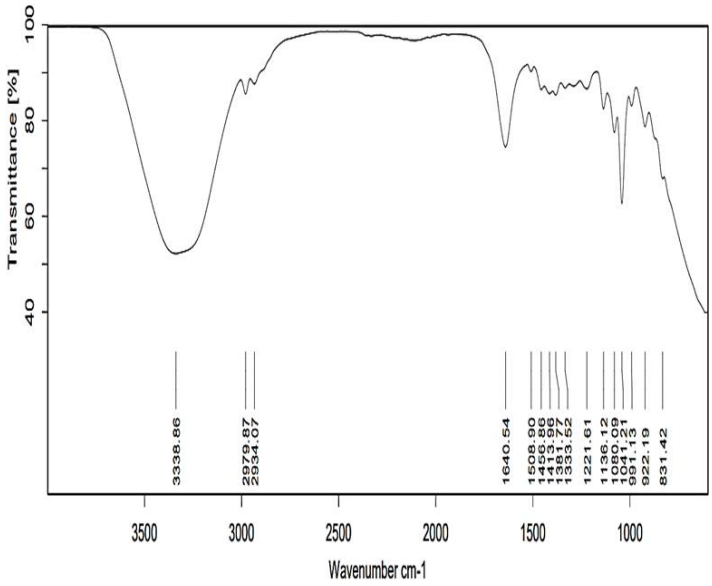

Figure 3: IR spectra of optimized batch.

pH measurement of ethosomal gel:

Human skin has a slightly acidic pH ranging from 4.1 to 5.8 [20]. The pH of prepared ethsomal gel was measure using digital pH meter (Systronic pH Meter) at room temperature and was found to be 5.5 ± 0.2.

Spreadability measurement:

Spreadability of gel was measured and found to be 15.27 g.cm/sec.

Physical appearance of ethosomal gel:

The alpha arbutin loaded ethosomal gel was physically observed for colour, texture and odour and results reported in Table 3.

Table 3: Physical appearance test

Characteristics	Results
Colour	Yellow transparent
Texture	Smooth
Odour	Characteristic

Comparative study by in vitro diffusion with marketed formulation:

In vitro drug diffusion study between prepared ethosomal gel and marketed product shown significant difference at the end of 8 h (Figure 4)

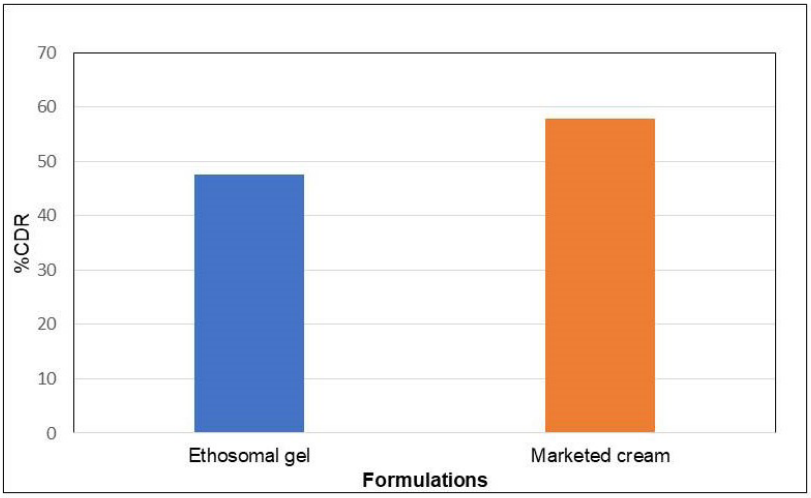

Figure 4: %CDR of ethosomal gel and marketed cream at 8 h.

Dermatokinetic modeling study:

A dermatokinetic study compared alpha arbutin-loaded ethosomal gel with a marketed cream (alpha arbutin 2 % w/w). The study evaluated the concentration of alpha arbutin in skin layers (epidermis and dermis) and retention time. Results indicated that the ethosomal gel achieved higher alpha arbutin concentrations and significantly increased drug retention in both the epidermal and dermal layers compared to the marketed cream. (Table 4)

Table 4: Dermatokinetic parameters of ethosomal gel and marketed cream

Parameters	Alpha arbutin loaded ethosomal gel		Marketed cream (Underated Alpha arbutin 2%)	
	Epidermis	Dermis	Epidermis	Dermis
(h)	4.9±1.32	3.53±1.10	0.83±0.75	0.68±2.12
(µg/)	60.33±0.98	138.37±1.75	55.10±0.61	53.70±0.04
	391.06±1.02	878.28±1.36	236.16±0.91	155.16±0.37
	690.49±1.4	1330.15±0.02	242.07±0.35	159.82±0.68
MRT(h)	8.42±0.99	7.07±1.64	2.44±2.5	2.46±0.53

Note: Cm_{ax} skin= the máximum drug concentration achieved, $T_{skinmax}$= time at which máximum concentration achieved, MRT= mean retention time, mean±SD, n=3

Stability study

Stability study of prepared ethosomal gel was carried out at two different temperatures (4 ± 2˚C and 30 ±2 ˚C). The formulations were evaluated for qualitative parameters and quantitatively by one way ANOVA test as shown in (Table 5). The calculated and theoretical F values at 4 °C were found to be

0.001289 and 4.4139 respectively, while at 30 °C they were found to be 0.00449 and 4.4139, respectively. As at both temperatures, calculated values where less than theoretical values indicate there was no significant difference between the stability data at stated temperatures and period.

Table 5: Stability study of ethosomal gel

Temperature	Identification test	Duration	
		0 Month	3 Month
Qualitative parameters			
4˚C±2˚C	Color	Yellow Transparent	Yellow Transparent
	Texture	Smooth	Smooth
	Odour	Characteristic	Characteristic
30˚C±2˚C	Color	Yellow Transparent	Yellow Transparent
	Texture	Smooth	Smooth
	Odour	Characteristic	Characteristic
Quantitative parameters			
4˚C±2˚C		**30˚C±2˚C**	
%CDR			
0 Month	3 Months	0 Month	3 months
0.17±0.51	0.21±0.37	0.23±0.15	0.219±0.21
4.76±1.03	5.12±0.75	5.23±0.67	5.43±0.34
9.86±1.26	10.29±1.07	10.51±0.51	10.71±0.77
15.69±1.14	16.31±1.61	16.47±0.86	15.92±0.89
21.3±0.95	20.75±1.28	21.56±1.32	20.57±1.25
23.15±1.03	23.32±1.73	24.03±1.53	23.49±1.13
27.51±1.86	26.93±1.57	27.19±1.78	26.62±1.65
35.63±1.62	34.52±1.78	35.68±1.46	35.12±1.98
42.74±1.43	42.09±1.22	41.98±1.95	40.95±2.16
49.36±2.21	48.08±2.04	48.53±2.77	47.72±2.43

Discussion

Increasing soy lecithin concentration from 1 % to 4 % w/w initially increased EE, but further increases in lecithin resulted in decreased EE likely due to reduced vesicle deformability. Vesicle size increased with higher ethanol concentrations up to 40 % w/w but decreased with higher soy lecithin concentration. A low PDI value implies the formation of bit polydisperse particles. The zeta potential value indicates that the prepared formulation has sufficient stability. The F3 formulation was selected as the optimized formulation based on the characterization results. FTIR data demonstrate that the drug and selected excipients are compatible. The pH of the formulated gel was comparable to that of human skin. The sufficiently large spreadability value suggests that the prepared gel can be easily applied to the skin and will reach to greater surface of skin. The *in vitro* study showed drug release from the prepared ethosomal gel was slower compared to the marketed product. The dermatokinetic study demonstrated that ethanol enhances the permeation of the drug, and the small particle size of ethosomes further facilitates the drug's penetration and better retention into the epidermis and dermis layers over marketed formulation. In vitro drug release from the prepared ethosomal gel was comparatively slow over marketed product. The formulation was found to be physically stable, and the in vitro drug release showed no significant difference after 3 months.

Conclusion

In present study, ethosomes was successfully prepared and incorporated into a gel using Sepineo P 600. Batch F3 exhibited the smallest vesicle size, acceptable PDI, and zeta potential value for stability; therefore, it is considered the optimized batch. The release of alpha arbutin from prepared ethosomal gel was a bit slow when compared to marketed product. The dermatokinetic study demonstrates better retention of the drug, with the MRT of the ethosomal gel increasing 3.45 and 2.87 folds over the marketed product in the epidermis and dermis layers, respectively. This study demonstrates that ethosomes have the potential to serve as an effective vehicle for topical delivery of alpha arbutin. In the current research, an alpha-arbutin-loaded ethosomal gel was successfully developed and evaluated.

References

1. Radmard A, Saeedi M, Morteza-Semnani K, Hashemi S, Nokhodchi A. An eco-friendly and green formulation in lipid nanotechnology for delivery of a hydrophilic agent to the skin in the treatment and management of hyperpigmentation complaints: Arbutin niosome (Arbusome). Colloids and Surfaces B: Biointerfaces. 2021;201:111616. Doi: 10.1016/j.colsurfb.2021.111616.

2. Rigopoulos D, Gregoriou S, Katsambas A. Hyperpigmentation and melasma. Journal of cosmetic dermatology. 2007;6(3):195-202. Doi: 10.1111/j.1473-2165.2007.00321.x.

3. Migas P, Krauze-Baranowska M. The significance of arbutin and its derivatives in therapy and cosmetics. Phytochemistry Letters. 2015;13:35-40. Doi: 10.1016/j.phytol.2015.05.015.

4. Saeedi M, Eslamifar M, Khezri K. Kojic acid applications in cosmetic and pharmaceutical preparations. Biomedicine & Pharmacotherapy. 2019;110:582-93. Doi: 10.1016/j.biopha.2018.12.006.

5. Ephrem E, Elaissari H, Greige-Gerges H. Improvement of skin whitening agents efficiency through encapsulation: Current state of knowledge. International Journal of Pharmaceutics. 2017;526(1-2):50-68. Doi: 10.1016/j.ijpharm.2017.04.020.

6. Garcia-Jimenez A, Teruel-Puche J, Berna J, Rodriguez-Lopez JN, Tudela J, Garcia-Canovas F. Action of tyrosinase on alpha and beta-arbutin: A kinetic study. PloS one. 2017;12(5):e0177330. Doi: 10.1371/journal.pone.0177330.

7. Smit N, Vicanova J, Pavel S. The hunt for natural skin whitening agents. International journal of molecular sciences. 2009;10(12):5326-49. Doi: 10.3390/ijms10125326.

8. Sugimoto K, Nishimura T, Nomura K, Sugimoto K, Kuriki T. Inhibitory effects of α-arbutin on melanin synthesis in cultured human melanoma cells and a three-dimensional human skin model. Biological and Pharmaceutical Bulletin. 2004;27(4):510-4. Doi: 10.1248/bpb.27.510.

9. Chandorkar N, Tambe S, Amin P, Madankar C. Alpha Arbutin as a Skin Lightening Agent: A Review. International Journal of Pharmaceutical Research (09752366). 2021;13(2). doi: 10.31838/ijpr/2021.13.02.446.

10. Wen A, Choi M, Kim D. Formulation of liposome for topical delivery of arbutin. Archives of pharmacal research. 2006;29:1187-92. Doi: 10.1007/BF02969312.

11. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. Journal of advanced pharmaceutical technology & research. 2010;1(3):274-82. Doi: 10.4103/0110-5558.72415.

12. Akhtar N, Akhtar N. Development of stable tocopherol succinate‐loaded ethosomes to enhance transdermal permeation: In vitro and in vivo characterizations. Journal of Cosmetic Dermatology. 2022;21(10):4942-55. Doi: 10.1111/jocd.14907.

13. Rakesh R, Anoop K. Ethosomes for transdermal and topical drug delivery. Int J Pharm Pharm Sci. 2012;4(3):17-24.

14. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of controlled release. 2000;65(3):403-18. Doi: 10.1016/s0168-3659(99)00222-9.

15. El-Shenawy A, Abdelhafez W, Ismail A, Kassem A. Formulation and characterization of nanosized ethosomal formulations of antigout model drug (febuxostat) prepared by cold method: In vitro/ex vivo and in vivo assessment. Aaps Pharmscitech. 2020;21:1-3. Doi: 10.1208/s12249-019-1556-z.

16. Satyam G, Shivani S, Garima G. Ethosomes: A novel tool for drug delivery through the skin. J Pharm Res. 2010;3(4):688-91.

17. Limsuwan T, Boonme P, Khongkow P, Amnuaikit T. Ethosomes of phenylethyl resorcinol as vesicular delivery system for skin lightening applications. Biomed research international. 2017;2017(1):8310979. Doi: 10.1155/2017/8310979.

18. Garg N, Sharma G, Singh B, Nirbhavane P, Tyagi K, Shukla R, Katare O. Quality by Design (QbD)-enabled development of aceclofenac loaded-nano structured lipid carriers (NLCs): An improved dermatokinetic profile for inflammatory disorder (s). International Journal of pharmaceutics. 2017;517(1-2):413-31. Doi: 10.1016/j.ijpharm.2016.12.010.

19. Raza K, Singh B, Singla S, Wadhwa S, Garg B, Chhibber S, Katare O. Nanocolloidal carriers of isotretinoin: antimicrobial activity against Propionibacterium acnes and dermatokinetic modeling. Molecular pharmaceutics. 2013;10(5):1958-63. Doi: 10.1021/mp300722f.

20. Proksch E. pH in nature, humans and skin. J dermatology. 2018;45(9):1044-52. Doi: 10.1111/1346-8138.14489.

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Artículos originales

Bacterias resistentes a antibióticos en aguas superficiales de la región pampeana, Argentina

Antibiotic resistant bacteria in surface waters of the pampeana region, Argentina

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Conflicto de intereses

Los autores dejan constancia que no existe ningún tipo de conflicto de intereses en el presente artículo.

Resumen

Introducción: Los antibióticos son ampliamente utilizados en medicina humana y veterinaria para el tratamiento terapéutico de enfermedades infecciosas.Pueden alcanzar el medioambiente acuático después de ser utilizados en la cría de animales como promotores del crecimiento y para fines terapéuticos.En este trabajo, se evaluó la presencia de bacterias resistentes a antibióticos en el sistema de canales naturales que conecta un feedlot de la Pampa Ondulada con el Arroyo Burgos, en la Provincia de Buenos Aires.

Método: Se determinó la prevalencia de bacilos Gram negativos resistentes a antibióticos de uso veterinario y humano. La sensibilidad a los antibióticos de bacterias Gram negativas seleccionadas se evaluó mediante el método de difusión en disco. Los aislamientos de bacterias resistentes a ceftazidima o a cefotaxima fueron designados como sospechosos productores de beta lactamasas de espectro extendido(BLEE). Potenciales aislamientos productores de BLEE fueron confirmados por el método de difusión en disco con ácido clavulánico.

Resultados: Entre las cepas aisladas encontramos: *Escherichia coli, Enterobacter cloacae, Pseudomonas fluorescens, Cedecea davisae y Pseudomonas oryzihabitans*. En el perfil de resistencia podemos observar una alta resistencia a cefalosporinas de tercera generación de uso clínico como ceftazidima y cefotaxima. De acuerdo a los ensayos fenotípicos, el 60 % de las cepas resistentes a ceftazidima son productoras de BLEE.

Conclusiones: Se pudo determinar una contaminación difusa con bacterias resistentes a antibióticos betalácticos tanto de uso veterinario como de uso clínico. Representando estos resultados un problema para la salud pública.

Palabras clave: Bacterias resistentes; betalactámicos; aguas superficiales; ganadería intensiva.

Abstract

Introduction: Antibiotics are widely used in human and veterinary medicine for the therapeutic treatment of infectious diseases. They can reach the aquatic environment after being used in animal husbandry as growth promoters and for therapeutic purposes. In this work, the presence of antibiotic-resistant bacteria in the natural canal system that connects a feedlot in the Pampa Ondulada with the Arroyo Burgos, in the Province of Buenos Aires, was evaluated.

Method: The prevalence of Gram-negative bacilli resistant to veterinary and human antibiotics was determined. The antibiotic sensitivity of selected Gram-negative bacteria was evaluated by the disk diffusion method. Bacterial isolates resistant to ceftazidime or cefotaxime were designated as suspected ESBL producers. Potential ESBL-producing isolates were confirmed by the disk diffusion method with clavulanic acid.

Results: Among the isolated strains we find: *Escherichia coli, Enterobacter cloacae, Pseudomonas fluorescens*, *Cedecea davisae* and *Pseudomonas oryzihabitans.* In the resistance profile we can observe high resistance to third-generation cephalosporins for clinical use such as ceftazidime and cefotaxime. According to phenotypic assays, 60% of ceftazidime-resistant strains are ESBL producers.

Conclusions: Diffuse contamination with bacteria resistant to beta-lactam antibiotics for both veterinary and clinical use could be determined. These results represent a problem for public health.

Keywords: Resistant bacteria; beta-lactams; surface waters; intensive livestock farming.

Puntos clave

Según la Organización Mundial de la Salud, “la resistencia a los antibióticos es hoy una de las mayores amenazas para la salud mundial”. La cría intensiva de animales para consumo humano se ha convertido en un reservorio para la propagación de la resistencia antimicrobiana principalmente en función de la cantidad de antimicrobianos utilizados en la producción animal.

Este trabajo resalta la importancia de estudiar la resistencia antimicrobiana desde el enfoque de *Una Salud* (OMS).

Con los datos obtenidos observamos cómo el ambiente acuático rural disemina la resistencia bacteriana, constituyendo una amenaza emergente para la salud pública y medioambiental.

Introducción

Los antibióticos son ampliamente utilizados en medicina humana y veterinaria para el tratamiento terapéutico de enfermedades infecciosas, y como promotores del crecimiento animal[1,2]. Se excretan como compuestos originales o metabolitos debido a una mala absorción intestinal o un metabolismo incompleto[3].En el medio acuático se detectan frecuentemente como consecuencia de su eliminación parcial durante el tratamiento de aguas residuales, derivando en una continua liberación al medioambiente[4].Una vez en el medioambiente, los residuos de antibióticos pueden tener efectos negativos sobre la biota de los diferentes niveles tróficos y sobre la salud humana por el consumo de alimentos y agua contaminados. La presencia de antibióticos y sus metabolitos contribuyen al aumento de la población bacteriana resistente y al mantenimiento de una presión selectiva capaz de provocar el desarrollo y/o la diseminación de dichas resistencia en los diferentes compartimentos del medioambiente[5,6].

La contaminación por antibióticos en agua dulce es ubicua y las concentraciones son sustanciales; muchos antibióticos son tóxicos para los organismos de agua dulce, desde bacterias hasta organismos multicelulares; incluso las concentraciones subletales tienen la capacidad de inducir cambios en las comunidades de agua dulce a través de la resistencia bacteriana[7]. El uso de antimicrobianos en la atención sanitaria, la agricultura, la horticultura, la acuicultura y los entornos industriales tiene un impacto en la expresión, selección, persistencia y transferencia de características de resistencia en poblaciones bacterianas[8,9]. Esta situación propicia la transferencia de bacterias resistentes a los antibióticos y genes de resistencia entre el medio ambiente, los animales y los seres humanos[10],lo que ha llevado al reconocimiento del papel del medio ambiente en la aparición y diseminación de la resistencia a los antimicrobianos (RAM) desde una perspectiva global de *Una Salud* (OMS). El enfoque desde *Una Salud* ayuda a lograr una comprensión más completa del problema de la RAM, permite soluciones eficientes, desarrolla pautas de uso apropiadas y proporciona comunicaciones de riesgos efectivas[11,12]. La RAM se aborda como un “tema de *Una Salud*”, desde las causas, hasta la búsqueda de soluciones que abarquen las interacciones entre los seres humanos, los animales y el medioambiente[13].

La persistencia de residuos antimicrobianos en los alimentos y los desechos animales que contaminan el suelo y el agua también afecta a los microbiomas acuáticos y ambientales[8,7]. Según Taylor et al.[14], el medio acuático se considera un entorno fundamental para la liberación ambiental, transformación, mezcla y persistencia de residuos de antibióticos, bacterias resistentes a antibióticos y genes de resistencia a antibióticos.

La RAM tanto en medicina humana como veterinaria ha alcanzado niveles alarmantes en la mayor parte del mundo y se ha reconocido como una importante amenaza emergente para la salud pública y la seguridad alimentaria a nivel mundial[8].

El sector ganadero es considerado uno de los principales contribuyentes de los problemas ambientales a nivel mundial, incluida la contaminación del agua. La producción ganadera genera una gran cantidad de estiércol y aguas residuales, que suelen contener altas concentraciones de contaminantes, como nutrientes (nitrógeno y fósforo compuestos), materia orgánica, metales pesados y productos farmacéuticos[15].

Esta liberación se da principalmente con la utilización de estiércol como fertilizante, cuando dicho estiércol proviene de animales tratados con antimicrobianos; asimismo, con el riego de cultivos con aguas contaminadas provenientes de granjas que no cumplen con las normas de utilización de aguas para riego de cultivos. Se estima que del 75 al 90 % de los antimicrobianos utilizados en los alimentos de los animales son excretados al medio ambiente, a través de la escorrentía agrícola[16]. El uso de cefalosporinas de tercera generación en el ganado se ha asociado con la aparición y propagación de betalactamasas de espectro extendido (BLEE) en bacterias Gram negativas, lo que plantea un grave riesgo para la salud pública[8].

La Región Pampeana, ubicada en el cono sur de América del Sur, es la más extensa pradera de Argentina. Se caracteriza por ser una gran llanura de tierras fértiles aptas para la agricultura y la ganadería. En respuesta a la creciente intensificación de la producción agrícola y la disminución de tierras disponibles para la ganadería en esta región, se han adoptado otras técnicas de producción ganadera. En los últimas décadas, los sistemas de engorde a corral (*feedlot*) con altas cargas animales por unidad de superficie, han experimentado un notable aumento en contraposición a la tradicional cría y engorde a campo en mayores extensiones de tierra[17]. Sin embargo, este cambio ha traído nuevas problemáticas en relación al impacto ambiental que generan, ya que representan una fuente de contaminación puntual tanto de aguas superficiales como subterráneas en el caso de no contar con un manejo adecuado[18].

El objetivo de este trabajo fue evaluar la presencia de bacterias resistentes a antibióticos presentes en el sistema de canales naturales que conecta un *feedlot* de la Pampa Ondulada con el Arroyo Burgos, en la provincia de Buenos Aires. Para cumplir con este objetivo se determinó la prevalencia de bacilos Gram negativos resistentes a antibióticos de uso veterinario y humano.

Métodos

Zona de estudio

Se evaluó un sector de la cuenca del arroyo Burgos. Dicho arroyo es tributario del Río Arrecifes. La cuenca del Río Arrecifes se encuentra ubicada en el norte de la provincia de Buenos Aires, en los partidos de San Pedro y Arrecifes (Argentina) y es tributaria de la Cuenca del Plata. Se encuentra a 180 Km al noroeste de la ciudad de Buenos Aires.El clima es templado, y la precipitación media anual oscila entre 800 y 1200 mm, con mínimas en julio y máximas en diciembre. La zona de estudio es predominantemente agrícola, con cultivos de soja, maíz y trigo. Existe en la región cría de ganado vacuno de forma intensiva y extensiva y de aves en menor escala.

Muestreo

Las muestras de agua fueron extraídas en la red de canales que conectan un *feedlot* ubicado en la localidad de Santa Lucia, San Pedro (33° 54’13,65”S/ 59° 50’12,15”W), con el cauce principal del Arroyo Burgos. Los sitios en los que se llevó adelante el muestreo fueron los siguientes: el punto 1 corresponde al efluente que sale del *feedlot* (con una capacidad de 15000 animales). Este canal atraviesa una zona con cultivos de soja, maíz y ganadería extensiva. Los puntos 2, 3, 4 se localizan en sitios alejados en forma creciente del punto 1. Los puntos 2 y 3 ubicados a 2,49 y 3,07 km con respecto al punto 1, corresponden a actividades agropecuarias consistentes en cultivos extensivos con predominio de soja y algunas instalaciones para la producción avícola intensiva. El punto 4 ubicado a 5,45 km con respecto al punto 1, corresponde a un área de ganadería extensiva. Los puntos 5 y 6 se ubican en el Arroyo Burgos, antes y después de la descarga del canal, respectivamente (Figura 1).

Figura 1. Ubicación de los sitios de muestreo

Ensayos de susceptibilidad antimicrobiana

A cada una de las muestras se les determinó el recuento de bacilos Gram negativos con y sin antibióticos (ampicilina (AMP), cefalotina (CFT), ceftriaxona (CRO), ceftazidima (CAZ), ceftiofur (C), oxitetraciclina (OT) y clortetraciclina (CTC) en medio Agar Violeta Rojo Bilis lactosa, con el objeto de calcular la prevalencia de bacilos Gram negativos resistentes a antibióticos. Los antibióticos fueron seleccionados considerando las principales clases de antimicrobianos utilizados en el ganado de las áreas de estudio e incluyendo algunos que son de importancia para la medicina humana Las placas fueron incubadas durante 24 hs a 35°C. Las pruebas de sensibilidad se han realizado por triplicado, siguiendo las normas del CLSI (Clinical&Laboratory Standards Institute, 2019)[19], utilizando los puntos de corte recomendados para definir la resistencia. La prevalencia de las bacterias resistentes se calculó como el número de bacterias que crecen en un medio con antibiótico dividido el número de bacterias que crecen en un medio sin antibiótico. Colonias morfológicas diferentes fueron adecuadamente aisladas de otros microorganismos para obtener subcolonias puras. La identificación bacteriana se realizó utilizando los sistemas de identificación API 20E y API 20NE (Biomérieux, France) y se utilizó como sistema de interpretación el APIWeb.

La sensibilidad a los antibióticos de bacterias Gram negativas seleccionadas se evaluó mediante el método de difusión en disco en agar Mueller-Hinton de acuerdo con el método estándar para pruebas de susceptibilidad a los antimicrobianos en disco. La densidad del inóculo se ajustó al estándar de turbidez de McFarland 0,5 y se incubó a 37°C durante 24 hs. Se determinó frente a 12 antibióticos; cefotaxima (CTX), ceftazidima (CAZ), cefalotina (CEF), cefepime (FEP), ciprofloxacina (CIP), gentamicina(GEN), trimetoprima-sulfametoxazol (TMS), imipenem (IMP), meropenem (MEM), amikacina (AKN), ampicilina-sulbactan (AMS) y piperacilina-tazobactam (TAZ).El diámetro de la zona de inhibición se mi-

dió con precisión después de 16 a 18 hs de incubación a 37°C.El diámetro se comparó con el diámetro de los estándares susceptibles, moderados y resistentes enumerados en CLSI 2019.

Los aislamientos resistentes a ceftazidima y a cefotaxima fueron designados como productores sospechosos de BLEE según lo define el CLSI.

Detección fenotípica de Betalactamasas de espectro extendido

Potenciales aislamientos productores de BLEE fueron confirmados por el método de difusión en disco de acuerdo con las guías de CLSI. Se utilizaron discos conteniendo cefotaxima (30 µg) frente a discos de cefotaxima +ácido clavulánico (30 +10 µg) y ceftazidima (30 µg) frente a ceftazidima + ácido clavulánico (30+10 µg). Se consideró como resultado positivo para la producción de BLEE, el aumento de 5 mm o más en la zona inhibitoria de los discos con ácido clavulánico con respecto al disco sin ácido clavulánico.

Análisis estadístico

Se transformaron los datos a log10 y se aplicó un ANOVA de un factor en cada punto muestra.

En todos los casos se evaluó la normalidad y la homoscedasticidad de los residuos. Al rechazarse la hipótesis de igualdad de medias entre antibióticos se evaluaron las medias de a pares mediante comparaciones a posteriori de Tuckey. Teniendo en cuenta que el tamaño de las muestras era chico se aplicó también la prueba no paramétrica de Kruskal Wallis obteniéndose las mismas conclusiones que con el test paramétrico. El procesamiento se realizó con el software InfoStat.

Resultados

En la Figura 2, se observan los valores medios de bacilos Gram negativos detectados en las muestras analizadas en los distintos puntos de muestreo. En todos los puntos de muestreo, se detectaron bacterias resistentes a los antibióticos ensayados.

En el punto 1(P1), no se puede decir que haya diferencia significativa entre los recuentos frente a los diferentes antibióticos. En el punto 2 (P2), no hay similitud en los recuentos frente a los antibióticos ensayados (P valor: 0,012), esto se debe a que hay una diferencia significativa entre cefalotina y ceftazidima (P valor: 0,04). Siendo la menor resistencia frente a ceftazidima y la mayor resistencia a cefalotina. En el punto 3 (P3) y en el punto 4 (P4), no se puede decir que haya diferencia significativa entre la resistencia a los antibióticos ensayados. En el punto 5 (P5), se observó una resistencia significativa frente a ampicilina y cefalotina (P valor: 0,001) con respecto a los demás antibióticos. En el punto 6 (P6), se puede afirmar que las bacterias resistentes a cefalotina presentan una resistencia significativa (P valor: 0,004) con respecto a ceftiofur, oxitetraciclina y clortetraclina.

Dentro de las cefalosporinas, ceftazidima presenta menor resistencia en todos los puntos de muestreo.

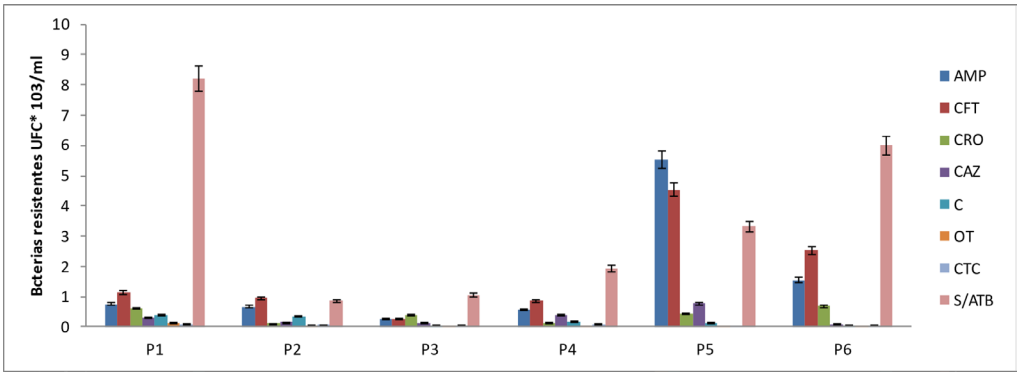

Figura 2. Recuento (UFC/ml) en medio Agar Violeta Rojo Bilis lactosa de bacilos Gram negativos resistentes a diferentes antibióticos. Puntos de muestreo: P1-P6.Antibióticos ensayados: AMP (ampicilina), CFT (cefalotina), CRO (ceftriaxona), CAZ (ceftazidima), C (ceftiofur), OT (oxitetraciclina), CTC (clortetraciclina); S/ATB (sin antibiótico).

En la Figura 3, se observa la prevalencia de las bacterias resistentes. Entre las cefalosporinas de 3ª generación, se observa una menor resistencia a ceftazidima, mientras que no hay diferencia entre ceftriaxona y ceftiofur. Tampoco, se observa diferencia entre las tetraciclinas.

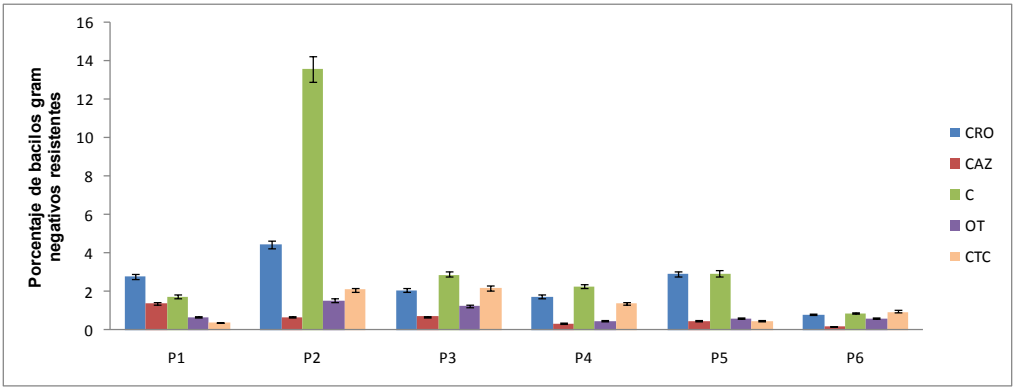

Figura 3. Prevalencia de bacilos Gram negativos resistentes a diferentes antibióticos, en medio Agar Violeta Rojo Bilis lactosa. Puntos de muestreo: P1-P6. Antibióticos ensayados: AMP (ampicilina), CFT (cefalotina), CRO (ceftriaxona), CAZ (ceftazidima), C (ceftiofur), OT (oxitetraciclina), CTC (clortetraciclina).

Entre las cepas aisladas encontramos: *Escherichia coli, Enterobacter cloacae, Pseudomonas fluorescens, Cedecea davisae y Pseudomonas oryzihabitans*. En el perfil de resistencia, podemos observar una alta resistencia a cefalosporinas de 3ª generación de uso clínico como ceftazidima y cefotaxima. De los 12 antibióticos ensayados, solo hallamos resistencia a 9antibióticos. No se observó Resistencia frente a ciprofloxacina, amikacina, piperacilina-tazobactam (Figura 4).

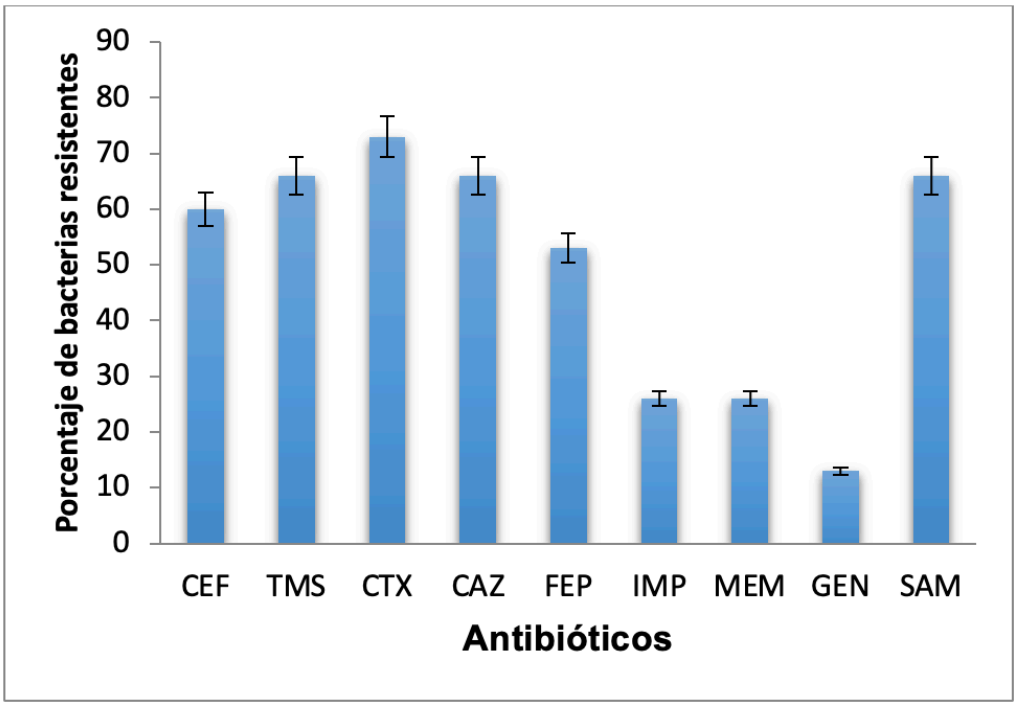

Figura 4. Perfil de resistenciade las diferentes cepas aisladas. Antibióticos de ensayo: CEF (cefalotina),TMS (trimetoprima-sulfametoxazol), CTX (cefotaxima), CAZ (ceftazidima), FEP(cefepime), IMP (imipenem),MEM (meropenem), GEN (gentamicina), SAM (ampicilina-sulbactam).

De acuerdo con los ensayos fenotípicos realizados con las cepas aisladas, el 60 % de las cepas resistentes a ceftazidima son productoras de BLEE.

Discusión

Este estudio evaluó la contribución de los efluentes rurales a la resistencia antimicrobiana. Se determinaron los bacilos Gram negativos resistentes a antibióticos beta-lactámicos y tetraciclinas, calculando la prevalencia fenotípica de las bacterias resistentes a los diferentes antibióticos ensayados.La ganadería se ha convertido en un foco de propagación de la RAM,principalmente en función de la cantidad de antimicrobianos utilizados en la producción de animales destinados al consumo humano[9].

Ceftiofur, una cefalosporina de 3ª generación (3GC), es uno de los tres antibióticos más utilizados para tratar y prevenir enfermedades del ganado. La ceftriaxona y la cefotaxima son antibióticos de 3GC similares que se utilizan para tratar infecciones graves causadas por cepas patógenas de Enterobacterias en humanos. El uso de cefalosporinas de la misma generación con similar estructura química, ingredientes activos y espectro de acción en la cría de ganado y en entornos de salud humana puede conducir a una resistencia cruzada que puede transferirse a los humanos o viceversa a través de vías directas e indirectas[20,21]. Schmidt *et al*[22] en 2013, demostraron mediante un estudio longitudinal que los tratamientos del ganado con ceftiofur condujeron a un aumento transitorio de la eliminación de *Escherichia coli* resistente a 3GC después del tratamiento con ceftiofur. Markland et al[23]en 2019 determinaron una prevalencia de bacterias resistentes a cefotaxima del 83% en ganado criado en corrales de engorde. Obtuvimos resultados similares a estos autores, en la resistencia a ceftriaxona y en el perfil de resistencia con cefotaxima; siendo que ambos antibióticos son de uso clínico.

Las BLEE pueden hidrolizar cefalosporinas de espectro extendido, incluidas cefotaxima, ceftriaxona, ceftazidima, o cefepime y antibióticos monobactámicos. Se han encontrado bacterias productoras de BLEE en recursos hídricos próximos al entorno de establecimientos de cría de ganado. En un estudio realizado por Noyes *et al*.[24], en 2016, se observó que el agua de los corrales de ganado vacuno de carne contenía genes de BLEE y carbapenemasas, lo que sugiere que el agua de los establecimientos de cría de ganado vacuno podría ser otra fuente debacterias productoras de BLEE.Las estrategias de mitigación eficaces, como la gestión de la granja, la bioseguridad y la higiene, podrían facilitar la reducción de las bacterias productoras de BLEE en el ganado[25].

El riesgo de ser colonizados por *E. coli* productora de betalactamasas aumenta en personas en contacto constante con el agua, como los arroyos analizados[26,27]. Entre la población general, los agricultores expuestos al ganado en contacto directo tienen mayores posibilidades de transmisión de bacterias resistentes a antimicrobianos[28].

Lepper *etal*.[29], en 2022, ilustraron el papel potencialmente importante del medio ambiente en la epidemiología de las infecciones bacterianas resistentes en los seres humanos.Destacaron la necesidad de considerar el papel del medio ambiente en el diseño de estrategias de control de la resistencia a antimicrobianos, ya que puede influir en la prevalencia humana de la resistencia, reduciendo la eficacia de las intervenciones que limitan el consumo de antibióticos en los animales, y puede ser un objetivo de intervención eficaz en sí mismo a través de una mejor infraestructura de saneamiento.

Conclusiones

Se pudo determinar una contaminación difusa con bacterias resistentes a antibióticos betaláctamicos tanto de uso veterinario como de uso clínico. Representando estos resultados un problema para la salud pública y medioambiental.

La cría intensiva de ganado puede contribuir a la diseminación de bacterias resistentes a antibióticos y representar un riesgo para la salud pública. Estos resultados resaltan la necesidad de centrarse en el concepto de *Una Salud*.

Bibliografía

1. Vanegas Múnera J, Jiménez Quiceno J. Resistencia antimicrobiana en el siglo xxi: ¿hacia una era postantibiótica? RevFacNac Salud Pública. 2019; 38(1):1-6.

2. Marti E, Variatza E, Balcazar JL. The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol. 2014; 22 (1): 36-41. doi: 10.1016/j.tim.2013.11.001.

3. Liu X, Lu S, Guo W, Xi B, Wang W. Antibiotics in the aquatic environments: A review of lakes, China. Science of the Total Environment. 2018; 627: 1195-1208. Doi: 10.1016/j.scitotenv.2018.01.271

4. Qiao M, Ying GG, Singer AC, Zhu YG. Review of antibiotic resistance in China and its environment. *Environ. Int*. 2018; 110: 160-172. Doi: 10.1016/j.envint.2017.10.016.

5. Danner MC, Robertson A, Behrends V, Reiss J.Antibiotic pollution in surface fresh waters: Occurrence and effects. Science of the Total Environment. 2019; 664: 793-804. Doi: 10.1016/j.scitotenv.2019.01.406.

6. Polianciuc SI, Gurzău AE, Kiss B, Georgia Ștefan M, Loghin F. Antibiotics in the environment: causes and consequences. Med Pharm Reports. 2020; 93(3):231-240.

7. Vaz-Moreira I, Nunes OC, Manaia CM. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. FEMS Microbiol Rev. 2014;38(4):761-778. Doi: 10.1111/1574-6976.12062.

8. Wall BA, Mateus AL P, Marshall L, Pfeiffer DU, Lubroth J, Ormel HJ. et al. Drivers, Dynamics and Epidemiology of Antimicrobial Resistance in Animal Production. *FAO* 2016. Roma Italia.

9. Agga GE, Arthur TM, Durso LM, Harhay DM, Schmidt JW. Antimicrobial-Resistant Bacterial Populations and Antimicrobial Resistance Genes Obtained from Environments Impacted by Livestock and Municipal Waste. PLoS ONE. 2015;10(7): e0132586. Doi: 10.1371/journal.pone.0132586.

10. Hernandes F, Henriques L, Pilz R, et al. Antibiotic resistance in aquatic environments of Rio de Janeiro, Brazil. In: Ahmad I, Ahmad Dar M, editors. Perspectives in water pollution. Croatia: InTech; 2013. Doi:10.5772/54638

11. Hanna N, Tamhankar AJ, Stålsby Lundborg C. Antibiotic concentrations and antibiotic resistance in aquatic environments of the WHO Western Pacific and South-East Asia regions: a systematic review and probabilistic environmental hazard assessment. Lancet Planet Health.2023;7: e45-54.

12. Scott HM, Acuff G, Bergeron G, Bourassa MW, Simjee S, Singer RS. Antimicrobial resistance in a One Health context: exploring complexities, seeking solutions, and communicating risks. Ann N Y Acad Sci. 2019; 1441(1):3-7. Doi: 10.1111/nyas.14057.

13. Bhushan C, Khurana A, Sinha R, Nagaraju M. Antibiotic Resistance in Poultry Environment: Spread of Resistance from Poultry Farm to Agricultural Field. Centre for Science and Environment: 2017 New Delhi, India

14. Taylor NGH, Verner-Jeffreys DW, Baker-Austin C. Aquatic systems: Maintaining, mixing and mobilizing antimicrobial resistance? Trends Ecol. Evol. 2011; 26: 278-284. Doi: 10.1016/j.tree.2011.03.004.

15. Cao ST, Tran HP, Le HTT, Bui HPK, Nguyen GTH, Nguyen LT. et al. Impacts of effluent from different livestock farm types (pig, cow, and poultry) on surrounding water quality: a comprehensive assessment using individual parameter evaluation method and water quality indices. Environ Sci Pollut Res Int.2021;(36):50302-50315. Doi: 10.1007/s11356-021-14284-9.

16. Rodríguez E, Jiménez JN. Resistencia bacteriana a antibióticos en ambientes acuáticos: origen e implicaciones para la salud pública. *Rev. Fac. Nac. Salud Pública*. 2023; 41(3):e351453. Doi: 10.17533/udea.rfnsp.e351453.

17. Chagas CI, Santanatoglia OJ, Moretton J, Paz M, Kraemer FB. Surface movement of cattle-borne biological contaminants in the drainage network of a basin of the Rolling Pampas. Ciencia del Suelo. 2010; 28(1):23-31.

18. Chagas CI, Kraemer FB, Santanatoglia OJ, Paz M, Moretton J. Biological water contamination in some cattle production fields of Argentina subjected to runoff and erosion. Span. J. Agric. Res. 2014; 12(4):1008-1017.

19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21, Clinical and Laboratory Standards Institute, Wayne, PA 2019

20. Gelalcha BD, KerroDego O. Extended-Spectrum Beta-Lactamases Producing Enterobacteriaceae in the USA Dairy Cattle Farms and Implications for Public Health. Antibiotics, 2022;11(10):1313. Doi:10.3390/antibiotics11101313

21. Kanwar N, Scott HM, Norby B, Loneragan, GH, Vinasco J,Mcgowan M. et al Effects of ceftiofur and chlortetracycline treatment strategies on antimicrobial susceptibility and on tet(A), tet(B), and bla CMY-2 resistance genes among *E. coli* isolated from the feces of feedlot cattle. PLoS One. 2013; 8: e80575 Doi: 10.1371/journal.pone.0080575.

22. Schmidt JW, Griffin D, Kuehn LA, Brichta-Harhay DM. Influence of therapeutic ceftiofur treatments of feedlot cattle on fecal and hide prevalences of commensal Escherichia coli resistant to expanded-spectrum cephalosporins, and molecular characterization of resistant isolates. Appl Environ Microbiol.2013; 79: 2273-2283. Doi: 10.1128/AEM.03592-12.

23. Markland S, Weppelmann TA, Ma Z, Lee S, Mir R, Teng L. et al. High prevalence of cefotaxime resistant bacteria in grazing beef cattle: a cross sectional study. Front Microbiol. 2019; 10:176. Doi: 10.3389/fmicb.2019.00176.

24. Noyes NR, Yang X, Linke LM, Magnuson RJ, Dettenwanger A, Cook S, et al. Resistome diversity in cattle and the environment decreases during beef production. Elife. 2016; 5:e13195. Doi: 10.7554/elife.13195.

25. Lee S, Mir RA, Park SH, Kim D, Kim HY, Boughton RK. et al. Prevalence of extended-spectrum β-lactamases in the local farm environment and livestock: challenges to mitigate antimicrobial resistance. Crit Rev Microbiol. 2020;46(1):1-14. Doi: 10.1080/1040841X.2020.1715339.

26. Leonard AFC, Zhang L, Balfour AJ, Garside R, Gaze WH . Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. Environ Int. 2015; 82:92-100. Doi: 10.1016/j.envint.2017.11.003.

27. Leonard AFC, Zhang L, Balfour AJ, Garside R, Hawkey PM, Murray AK. et al. Exposure to and colonisation by antibiotic-resistant *E. coli* in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey*).* Environ. Int. 2017; 114:326-333. Doi: 10.1016/j.envint.2017.11.003.

28. Klous G, Huss A, Heederik DJ, Coutinho RA. Human–livestock contacts and their relationship to transmission of zoonotic pathogens, a systematic review of literature. One Health. 2016; 2:65-76. Doi: 10.1016/j.onehlt.2016.03.001.

29. Lepper HC, Woolhouse MEJ, Van Bunnik BAD. The Role of theEnvironment in Dynamics of Antibiotic Resistance in Humans and Animals: A Modelling Study. Antibiotics. 2022; 11: 1361. Doi:10.3390/antibiotics11101361

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Artículos de revisión

Foeniculum vulgare Miller en el tratamiento de la sintomatología asociada a la menopausia

Foeniculum vulgare Miller for the treatment of symptoms associated with the menopause

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Resumen

Introducción: Diversos estudios han puesto de manifiesto que los preparados del fruto de hinojo (*Foeniculum vulgare* Miller) pueden ser útiles para aliviar los síntomas asociados a la menopausia. El objetivo de este trabajo es realizar una revisión de los artículos publicados sobre la eficacia y la seguridad del hinojo en el tratamiento de los síntomas menopáusicos.

Método: Se realizó una búsqueda de los artículos publicados desde enero de 2011 a julio de 2024 en el buscador de recursos de la Universidad de La Laguna (ULL), "PuntoQ". Se seleccionaron los ensayos clínicos aleatorizados y controlados (con placebo u otra medicación), que tuvieran las palabras claves en el resumen, texto o título, tanto en inglés como en español.

Resultados: Se identificaron 11 artículos que incluían los criterios de inclusión. Se encontró que los preparados de hinojo, especialmente su aceite esencial, pueden resultar útiles para aliviar la frecuencia e intensidad de los síntomas de la menopausia, principalmente síntomas vegetativos (sofocos y sudores nocturnos) y síntomas psicológicos. Además, se observó mejoría en el nivel hormonal (aumento de estradiol), en la calidad de vida, en la función sexual, así como en los síntomas de atrofia vaginal. No se observaron efectos adversos significativos a las dosis administradas.

Conclusiones: Los preparados de hinojo (especialmente su aceite esencial) mostraron ser eficaces en el tratamiento de la sintomatología menopáusica, siendo bien tolerados. No obstante, debido a las limitaciones de los estudios revisados, se requieren más ensayos clínicos de calidad para confirmar estos resultados.

Palabras clave: Hinojo; *Foeniculum vulgare*; menopausia; síntomas menopáusicos; revisión.

Abstract

Introduction: Several studies have shown that preparations of fennel (*Foeniculum vulgare* Miller) may be useful in relieving symptoms associated with the menopause. The aim of this paper is to review published articles on the efficacy and safety of fennel in the treatment of menopausal symptoms.

Method: Articles published from January 2011 to July 2024 were searched in the University of La Laguna (ULL) search engine, "PuntoQ". Randomised and controlled clinical trials (with placebo or other drugs) with the keywords in the abstract, text or title, both in English and Spanish, were selected.

Results: Eleven articles that met the inclusion criteria were identified. We found that fennel preparations, especially its essential oil, may be useful in reducing the frequency and intensity of menopausal symptoms, mainly vegetative symptoms (hot flushes and night sweats) and psychological symptoms. In addition, improvements in hormone levels (increased levels of estradiol), quality of life, sexual function and symptoms of vaginal atrophy have been observed. No significant adverse effects were observed at the doses administered.

Conclusions: Fennel preparations (especially its essential oil) have been shown to be effective and well tolerated in the treatment of menopausal symptoms. However, due to the limitations of the trials reviewed, more high-quality clinical research is needed to confirm these findings.

Keywords: Fennel; *Foeniculum vulgare*; menopause; menopausal symptoms; review.

Puntos clave

En los últimos años, diversos ensayos clínicos han demostrado la eficacia de los preparados del fruto de *Foeniculum vulgare* Miller, especialmente su aceite esencial, en el tratamiento de los síntomas menopáusicos. No obstante, los estudios siguen siendo limitados y no concluyentes.

Se propone realizar en este trabajo una revisión de los artículos publicados hasta el momento sobre la eficacia y seguridad del hinojo en el tratamiento de los síntomas de la menopausia.

Se pretende obtener un mayor conocimiento actualizado de la eficacia y seguridad del hinojo en el tratamiento de la menopausia y esclarecer si pudiese constituir una alternativa para aquellas mujeres que no deseen o no puedan ser tratadas con terapia hormonal.

Introducción

La menopausia se define como el cese natural del ciclo menstrual durante más de un año debido a la deficiencia de estrógenos, lo que suele ocurrir en las mujeres alrededor de los 45 a 55 años. Este déficit estrogénico puede ocasionar una serie de síntomas como alteraciones vasomotoras (sofocos, sudoración), cambios en el estado de ánimo o alteraciones genitourinarias, que pueden causar molestias y comprometer la calidad de vida[1,2,3,4].

Uno de los tratamientos más habituales para los síntomas vasomotores de la menopausia es la terapia hormonal sustitutiva. Sin embargo, debido al posible riesgo a largo plazo de este tratamiento (incremento del riesgo de cáncer de endometrio, infarto de miocardio), su uso no está recomendado para todas las mujeres, siendo necesario una valoración individual pormenorizada que considere los riesgos y beneficios, reduciendo la dosis a la mínima eficaz durante el menor tiempo posible[2-4,5]. De hecho, en los últimos años se ha observado un aumento del uso de preparados a base de plantas medicinales (especialmente las que contienen fitoestrógenos) en aquellas mujeres que no desean el tratamiento hormonal o que se encuentra contraindicado[1,6].

Entre estas plantas medicinales con fitoestrógenos, tenemos el hinojo (*Foeniculum vulgare* Mill., Apiaceae), planta herbácea cuyo fruto se ha utilizado como especia y también tradicionalmente para el tratamiento sintomático de trastornos digestivos espasmódicos leves y de espasmos menores asociados a la menstruación, así como para el tratamiento de catarros de vías respiratorias altas[7,8,9,10,11].

El principal constituyente de esta droga y al que se le atribuye sus acciones farmacológicas es el aceite esencial, que está constituido mayoritariamente por anetol, estragol y fenchona (su proporción varía según se trate del fruto de hinojo amargo o dulce). También contiene flavonoides, ácidos fenólicos, hidroxicumarinas y furocumarinas, entre otros[9,11].

Diversos estudios han puesto de manifiesto que el aceite esencial de hinojo presenta actividad estrogénica y puede ser útil para aliviar los síntomas asociados a la menopausia, demostrando en varios ensayos sus efectos terapéuticos en los sofocos, el estrés, los trastornos del sueño y la sequedad vaginal[1,7,8,11,12]. Se considera que esta actividad estrogénica es debida principalmente al anetol y sus polímeros, dianetol y fotoanetol[10]. No obstante, los datos siguen siendo limitados y no concluyentes.

El objetivo de este trabajo es realizar una revisión de los artículos publicados sobre la eficacia y la seguridad del hinojo en el tratamiento de los síntomas menopáusicos.

Métodos

Se ha realizado una búsqueda de los artículos publicados desde enero de 2011 a julio de 2024 en el buscador de recursos de la Universidad de La Laguna (ULL), “PuntoQ”, sobre la eficacia de los preparados de hinojo en los síntomas de la menopausia. PuntoQ es el portal de búsqueda de recursos electrónicos de información con fines académicos de la ULL, el cual permite hacer búsquedas desde un solo punto de acceso a 185 bases de datos, entre las que se puede destacar para este trabajo: EBSCO,

Biblioteca Cochrane Plus, BioMedCentral, CINAHL, PubMed, ScienceDirect Journals, Scopus, WOS o Wiley Online Library, entre otras.

Se introdujeron las siguientes palabras claves: (foeniculum OR fennel) en el título y (menopaus* OR climacteric OR perimenopaus* OR postmenopaus* OR vaginal OR vasomotor OR sweat* OR "hot flush*" OR "hot flash*" OR urogenital OR irritability OR genitourinary) y (trial OR study OR review) en todos los campos.

Se seleccionaron los ensayos clínicos que cumplieran los siguientes criterios de inclusión: estudios en mujeres peri-menopáusicas, menopáusicas o postmenopáusicas con edad superior a 45 años y que usaran preparados de hinojo para tratar la sintomatología menopaúsica. Se incluyeron ensayos clínicos aleatorizados y controlados (con placebo u otra medicación), que tuvieran las palabras claves en el resumen, texto o título, tanto en inglés como en español. Se excluyeron los estudios en animales, ensayos clínicos no controlados o no aleatorizados, estudios de casos, resúmenes de congreso o aquellos que estuvieran en otro idioma. La adecuación de los estudios incluidos fue evaluada de forma independiente por dos investigadores, resolviendo cualquier discrepancia por consenso.

Los estudios se agruparon en una tabla según las variables a estudiar, con el fin de sistematizar y facilitar la compresión de los resultados, considerando los siguientes datos: primer autor y año de publicación, tipo de estudio, población, duración del estudio y dosis, síntomas evaluados y resultados obtenidos.

La calidad de los estudios incluidos fue evaluada de forma independiente por dos investigadores mediante la escala de Jadad, resolviéndose las discrepancias por consenso. Las puntuaciones de esta escala oscilan entre 0 y 5 puntos, evaluándose tres criterios: aleatorización (dos puntos), enmascaramiento o cegamiento (2 puntos) y el control de las pérdidas en el seguimiento (1 punto). Se considera que un ensayo clínico es de pobre calidad metodológica si su puntación es inferior a 3 puntos.

La evaluación del riesgo de sesgo de cada estudio incluido se realizó de forma independiente por dos investigadores siguiendo la metodología recomendada por la Colaboración Cochrane (Manual Cochrane de revisiones sistemáticas de intervenciones, versión 5.1.0 marzo de 2011), resolviéndose las discrepancias por consenso. Se evaluaron los siete dominios propuestos: generación de la secuencia aleatoria, ocultación de la asignación, cegamiento de los participantes y el personal, cegamiento de los evaluadores de resultado, datos de resultado incompletos, notificación selectiva, otros sesgos. Cada dominio puede tener tres clasificaciones (alto riesgo, bajo riesgo, riesgo dudoso), considerándose que un estudio tiene un "sesgo bajo" cuando tiene una calificación de "bajo riesgo" en todos los dominios y un "sesgo alto" cuando tiene por lo menos un dominio con una calificación de "alto riesgo".

Resultados

Al realizar la búsqueda con las palabras clave seleccionadas, se encontraron inicialmente 51 artículos. Aplicando los criterios de inclusión y exclusión, se excluyeron 27 artículos tras la lectura del título y resumen por los motivos reflejados en el diagrama de flujo (Figura 1). De los 24 artículos relevantes identificados, se excluyeron 13 tras lectura de texto completo (revisiones de artículos, escritos en idioma distinto al inglés o español, protocolo, estudio piloto, resumen de congreso, artículo no disponible). Finalmente, quedaron 11 artículos que cumplían los criterios de inclusión, recogiéndose su información en la Tabla 1[13-23].

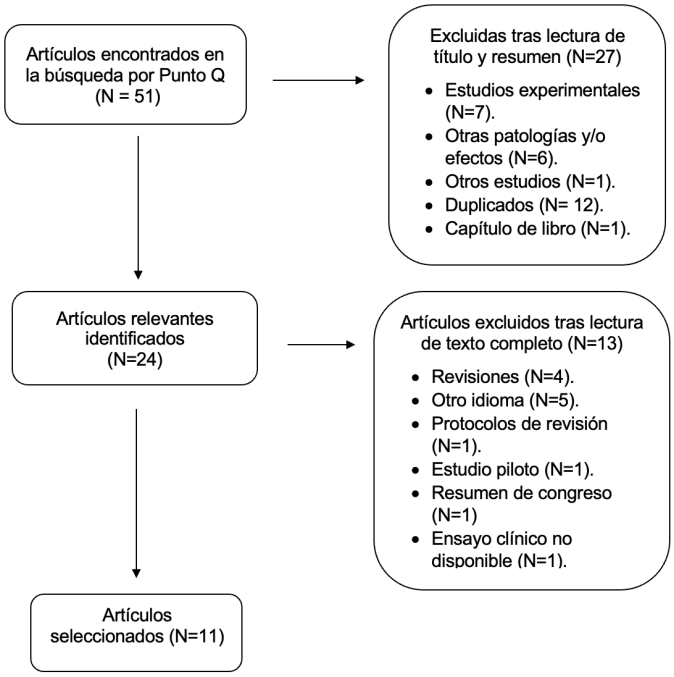

Figura 1. Diagrama de flujo de la búsqueda (elaboración propia, siguiendo las recomendaciones de la declaración PRISMA).

Tabla 1. Ensayos clínicos con el fruto de hinojo en el tratamiento de los síntomas de la menopausia.

Autor, año, referencia, diseño	Pacientes	Duración Dosis	Síntomas Evaluados	Resultados
Ghavi et al., 2023[13]. Ensayo clínico aleatorizado, triple ciego, controlado con placebo.	Mujeres (N=125), edad 45-60 años, postmenopáusicas (≥12 meses).	Duración: 8 s. G1 (N = 43): 2 cáps/ día aceite esencial de fruto hinojo (60 mg/ día). G2 (N = 42): 2 cáps/ día aceite de onagra (2000 mg/ día). G3 (N = 40): 2 cáps/ día almidón soja (200 mg/día).	Medida niveles plasmáticos de hormonas (FSH y estradiol) al inicio y final tratamiento. Evaluación de síntomas menopáusicos mediante cuestionario MRS al inicio y final tratamiento.	↓FSH y ↑estradiol en G1 y G2 frente placebo ($p< 0.001$); > ↑ estradiol en G1frente G2 ($p<0.05$). Mejoría síntomas menopáusicos en G1 y G2, pero sólo significativo en síntomas psicológicos ($p<0.05$) frente placebo.

Autor, año, referencia, diseño	Pacientes	Duración Dosis	Síntomas Evaluados	Resultados
Jenabi et al., 2023[14]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo.	Mujeres (N = 70), edad 45-55 años, postmenopáu-sicas (≥12 meses) con bochornos y trastornos del sueño.	Duración: 8 s. G1 (N = 35): 2 cáps/día extracto alcohólico (1:1) hinojo (86.86% anetol) y valeriana (1000 mg/día). G2 (N = 35): 2 cáps/día placebo (1000 mg/día almidón).	Evaluación de severidad, duración y frecuencia de sofocos mediante KI y de la calidad del sueño mediante PSQI al inicio, 4 y 8 semanas tratamiento.	↓frecuencia y severidad de sofocos en G1 frente G2 ($p<0.05$) a 4ª y 8ª s. de tratamiento Mejoría G1 en calidad del sueño frente placebo ($p<0.05$) tras 2 m. de tratamiento. No E.A.
Mahdavian et al., 2019[15]. Ensayo clínico aleatorizado, triple ciego, controlado con placebo.	Mujeres (N = 109), edad entre 45-65 años, peri-y postmenopáusicas (≥12 meses).	Duración: 12 s. G1 (N= 26): 2 ml/día extracto alcohólico de: 250 mg/día flores manzanilla + 30 mg/día fruto hinojo + 15 mg/día estigma azafrán. G2 (N= 29): 2 ml/día extracto (500 mg manzanilla + 60 mg hinojo + 30 mg azafrán). G3 (N= 29): 2 ml/día extracto alcohólico (1000 mg manzanilla + 120 mg hinojo + 60 mg azafrán). G4 (N =25): placebo (2 ml/día agua esterilizada).	Evaluación de síntomas menopáusicos (neurovegetati-vos, psicológicos y urogenitales) mediante el cuestionario MRS al inicio, 6 y 12 s. de tratamiento.	Mejoría significativa ($p<0.001$) síntomas menopáusicos neurovegetativos, psicológicos y urogenitales G2 y G3 frente placebo a las 6 y 12 s. de tratamiento (> con G3). No E.A.
Abedi et al., 2018[16]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo.	Mujeres (N = 60), edad 45-65 años, postmenopáusicas (≥ 12 meses o FSH>40 UI) con disfunción sexual (puntuación <26 en cuestionario FSFI).	Duración: 8 s. G1 (N = 30): 5 g/día crema vaginal de fruto hinojo (extracto seco etanólico mezclado con crema base en emulsión al 5%). G2 (N= 30): placebo.	Evaluación de función sexual (excitación, lubricación, orgasmo, dolor, satisfacción sexual) mediante cuestionario FSFI al inicio y 8 s. tratamiento	Mejoría de función sexual en ambos grupos, pero significativamente superior en todas las áreas estudiadas con hinojo ($p < 0.001$) frente placebo. No E.A.
Afiat et at., 2018a[17]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo.	Mujeres (N = 60), edad media 56 años, postmenopáusicas (≥12 meses).	Duración: 3 m. G1 (N=25): 3 cáps/día hinojo (cada cáps de 100 mg contiene 30% aceite esencial de hinojo (21-27 mg anetol) y aceite girasol). G2 (N=29): placebo (aceite de girasol).	Evaluación del perfil lipídico (triglicéridos, colesterol total, LDL, HDL) al inicio y a los 3 m. tratamiento.	No se observaron diferencias significativas en el perfil lipídico entre ambos grupos, excepto una mejoría leve para HDL ($p = 0.052$) en el hinojo frente valor inicial. No E.A.

Autor, año, referencia, diseño	Pacientes	Duración Dosis	Síntomas Evaluados	Resultados
Afiat et at., 2018b[18]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo.	Mujeres (N = 50), edad 45-65 años, postmenopáusicas (≥12 meses).	Duración: 12 s. G1 (N=25): 3 cáps/ día hinojo (cada cáps de 100 mg contiene 30% de aceite esencial hinojo (21-27 mg de anetol) y aceite de girasol). G2 (N=25): placebo (aceite de girasol).	Evaluación de la calidad del sueño mediante índice de PSQI al inicio y al final del tratamiento.	No se observó mejoría significativa en la puntuación total de la calidad del sueño comparado con placebo, excepto una ligera tendencia a la mejoría en la duración del sueño (P = 0.059). No E.A.
Ghazanfarpour et al., 2018[19]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo.	Mujeres (N = 50), edad 45-65 años, postmenopáusicas (≥12 meses).	Duración: 3 m. G1 (N=25): 3 cáps/ día hinojo (cada cáps de 100 mg contiene 30% de esencia hinojo (21-27 mg anetol) y aceite girasol). G2 (N= 25): placebo.	Evaluación de calidad de vida mediante el cuestionario MENQOL al inicio y a los 3 meses de tratamiento.	Mejora síntomas menopáusicos (sofocos, sudores, psicológicos) en ambos G (p<0.001) frente valor inicial; sin diferencias significativas entre grupos. Efecto placebo ↑↑. No E.A.
Ghazanfarpour et al., 2017[20]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo.	Mujeres (N = 60), edad media 56 años, postmenopáusicas (≥12 meses).	Duración: 12 s. G1 (N=25): 3 cáps/ día hinojo (100 mg por cáps.: 30% esencia hinojo (21-27 mg anetol) y aceite girasol). G2 (N=25): placebo.	Medida de densidad mineral ósea y contenido mineral óseo mediante absorciometría con rayos X de doble energía al inicio y al final.	No se observó mejoría en la densidad y contenido mineral óseo (diferencias no significativas entre ambos grupos). No E.A. serios.
Rahimi-kian et al., 2017[21]. Ensayo clínico aleatorizado, triple ciego, controlado con placebo.	Mujeres (N = 80), edad 45-60 años, postmenopáusicas (≥ 12 meses y < 5 años).	Duración: 8 s. G1 (N = 40): 2 cáps./ día esencia de hinojo (100 mg por cáps. con 71-90 mg anetol). G2 (N= 40): placebo.	Evaluación de calidad de vida mediante el cuestionario MENQOL al inicio y final de tratamiento.	Mejora de la calidad de vida y disminución de síntomas de menopausia de G1 frente placebo (p<0.001). No E.A. serios.
Saghafi et al., 2017[22]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo.	Mujeres (N = 47), edad media 57 años, postmenopáusicas con sobrepeso y obesidad.	Duración: 3 m. G1 (N= 22): 3 cáps/ día esencia de hinojo (100 mg/ cáps., 71-90 mg anetol). G2 (N= 25): placebo.	Medida del peso, índice de masa corporal y distribución de la grasa al inicio y al final del tratamiento.	No se observaron efectos significativos en ninguno de los parámetros medidos entre los grupos.
Yaralizadeh et al., 2016[23]. Ensayo clínico aleatorizado, doble ciego, controlado con placebo	Mujeres (N = 60), edad 45-65 años, postmenopáusicas (≥ 12 meses) con disfunción sexual.	Duración: 8 s. G1 (N = 30): 5 g/día crema vaginal de fruto hinojo (extracto seco etanólico mezclado con crema base en emulsión al 5%). G2 (N= 30): placebo.	Medida del pH y del índice de maduración vaginal al inicio y 8 s. Medida de síntomas atrofia vaginal al inicio, 2, 4 y 8 s.	↑nº células superficiales de la vagina, ↓pH vaginal y síntomas de atrofia vaginal (palidez, picazón, sequedad, dispareunia) frente placebo (p<0.001) a las 8 s. No E.A.

↑: aumento; ↓: disminución; cáps: cápsula; E.A: efectos adversos; G: grupo; m: mes; s: semana

FSFI: Female Sexual Function Index; KI: Kupperman Menopausal Index; MENQOL: Menopause-Specific Quality of Life; MRS: Menopause Rating Scale; PSQI: Pittsburg Sleep Quality Index.

Los 11 estudios incluidos en esta revisión fueron ensayos clínicos aleatorizados doble ciego controlados con placebo, excepto tres que fueron triple ciego[13,15,21].

El número de pacientes incluidos en los ensayos clínicos oscilaba entre 47 y 80, menos en dos estudios que fue de 109[15] y 125[13]. Se seleccionaron pacientes postmenopáusicas con edades comprendidas entre 45 y 65 años con presencia de síntomas menopáusicos naturales de al menos durante un año.

La duración de los estudios osciló entre 8 y 12 semanas. Se administraron cápsulas de aceite esencial de hinojo en siete ensayos a diferentes dosis: 60 mg/día[13], 200 mg/día[21] y 300 mg/día[17,18,19,20,22]. Además, en un ensayo[14] se administró una combinación de extractos alcohólicos de hinojo y valeriana (1000 mg/día) en cápsulas, mientras que en otro[15] se administró vía oral una combinación de tres extractos alcohólicos: flores de manzanilla (250-1000 mg/día), fruto de hinojo (30-120 mg/día), estigma de azafrán (15-60 mg/día). También se aplicó una crema vaginal de extracto seco etanólico de fruto de hinojo (5 g/día) en dos ensayos[16,23].

Se evaluaron los síntomas menopáusicos mediante diferentes cuestionarios y escalas: índice de Kupperman[14], cuestionario MRS[13,15]. También se evaluó la calidad de vida mediante cuestionario MENQOL[19,21], la calidad del sueño mediante el índice PSQI[14,18], la función sexual mediante cuestionario FSFI[16] y los síntomas de atrofia vaginal[23]. Además, se midió los niveles plasmáticos de las hormonas FSH y estradiol[13], la densidad y el contenido mineral óseo[20], el perfil lipídico[17], así como el peso y el índice de masa corporal[22].

Tras el análisis de los resultados, se observó que la administración en cápsulas del aceite esencial de hinojo produjo una reducción significativa de los síntomas vegetativos (sofocos, sudores) y los síntomas psicológicos comparado con el grupo placebo[13,21], excepto en un ensayo que se observó un importante efecto placebo[19]. Además, se observó una mejoría de la calidad de vida[21], así como una disminución de la hormona folículo estimulante (FSH) y un aumento del estradiol[13]. No se encontró mejoría significativa en la calidad del sueño[18], el perfil lipídico[17], la densidad y contenido mineral óseo[20] ni en el peso e índice de masa corporal[22].

Cuando se administró conjuntamente los extractos alcohólicos de frutos de hinojo con los de raíz de valeriana[14] o con los extractos de manzanilla y azafrán[15] se encontró también una mejoría significativa de los síntomas menopáusicos comparado con el placebo, observándose además una mejoría en la calidad del sueño[14].

Por otro lado, su administración por vía tópica produjo una mejoría de la función sexual y los síntomas de atrofia vaginal[16,23].

No se observaron efectos adversos serios durante el tratamiento con los diferentes preparados de hinojo a las dosis usadas en estos estudios.

Con respecto a la evaluación de la calidad, todos los estudios obtuvieron la puntuación más alta posible (5 puntos) en la escala de Jadad, excepto un ensayo que obtuvo una calificación de 4[20] porque no mencionó las pérdidas por seguimiento, aunque sí las menciona en un artículo publicado posteriormente[17].

Por otro lado, cuatro de los ensayos clínicos[13,14,15,21] tuvieron un nivel de sesgo bajo en todos los dominios evaluados según la metodología recomendada por la Colaboración Cochrane, pero 7 de ellos[16-20,22,23] tuvieron un nivel de riesgo dudoso (poco claro) en el sesgo de notificación (notificación selectiva), ya que se publicaron diferentes resultados de un mismo ensayo clínico en varias revistas (Tabla 2).

Tabla 2. Evaluación del riesgo de sesgo de los ensayos clínicos aleatorizados siguiendo la metodología Cochrane Risk of bias

Autor, año, referencia	1	2	3	4	5	6	7
Ghavi et al., 2023[13]	BR	BR	BR	BR	BR	BR	BR
Jenabi et al., 2023[14]	BR	BR	BR	BR	BR	BR	BR
Mahdavian et al., 2019[15]	BR	BR	BR	BR	BR	BR	BR
Abedi et al., 2018[16]	BR	BR	BR	BR	BR	RD	BR
Afiat et al., 2018a[17]	BR	BR	BR	BR	BR	RD	BR
Afiat et al., 2018b[18]	BR	BR	BR	BR	BR	RD	BR
Ghazanfarpour et al., 2018[19]	BR	BR	BR	BR	BR	RD	BR
Ghazanfarpour et al., 2017[20]	BR	BR	BR	BR	RD	RD	BR
Rahimi-kian et al., 2017[21]	BR	BR	BR	BR	BR	BR	BR
Saghafi et al., 2017[22]	BR	BR	BR	BR	BR	RD	BR
Yaralizadeh et al., 2016[23]	BR	BR	BR	BR	BR	RD	BR

AR: Alto riesgo, BR: Bajo riesgo; RD: Riesgo dudoso (poco claro)

1. Generación de la secuencia aleatoria; sesgo de selección
2. Ocultación de la asignación; sesgo de selección
3. Cegamiento (enmascaramiento) de los participantes y del personal; sesgo de realización
4. Cegamiento (enmascaramiento) de los evaluadores de resultado; sesgo de detección
5. Datos de resultado incompletos; sesgo de desgaste
6. Notificación selectiva; sesgo de notificación
7. Otros sesgos

Discusión

Tras el análisis de los resultados obtenidos, podemos afirmar que los preparados de hinojo, especialmente su aceite esencial, pueden resultar útiles para aliviar la frecuencia e intensidad de los síntomas de la menopausia, principalmente síntomas vegetativos (sofocos y sudores nocturnos) y síntomas psicológicos[13,21]. Además, se observó una mejoría en el nivel hormonal (aumento de estradiol)[13] y en la calidad de vida[21], así como una mejoría de la función sexual[16] y los síntomas de atrofia vaginal[23] cuando se administró tópicamente en forma de crema. Asimismo, estos preparados fueron bien tolerados, no observándose efectos adversos significativos a las dosis administradas.

Cabe mencionar que la combinación de un extracto alcohólico de hinojo con un extracto alcohólico de valeriana[14] o con los extractos de manzanilla y azafrán[15] también produjo una mejoría de los síntomas menopáusicos.

Aunque todavía no está claro el mecanismo por el cual el fruto del hinojo tiene efectos beneficiosos en los síntomas menopáusicos, se ha relacionado con la actividad estrogénica observada para el anetol y sus polímeros (dianetol, fotonaetol), constituyentes del aceite esencial[10]. Otras drogas vegetales, como las semillas de soja (*Glycine max* (L.) Merr.) o la sumidad florida del trébol rojo (*Trifolium pratense* L.) presentan actividad estrogénica por su contenido en isoflavonas, siendo útiles principalmente en el tratamiento de los síntomas vegetativos de la menopausia[24].

No obstante, la comparación e interpretación de los resultados obtenidos en los estudios resultó difícil debido a varios factores. Así, si bien el diseño de los ensayos tenía un grado aceptable de calidad (aleatorizado, doble o triple ciego, controlado con placebo), se observaron diferencias en el número de pacientes incluidos, siendo muy pequeño en algunos casos (47), y los síntomas evaluados, así como

en las dosis y los preparados de hinojo usados (aceite esencial, extracto alcohólico), lo cual dificultó la valoración correcta de los resultados obtenidos.

Sin embargo, cabe mencionar que en nuestra revisión predominó la administración del aceite esencial de hinojo en forma de cápsula a una dosis de 60-300 mg/día[13,17-22] y estandarizado en su contenido en anetol[14,17-22], constituyente al cual se le atribuye la mayoría de las acciones farmacológicas del aceite esencial[9,11].

Por otro lado, debe comentarse el bajo número de ensayos clínicos encontrados, la corta duración de los mismos (8-12 semanas) y el importante efecto placebo observado en alguno de los ensayos[13,21], lo que supuso una limitación a la hora de analizar los resultados.

Además, encontramos tras la lectura de los 11 artículos incluidos en nuestra revisión que, en realidad, correspondían a 7 ensayos clínicos[13-18,21], ya que se habían publicado por separado diferentes resultados del mismo ensayo clínico, presentando un nivel de riesgo dudoso en el sesgo de notificación (notificación selectiva). Así, los artículos de Abedi et al.[16] y Yaralizadeh et al.[23] son publicaciones separadas de diferentes síntomas del mismo ensayo clínico, ocurriendo lo mismo con las publicaciones de Afiat et al.[18] y Ghazanfarpour et al.[19], así como los trabajos publicados por Afiat et al.[17], Ghazanfarpour et al.[20] y Saghafi et al.[22]. Todo esto dificultó aún más el análisis de los resultados.

Teniendo en cuenta las limitaciones anteriormente expuestas, sería necesario realizar en el futuro más ensayos clínicos rigurosos, evitando la notificación selectiva y la duplicidad de publicaciones, con mayor número de pacientes y duración más prolongada, así como con un diseño homogéneo en términos de preparaciones, dosis, duración y métodos de evaluación, para facilitar las comparaciones y confirmar el potencial efecto beneficioso del hinojo en el tratamiento de los síntomas menopáusicos.

Asimismo, debemos resaltar que todos los ensayos clínicos encontrados se realizaron en Irán, lo que introduce un sesgo geográfico y cultural significativo que limita la aplicabilidad global de los resultados. Por ello, deberían realizarse ensayos clínicos en otras regiones y poblaciones con características genéticas, culturales y alimentarias diferentes para poder generalizar los resultados obtenidos en nuestra revisión.

Conclusión

Los datos obtenidos en los estudios revisados permiten concluir que los preparados de *Foeniculum vulgare* Mill., y especialmente su aceite esencial, mostraron ser eficaces en el tratamiento de la sintomatología menopáusica (síntomas vegetativos, síntomas psicológicos, función sexual, atrofia vaginal), siendo bien tolerados.

Dado que estos preparados han sido eficaces y seguros a las dosis administradas, podrían constituir una alternativa para aquellas mujeres que no deseen o no puedan ser tratadas con terapia hormonal.

Sin embargo, debido a las limitaciones encontradas en los estudios revisados, los resultados obtenidos hasta el momento no son concluyentes. Se precisa realizar más ensayos clínicos de calidad, en otras zonas geográficas, con mayor número de pacientes y más extensos, así como el uso de las mismas preparaciones estandarizadas en cuanto a su composición, para confirmar la eficacia y seguridad de estos preparados en el tratamiento de los síntomas menopáusicos.

Bibliografía

1. De Franciscis P, Colacurci N, Riemma G, Conte A, Pittana E, Guida M, Schiattarella A. A nutraceutical approach to menopausal complaints. Medicina (Kaunas). 2019; 55(9): 544. doi: 10.3390/medicina55090544.

2. Magraith K, Jang C. Management of menopause. Aust Prescr 2023; 46: 48–53. doi: 10.18773/austprescr.2023.014.

3. O'Neill S, Eden J. The pathophysiology and therapy of menopausal symptoms. Obstet Gyanecol Reprod Med. 2020; 30(6): 175-183. doi.org/10.1016/j.ogrm.2020.03.005.

4. Vigneswaran K, Hamada H. The pathophysiology and management of menopausal sympotms. Obstet Gynaecol Reprod Med 2023; 33(5): 135-140. doi: 10.1016/j.ogrm.2023.02.003.

5. Madsen TE, Sobel T, Negash S, Shrout Allen T, Stefanick ML, Manson JE, Allison M. A review of hormone and non-hormonal therapy options for the treatment of menopause. Int J Womens Health. 2023; 15: 825-836. doi: 10.2147/IJWH.S379808.

6. Al Wattar BH, Talaulikar V. Non-oestrogen-based and complementary therapies for menopause. Best Pract Res Clin Endocrinol Metab. 2024; 38(1): 101819. doi: 10.1016/j.beem.2023.101819.

7. Khadivzadeh T, Najafi MN, Kargarfard L, Ghazanfarpour M, Dizavandi FR, Khorsand I. Effect of fennel on the health status of menopausal women: A systematic and meta-analysis. J Menopausal Med. 2018; 24(1): 67-74. doi: 10.6118/jmm.2018.24.1.67.

8. Lee HW, Ang L, Kim E, Lee MS. Fennel (*Foeniculum vulgare* Miller) for the management of menopausal women's health: A systematic review and meta-analysis. Complement Ther Clin Pract. 2021; 43: 101360. doi: 10.1016/j.ctcp.2021.101360.

9. Noreen S, Tufail T, Ul Ain HB, Awuchi CH. Pharmacological, nutraceutical, functional and therapeutic properties of fennel (*Foeniculum vulgare*). Int J Food Prop. 2023; 26(1): 915-927. doi: 10.1080/10942912.2023.2192436.

10. Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi, MA. *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arab J Chem. 2016; 9(2): S1574-1583. doi: 10.10167j.arabjc.2012.04.011.

11. Vanaclocha B, Cañigueral S. Fitoterapia. Vademécum de prescripción. 5ª ed. Barcelona: Elsevier España, S.U.; 2019. p. 366-367. ISBN: 978-84-9113-299-8.

12. Mahboubi. *Foeniculum vulgare* as a valuable plant in a management of women's health. J Menopausal Med. 2019; 25(1):1-14. doi: 10.6118/jmm.2019.25.1.1.

13. Ghavi F, Shakeri F, Farahnaz H, Abdolahian S. Comparison of the effect of fennel and evening primrose oil on menopausal problems and hormonal levels: A randomized controlled trial. Iran J Nurs Midwifery Res. 2023; 28: 430-435. doi: 10.4103/ijnmr.ijnmr_149_22.

14. Jenabi E, Khazaei S, Aghababaei S, Moradkhani S. Effect of Fennel-Valerian Extract on Hot Flashes and Sleep Disorders in Postmenopausal Women: A Randomized Trial. J Menopausal Med. 2023; 29(1): 21-28. doi: 10.6118/jmm.22026.

15. Mahdavian M, Mirzaii Najmabadi K, Hosseinzadeh H, Mirzaeian S, Badiee Aval S, Esmaeeli H. Effect of the mixed herbal medicines extract (fennel, chamomile, and saffron) on menopause syndrome: A randomized controlled clinical trial. J Caring Sci. 2019; 8(3): 181-189. doi: 10.15171/jcs.2019.026.

16. Abedi P, Najafian M, Yaralizadeh M, Namjoyan F. Effect of fennel vaginal cream on sexual function in postmenopausal women: A double blind randomized controlled trial. J Med Life. 2018; 11(1): 24-28. PMID: 29696061.

17. Afiat M, Amini E, Ghazanfarpour M, Nouri B, Mousavi MS, Babakhanian M, Rakhshandeh H. The effect of short-term treatment with fennel on lipid profile in postmenopausal women: A randomized controlled trial. J Menopausal Med. 2018a; 24(1): 29-33. doi: 10.6118/jmm.2018.24.1.29.

18. Afiat M, Dizavandi FR, Kargarfard L, Vahed SHM, Ghazanfarpour M. Effect of *Foeniculum vulgare* (fennel) on sleep quality of menopausal women: A double-blinded, randomized placebo-controlled trial. J Menopausal Med. 2018b; 24(3): 204-209. doi: 10.6118/jmm.2018.24.3.204.

19. Ghazanfarpour M, Najafi MN, Sharghi NB, Mousavi MS, Babakhanian M, Rakhshanded H. A double-blind, placebo-controlled trial of fennel *(Foeniculum vulgare)* on menopausal symptoms: A high placebo response. J Turk Ger Gynecol Assoc. 2018; 19(3): 122-127. doi: 10.4274/jtgga.2017.0124.

20. Ghazanfarpour M, Amini E, Khadivzadeh T, Babakhanian M, Nouri B, Rakhshandeh H, Afiat M. The effect of short-term treatment with fennel on bone density in postmenopausal women: A randomized controlled trial. J Menopausal Med. 2017; 23(2): 124-130. doi: 10.6118/jmm.2017.23.2.124.

21. Rahimi-kian F, Bekhradi R, Rahimi R, Golzareh P, Mehran A. Evaluating the effect of fennel soft capsules on the quality of life and its different aspects in menopausal women: A randomized clinical trial. Nurs Pract Today. 2017; 4(2): 87-95. https://npt.tums.ac.ir/index.php/npt/article/view/229

22. Saghafi N, Ghazanfarpour M, Khadivzadeh T, Babakhanian M, Afiat M. The effect of *Foeniculum vulgare* (fennel) on body composition in postmenopausal women with excess weight: a double-blind randomized placebo-controlled trial. J Menopausal Med. 2017; 23(3):166-171. doi: 10.6118/jmm.2017.23.3.166.

23. Yaralizadeh M, Abedi P, Najar S, Namjoyan F, Saki A. Effect of *Foeniculum vulgare* (fennel) vaginal cream on vaginal atrophy in postmenopausal women: A double-blind randomized placebo-controlled trial. Maturitas. 2016; 84: 75-80. doi: 10.1016/j.maturitas.2015.11.005.

24. Dietz BM, Hajirahimkhan A, Dunlap TL, Bolton JL. Botanicals and their bioactive phytochemicals for women's health. Pharmacol Rev. 2016; 68 (4):1026-1073. doi: 10.1124/pr.115.010843.

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Artículos de revisión

Identificación y caracterización de eventos adversos asociados a la utilización de Cannabis: Revisión estructurada

Identification and characterization of adverse events associated with Cannabis use: A comprehensive review

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Resumen

Introducción: La disponibilidad de información estructurada de eventos adversos asociados al cannabis puede contribuir a la utilización adecuada del cannabis medicinal.

Objetivo: Sintetizar y caracterizar los eventos adversos asociados a la utilización de cannabis.

Método: Revisión estructurada de eventos adversos asociados a la utilización del cannabis en PubMed, utilizando términos Mesh ("Cannabis" And ("Drug-Related Side Effects or Adverse Reactions"), en humanos, publicados en inglés o español y acceso a texto completo, hasta noviembre 1 de 2024. Dos investigadores revisaron los artículos identificados y definieron su inclusión. Adicionalmente, se incluyeron referencias de artículos consideradas de interés.

Resultados: Se identificaron 47 eventos adversos asociados al uso de cannabis, afectando principalmente el sistema nervioso central y cardiovascular, en especial desórdenes mentales, somnolencia y afecciones gastrointestinales. No se identificaron eventos adversos mortales relacionados al uso de cannabis.

Conclusiones: La utilización de cannabis se asocia con la presentación de eventos adversos, en especial en los sistemas nervioso central y cardiovascular. Es necesario más estudios orientados a identificar y caracterizar eventos adversos asociados al cannabis.

Palabras claves: Cannabis medicinal; cannabis recreacional; cannabinoides; eventos adversos; revisión estructurada.

Abstract

Introduction: Available structured information about adverse events related to cannabis can contribute to appropriate use medical cannabis.

Objective: Summarize and characterize the adverse events related to cannabis use.

Method: Comprehensive review regarding to adverse events associated to cannabis use in PubMed. Mesh terms ("Cannabis" and "Drug-Related Side Effects and Adverse Reactions") were employed, focusing on human studies published in English or Spanish with full-text access, up to November 1, 2024. Two researchers reviewed the identified articles and reached a consensus on their inclusion. Additionally, relevant references from these articles were considered.

Results: A total of 47 adverse events associated to cannabis use were identified, mainly affecting the central nervous and cardiovascular systems, especially mental disorders, somnolence, and gastrointestinal conditions. No fatal adverse events were identified related to cannabis.

Conclusions: The use of cannabis is associated to adverse events, particularly in the central nervous and cardiovascular systems. More studies are needed to identify and characterize adverse events associated to cannabis.

Keywords: Medical cannabis; recreational cannabis; cannabinoids; adverse drug events; comprehensive review.

Puntos clave

En la actualidad, se reconocen los beneficios terapéuticos del cannabis en ciertos problemas de salud, caso del dolor crónico y trastornos neurológicos; sin embargo, la información disponible de sus efectos adversos es limitada. La variabilidad farmacológica, genera mayor complejidad en la evaluación de causalidad.

La revisión identifica 47 reacciones adversas asociadas a la utilización del cannabis. Se presenta una clasificación de estos efectos en los sistemas del cuerpo humano, especialmente en el sistema nervioso central y cardiovascular.

La identificación y caracterización de 47 eventos adversos asociados al uso de cannabis son un soporte para programas de farmacovigilancia de los medicamentos y productos a base de cannabis. Además, se muestra la necesidad del seguimiento de resultados alcanzados en los pacientes, en especial en los sistemas nervioso central y cardiovascular.

Introducción

El cannabis es una planta que se ha utilizado durante cinco milenios con fines espirituales, medicinales y recreativos[1]. En su composición se han identificado aproximadamente 140 cannabinoides, entre los cuales se destacan el delta-9 -tetrahidrocannabinol (THC), conocido por sus efectos psicoactivos, y el Cannabidiol (CBD), asociado a efectos benéficos en los sistemas biológicos[2]. A diferencia del THC, el CBD no actúa como agonista del receptor cannabinoide tipo 1 (CBR1), lo que explica el menor riesgo de efectos psicoactivos[3].

El cannabis pertenece a la familia Cannabaceae y se encuentra disponible en la mayor parte del mundo, debido a que puede crecer en diversos climas[4]. Se reconoce fácilmente por la disposición característica de sus hojas; siendo la *Cannabis sativa* y la *Cannabis indica* las dos variantes botánicas más comunes. Desde la década de 1970, la planta ha sufrido una hibridación y cruzamiento selectivos, generando una diversidad significativa y la presencia de cepas escasamente caracterizadas. Por tanto, existe una circulación amplia de variedades de cannabis[4].

De forma general, cada vez se dispone de mayor información de los beneficios terapéuticos de los cannabinoides en ciertos problemas de salud[5]. En este contexto, el cannabis medicinal hace referencia al uso de productos o medicamentos basados en cannabis en el tratamiento de enfermedades o para aliviar síntomas, en diversas preparaciones, por ejemplo, en productos vegetales o aceites en extractos manufacturados, y por diferentes vías de administración, por ejemplo, inhalada, vaporizada u oral[3]. En este contexto, en las últimas 2-3 décadas se han producido avances científicos significativos que avalan el uso de productos derivados del cannabis en el tratamiento de la epilepsia, el dolor crónico y diversas afecciones neurológicas y mentales[3]. Para el año 2022, se estimó que unos 192 millones de personas (3,9% de la población mundial) consumió *Cannabis sativa* con fines médicos y/o recreativos[3]. Dato que señala la importancia del cannabis como una de las sustancias más utilizadas globalmente, tanto con fines medicinal como recreacional[3].

Aunque el cannabis se ha utilizado desde la antigüedad, la regulación con fines medicinales data del siglo XX y puede variar entre regiones y países. Por ejemplo, en Estados Unidos de América, la Food and Drugs Administration (FDA, por sus iniciales en inglés) ha aprobado la Nabilona, el CBD y el Dronabinol para uso médico[4]. Adicionalmente, para el 2022, Estados Unidos de América, Canadá, Reino Unido, Australia y la mayoría de los países miembros de la Unión Europea habían autorizado el acceso a una gama de productos de CBD sin prescripción médica[3].

De forma general, se dispone de estudios y revisiones de los cannabinoides focalizados en establecer la eficacia[6]. Los resultados de la evaluación de la seguridad de los productos a base de cannabis son controvertidos, entre otras causas, por la confusión con el uso recreativo, los diferentes diseños de estudio, al igual que las indicaciones, dosificación, composición y vías de administración[6].

La dosis empleada de THC, las limitaciones en el conocimiento de las múltiples composiciones químicas del cannabis, los posibles contaminantes químicos asociados al cultivo y la extracción-preparación de productos, son factores de riesgo para la seguridad y salud de los usuarios de productos de cannabis[4]. Estos efectos frecuentemente son ignorados, debido a los beneficios como la reducción de ansiedad mejora del sueño, alivio del dolor y disminución de náuseas[4].

Adicionalmente, la legalización del uso médico y recreativo también se ha asociado con una disminución de las percepciones de riesgo del cannabis y un aumento del uso médico en adultos[7]. Sin embargo, la profesión médica mantiene sus reservas con el uso terapéutico del cannabis, debido a la probabilidad de eventos adversos asociados a la utilización de este tipo de productos[8]. A lo que se suma que, en esencia, la información disponible de eventos adversos asociados al uso del cannabis[7], podría ser de mayor utilidad si es sintetizada y organizada por sistemas; por tanto, el objetivo de esta revisión fue identificar, sintetizar y caracterizar la información disponible sobre los eventos adversos asociados a la utilización del cannabis.

Métodos

Obtención de los datos.

Se realizó una revisión estructurada de la literatura sobre los eventos adversos asociados a la utilización del cannabis en PubMed, utilizando términos Mesh ("Cannabis" y "Drug-Related Side Effects and Adverse Reactions").

Criterios de inclusión: Se incluyó todo tipo de artículos con información sobre eventos adversos en humanos, asociados a la utilización del cannabis con fines recreativos o medicinales, publicados en inglés o español, con acceso a texto completo, sin límites en fecha inicial de su publicación y hasta noviembre de 2024.

Criterios de exclusión: (a) Estudios sin relación con eventos adversos del cannabis; (b) estudios sin asociación clara del evento adverso con el cannabis, (c) estudios in-vitro o en animales; (d) estudios sin información específica de los eventos adversos, (e) estudios con información de eventos adversos sin el soporte científico adecuado (no se puede determinar que los eventos adversos descritos sean exclusivamente atribuibles al uso de cannabis), (f) estudios relacionados con cannabis sintéticos, (g) estudios con efectos benéficos del cannabis.

Registro de la información.

Siguiendo la metodología PRISMA[9], dos revisores evaluaron de forma independiente todos los títulos y resúmenes basados en los criterios de elegibilidad definidos. Si el resumen cumplía con los criterios de inclusión, se leyó y analizó el texto completo y, con ello, se definió su inclusión o no en la revisión. Las diferencias en las opiniones de los revisores se resolvieron, por consenso con la participación de un tercer revisor. Además, se incluyeron referencias relevantes de los artículos incluidos. La información se registró en un archivo en Microsoft Excel® con los siguientes ítems:

• **Información general:** nombre del primer autor, año de publicación, país, título del artículo, tipo de estudio (reporte de caso, observacional descriptivo, observacional analítico, intervención -estudio clínico), decisión de inclusión (incluido o se excluido) y observaciones.

• **Información específica:** cantidad de pacientes, características del o de los pacientes (sexo, edad, características clínicas), tipo de uso del cannabis (recreativo o medicinal), descripción de los eventos adversos, sistemas u órganos afectados y observaciones o comentarios.

Además, los eventos adversos identificados se clasificaron acorde con el tipo de estudio en el que lo soporta (revisiones sistemáticas, estudios clínicos, estudios analíticos, reportes de casos y revisión narrativa), acorde con el sistema (órgano) afectado y la frecuencia reportada.

Presentación de datos

La información identificada en esta revisión estructurada, relacionada con los eventos adversos asociados a la utilización del cannabis, se presenta con un enfoque sintético y narrativo en texto, tablas y figuras.

Resultados

Artículos incluidos en la revisión

Se identificaron 49 artículos, de los cuales se excluyó uno por no tener texto completo disponible, y 34 por no estar relacionados con el objetivo de la revisión o por criterios de exclusión. Además, en las referencias de los 14 artículos incluidos por la búsqueda[6,7,10-21], se identificaron 22 relacionados y con información objetivo de la revisión[1,4,22-41], de las cuales la referencia 1 y 4 se habían utilizado en la introducción. Por tanto, los resultados de esta revisión se soportan en 36 publicaciones (figura 1)[9].

En la revisión, se identificaron 47 eventos adversos, en la tabla 1 se detallan los sistemas (órganos) y los principales eventos adversos identificados; además, en la figura 2 se ilustran los sistemas afectados, los eventos asociados a la utilización de cannabis, acorde con el tipo de estudio que lo soporta. Por su parte, en la tabla 2 se especifica la frecuencia de aparición de los eventos adversos por cada sistema afectado.

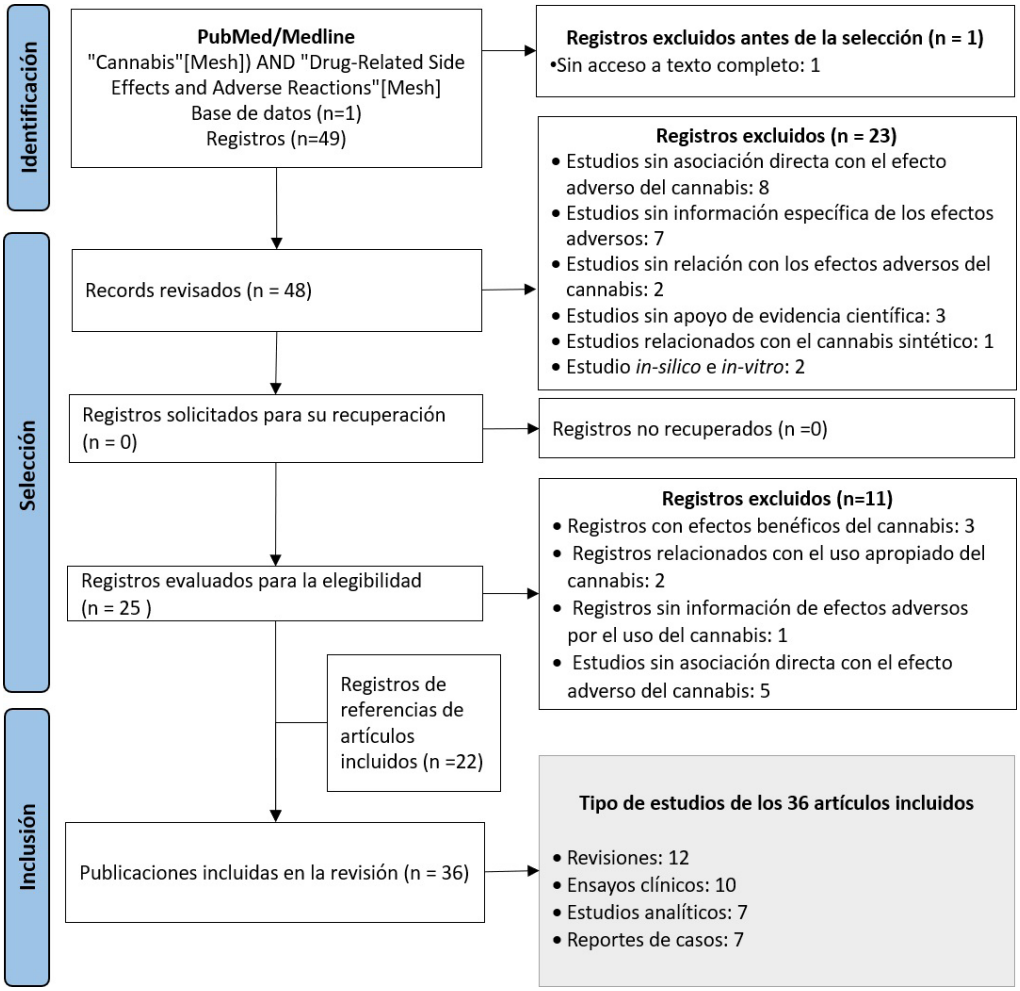

Figura 1. Diagrama *PRISMA* (Prefered report for systematic review and meta-analysis)[9].

Tabla 1. Eventos adversos asociados con la utilización de cannabis en diversos sistemas (órganos).

Sistema afectado	Eventos adversos	Comentarios
Sistema nervioso central	**Alteraciones mentales:** Pensamientos disociativos o trastornos de la percepción[7], alucinaciones[10,11,20], delirios[11], pensamientos acelerados[11], aislamiento social[11], sensación de euforia[12] y esquizofrenia[11,22,23,24,25]	Estudios epidemiológicos han demostrado que el uso del cannabis está asociado con un aumento en la probabilidad de desarrollar cuadros psicóticos similares a la esquizofrenia. Este riesgo varía según la dosis y frecuencia del uso, así como la edad y factores genéticos, incluyendo una predisposición genética parcialmente relacionada con la esquizofrenia[11]. En relación con este aspecto, un estudio retrospectivo de casos y controles realizado en 2010, con el objetivo de explorar la sensibilidad individual al cannabis en 121 pacientes con esquizofrenia, encontró que, 44 de los 121 participantes, eran sensible a los efectos sicotógenos del cannabis[26]. Esta sensibilidad podría estar relacionada con la vulnerabilidad genética a la esquizofrenia y con la influencia del cannabis en la maduración cerebral[25]. Un estudio encontró que, el consumo intenso (una vez por semana durante un año) de cannabis en mayores de 18 años, aumenta en 6 veces el riesgo de esquizofrenia 15 años después[23]. La exposición de jóvenes al cannabis puede causar alteraciones no reversibles en el funcionamiento del sistema endocannabinoide[23]. Una revisión narrativa del 2005, en pacientes con psicosis inducida por el cannabis reportó que, a los 3 años siguientes, un 40-50% de los pacientes con psicosis asociada cannabis, desarrollará esquizofrenia[22]. En el 2019, un estudio clínico en pacientes con cáncer avanzado y evidencia histológica de neoplasia maligna incurable con esperanza de vida estimada ≥3 meses, reportó que las alucinaciones fueron el evento adverso más notorio, tanto con dosis de 10 mg (9,5 mg THC y 0,5 mg CBD) como con 5 mg (4,75 mg THC y 0,25 mg CBD)[10].
	Pérdida de concentración[7,26,27], somnolencia[4,7,26,27,28], confusión[26,27], mareo[4,7,10,29]	En un estudio realizado con 329 pacientes con esclerosis múltiple, la utilización diaria de 28 mg/día de THC por 3 años, se asoció con la presentación de pensamientos disociativos, somnolencia y mareos[27]. A su vez, en dos estudios con pacientes con cáncer y con esclerosis múltiple, el mareo y la pérdida de concentración fueron reportados como eventos adversos[4,10]. De forma global, los eventos adversos aparecieron entre 1 a 2 horas después de la ingesta de la cápsula de cannabis, con una duración entre 2 y 3 horas y causaron incapacidad para realizar actividades físicas durante estas horas[10].
	Convulsiones[4]	En 4 de 137 pacientes con esclerosis múltiple incluidos en un estudio clínico, la utilización de un aerosol basado en cannabis (THC 27 mg/ml y CBD 25 mg/ml), se presentó convulsiones por primera vez[4].
	Cefaleas[7,12,26,27], dificultad para dormir[12]	En personas de unos 48 años y con cáncer, la utilización de cannabis se asoció con la presentación de dolor de cabeza, dificultad para dormir y euforia[12].
	Disminución del volumen de materia gris[11,22,30,31]	En un estudio de casos y controles, en personas sin antecedentes de enfermedades médicas crónicas o afecciones neurológicas ni trastorno psiquiátrico, la exposición crónica al cannabis se asoció con alteraciones cerebrales. Además, se identificó disminución en el volumen de la materia gris, especialmente en las regiones del cerebro con una alta concentración de receptores CB1 (hipocampo, corteza prefrontal, amígdala y cerebelo)[30]
	Movimientos involuntarios[21]	Un reporte de caso muestra que la ingesta de aceite de cannabis de forma oral puede causar movimientos involuntarios[21].

Sistema afectado	Eventos adversos	Comentarios
Sistema cardiovascular	Taquicardia[7,13,14,15,16,20,21,25,30,31,32,33,34]	Un metaanálisis informó un aumento promedio en la frecuencia cardiaca en 8 latidos por minuto, asociado al consumo agudo de THC, efecto adverso que es dosis dependiente[7]. En este sentido, se reportó que el fumar el cannabis provoca un aumento inmediato de la frecuencia cardiaca, aproximadamente durante 1 hora, efecto que se atribuye a un aumento sustancial en la concentración sérica de la noradrenalina a los 30 minutos[13].
	Hipertensión arterial[6]	En un estudio con 1.213 pacientes sin especificar antecedentes clínicos, en usuarios consumidores de marihuana se identificó un mayor riesgo de hipertensión arterial y mortalidad asociada. A mayor duración de consumo mayor riesgo de muerte relacionado con la hipertensión arterial[6].
	Vasculopatía[6], vasoespasmo coronario[6] y agregación plaquetaria[6]	La utilización del cannabis en un paciente masculino de 30 años se asoció con lesiones arteriales segmentarias distales que ocluían la arteria poplítea, lo que desencadenaron una necrosis digital del dedo del pie derecho de este[6]. En otro reporte de caso, en un paciente de 29 años, se identificó síndrome coronario agudo, con elevación dinámica del segmento ST en diferentes derivaciones y con un aumento notorio de la troponina I de alta sensibilidad[6]. Además, en otro caso se reportó un aumento en la de agregación plaquetaria en un paciente de 20 años, lo que desencadeno un infarto de miocardio. Las concentraciones elevadas de cannabinoides se asocian con un aumento irreversible en la agregación plaquetaria[6].
	Aumento de troponinas séricas (isquemia)[14]	Un reporte de caso, en joven atlético de 17 años, la utilización de cannabis con fines recreativos se asoció con un aumento en los niveles de troponinas séricas elevadas, posible isquemia y taquicardia persistente. De forma global, se acepta que los niveles elevados de THC, pueden causar agitación y daños en los órganos diana, mediados por estimulo simpático y serotoninérgico[14].
	Hemorragia[17]	Hemorragia hepática y timal en neonato (11 días de nacimiento) asociado al consumo de marihuana en la madre[17].

Sistema afectado	Eventos adversos	Comentarios
Sistemas sensoriales	OIDOS Tinnitus y vértigo[11]	Un metaanálisis de 7 revisiones identificó la presentación de tinnitus, zumbido en oídos y vértigo, como eventos adversos asociados a la utilización de cannabis[11].
	BOCA Trastorno de la mucosa bucal[4,12,16,29] y dolor bucal[4]	En un estudio retrospectivo, realizado con pacientes con cáncer, la sequedad bucal fue el evento adverso más notorio[11]. De forma similar, en un estudio controlado en pacientes con esclerosis múltiple, los trastornos de la mucosa bucal y dolor oral fueron los eventos adversos asociados con la utilización de cannabis medicinal[4]. Por su parte, en un reporte de caso de un hombre de 18 años de edad, la primera exposición al cannabis recreativo se asoció efectos anticolinérgicos, los cuales fueron la explicación para la presentación de boca seca y sed excesiva[16].
	PIEL Reacciones cutáneas, anafilaxia[1] y pirexia[4].	La utilización de cannabis (fumar, ingerir o inhalar cannabis), al igual que el contacto dérmico, se asoció con alergias ocupacionales, tales como las reacciones cutáneas y anafilaxias (estas producidas por las semillas de cáñamo)[1]. Por ello, el cannabis puede provocar reacciones alérgicas tipo 1 y tipo 4. Los alérgenos oficialmente reconocidos incluyen un alérgeno de clase 10 relacionado con la patogénesis, la profilina y una proteína de transferencia de lípidos no específica[1]. En este contexto, un estudio clínico, el grupo expuesto al cannabidiol, la pirexia fue el evento adverso más frecuente[4].
	OJOS Midriasis[15], lagrimeo[18] y conjuntivitis[1]	Un reporte de caso, en un joven de 16 años, la utilización por primera vez de cannabis con fines recreativos se asoció con midriasis de 5 mm[15]. Por su parte, en otro reporte de caso, en un joven de 16 años, la utilización de cannabis con fines recreativos se asoció con lagrimeo[18]. Además, las reacciones alérgicas al cannabis pueden presentarse con síntomas de conjuntivitis[1].
	NARIZ Síntomas de purito, congestión nasal[18] y rinitis[1]	Un reporte de caso, en un hombre de 29 años, la utilización por primera vez de cannabis con fines recreativos se asoció con purito nasal y faríngeo, congestión nasal, disnea y sibilancias, los cuales persistieron de 20 a 30 minutos[18]. Luego, presentó una recurrencia de los síntomas anteriores después de fumar una segunda vez[18]. Además, la exposición al cannabis puede causar rinitis[1].
Sistema respiratorio	Neumonía[4,19,36,37,38]	Un estudio longitudinal en pacientes con y sin VIH evidenció que hay un mayor riesgo de enfermedad pulmonar infecciosa (incluida la neumonía) entre los hombres que fumaban cannabis y que viven con VIH[38].
	Disnea[12,18], síntomas de purito faríngeo y sibilancias[18]	En un estudio realizado en el 2022, orientado a caracterizar los beneficios del cannabis medicinal en 163 pacientes, la disnea se reportó como un evento adverso[12]. Por otra parte, en un reporte de caso se identificó purito faríngeo y sibilancias como eventos adversos asociadas a la primera exposición al cannabis recreativo[18].
	Deterioro funcional de los macrófagos alveolares[19,36,39]	La utilización del cannabis tiene diversos efectos sobre el sistema inmunitario, incluido el deterioro funcional de los macrófagos alveolares en los fumadores de cannabis, lo cual genera que se aumente la susceptibilidad a la infección[39].
	Asma[1]	La exposición al cannabis puede provocar reacciones alérgicas de tipo 1 y tipo 4. Esta exposición puede comprender desde fumar, comer, inhalar polen o humo de cannabis y el contacto con la piel. Además, el desarrollo de asma es uno de los eventos adversos asociado a la exposición con el cannabis[1].
Sistema gastrointestinal	Náuseas[4,11,28,29,40,41], vómito[4,11,28,40,41], diarrea[4,7,28,29] e inapetencia[4,28]	En estudios clínicos, la utilización de cannabis se ha asociado con la presentación de náuseas, mareos, diarrea e incluso gastroenteritis[4]. Por su parte, en ensayo aleatorizado doble ciego, 74 de 86 pacientes del grupo expuesto al cannabidiol en comparación con 59 de 85 pacientes del grupo de placebo, presentaron diarrea y vómito[4].
Sistema renal	Dificultad para orinar[16]	Un reporte de caso mostró que la exposición por primera vez al cannabis puede causar síntomas anticolinérgicos, los cuales se pueden manifestar en dificultad para orinar[16].

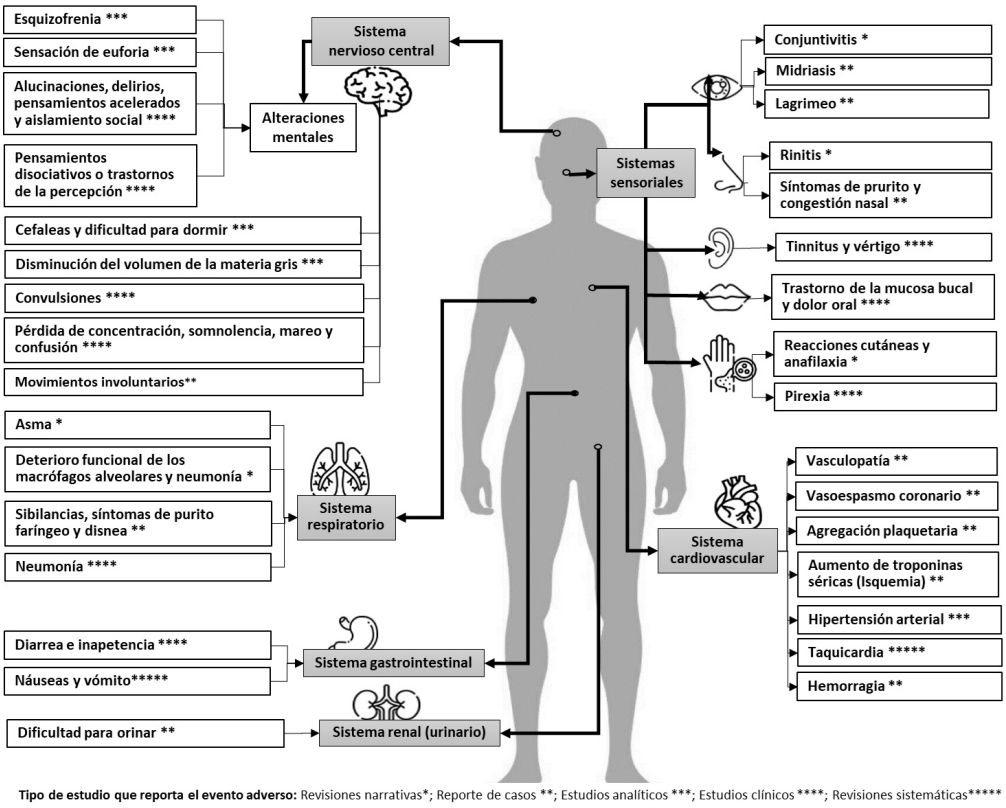

Figura 2. Eventos adversos y sistemas afectados asociados a la utilización de cannabis, acorde con el tipo de estudio que lo soporta.

Fuente: Elaboración propia

Tabla 2. Determinación cuantitativa de los eventos adversos y frecuencia de aparición por cada sistema afectado.

Sistema	Eventos adversos identificados	Frecuencia de aparición del sistema por artículo (%), n=55*
Nervioso central	15	15 (27,3)
Cardiovascular	7	16 (29,1)
Respiratorio	6	9 (16,4)
Sensorial	13	8 (14,5)
Gastrointestinal	4	6 (10,9)
Renal	1	1 (1,82)

* La suma de las frecuencias es superior a 36, debido a que algunos eventos adversos pueden ser reportados en un mismo artículo.
Fuente: Elaboración propia

Discusión

La utilización con fines terapéuticos del cannabis está asociada al riesgo de la presentación de eventos adversos y problemas de seguridad en general. En este sentido, similar al caso de otros medicamentos, en un paciente específico, se debe analizar los posibles riesgos y beneficios y, con ello, establecer una relación adecuada riesgo/beneficio. Evaluación que requiere establecer el perfil de seguridad y generar información para su uso adecuado, minimizando el riesgo de ocurrencia de problemas relacionados con medicamentos y eventos adversos en general[43]. Dentro del perfil de seguridad, además de las interacciones entre el cannabis (incluyendo la particularidad de las diferentes moléculas) y otros fármacos[44], es clave la identificación y caracterización de los eventos adversos, lo cual fue el objetivo de la presente revisión.

Esta revisión estructurada identifica y caracteriza los eventos adversos que se presentan con el uso del cannabis recreativo o medicinal. En la tabla 2 se presenta una determinación cuantitativa de los eventos adversos asociados al uso de cannabis, desglosando la frecuencia de aparición según el sistema afectado. Esta información identifica los sistemas más afectados y los eventos adversos más comunes en los mismos (tabla 1). En este sentido, el sistema nervioso central (SNC) es el más destacado, con efectos como esquizofrenia[11,22,23,24,25], pérdida de concentración[7,26,27] somnolencia[4,7,26,27,28], dolor de cabeza[7,12,26,27], confusión[26,27], mareos[4,7,10,29], pensamientos disociativos[7] y alucinaciones[10,11,20], entre otros, los cuales, para pacientes con antecedentes de trastornos mentales, podrían estar en mayor riesgo (tabla 1). Adicionalmente, en personas sin antecedentes de enfermedades médicas crónicas y sin afecciones neurológicas ni trastornos psiquiátricos, la utilización crónica al cannabis se ha asociado con la disminución del volumen de la materia gris, especialmente en el hipocampo, la corteza prefrontal, la amígdala y el cerebelo[11,22,30,31]; efectos adversos de mayor relevancia en adolescentes, debido a que sus cerebros pueden estar aún en desarrollo y, por tanto, son más susceptibles a los impactos negativos del cannabis[23].

Por otra parte, el sistema cardiovascular, es el segundo sistema con mayor afectación por eventos adversos asociados al uso del cannabis (tabla 2). En el caso del corazón, se identificó taquicardia[7,13,14,15,16,20,21,27,32,33,34,35,36], e hipertensión arterial[6] (tabla 1). En este contexto, es importante destacar que, la hipertensión arterial, se ha convertido en uno de los principales factores de riesgo global para la mortalidad y discapacidad. Entre 1990 y 2019 el número de personas con hipertensión aumentó significativamente, pasando de 650 millones a 1,3 mil millones[45]. Esta condición es el factor de riesgo más relevante para prevenir muertes tempranas, responsable de 8,5 millones de muertes anuales por accidentes cerebrovasculares, enfermedades isquémicas del corazón, otras enfermedades vasculares y enfermedades renales en todo el mundo[46]. Adicionalmente, relacionada con los vasos sanguíneos, se identificó vasculopatía, vasoespasmo coronario y mayor agregación plaquetaria[6].

En tercer lugar, la utilización de cannabis también se asocia con afectaciones de órganos como los ojos, los oídos, la nariz, boca, pulmones, estómago y riñón (tabla 1). En este sentido, algunos reportes de casos han descrito midriasis hasta de 5 mm[15], lagrimeo[18], prurito nasal y faríngeo[14]. Además, en un reporte de casos, en un paciente masculino de 18 años, con su primera exposición al cannabis de manera recreativa, presentó boca seca, sed excesiva y dificultad para orinar, asociados a efectos anticolinérgicos[16]. En estudios analíticos se identificó deterioro funcional de los macrófagos alveolares, sibilancias, disnea[19,36,38], y eventos adversos gastrointestinales, caso de náuseas intensas, vómitos, diarrea y malestar abdominal[4,7,28,29]; mientras que, en estudios clínicos, se identificó evidencia de tinnitus y vértigo[11]. Aunque para este tipo de eventos adversos se identificó una menor frecuencia, de igual forma podrían afectar la calidad de vida de quienes utilizan el cannabis, ya sea de forma recreativa o medicinal.

La prevalencia de eventos adversos varía dependiendo del tipo de estudio; en este sentido, los eventos adversos de mayor prevalencia reportados por algunos estudios incluidos fueron: desordenes del sistema nervioso central, incluyendo alteraciones mentales[11], somnolencia[28], mareo[4]; afecciones gastrointestinales[40], caso de la hiperémesis[41], diarrea[4], náuseas y vómitos[33]. Además, un resumen de revisiones sistemáticas (revisión paraguas) identificó a la diarrea, la somnolencia y disminución en el apetito como los eventos adversos más asociados al tratamiento con cannabis[45]. Por su parte, la

prevalencia de efectos adversos respiratorios relacionados con uso de cannabis (no necesariamente medicinal) podría ser similar a la reportada para el tabaquismo[1,37].

Algunas revisiones han reportado que el CBD (aislado) es excepcionalmente seguro, incluso en el rango de dosis consideradas como altas (> 300–400 mg) [2]. Por ello, el CBD se considera más seguro que el THC[7], en especial en los sistemas nervioso central y cardiovascular[19]. De forma general, se acepta que, ambas moléculas, pueden generar efectos opuestos; el CBD tiene cierta evidencia de su potencial como ansiolítico; mientras que el THC puede generar ansiedad[2,7]. Incluso, se estima que, el THC, a diferentes dosis, puede generar efectos contrarios. Por ejemplo, a dosis bajas puede ser antiemético; mientras que, a altas dosis, podría ser pro emético[11]. En otros casos, como las alergias, las reacciones adversas se asocian a otro tipo de sustancias, siendo las proteínas de alto peso molecular las que pueden actuar como alérgenos[1].

De forma global, los eventos adversos identificados en la presente revisión (tabla 1) están relacionados con efectos atribuidos al cannabis. En este contexto, los cannabinoides, caso del THC y el CBD, activan los receptores cannabinoides tipo 1 (CB1) y tipo 2 (CB2) en el sistema endocannabinoide. En este sentido, el sistema endocannabinoide cumple funciones neuro-moduladoras y homeostáticas[48]. En ello, los endocannabinoides mediante neurotransmisión retrógrada, puede causar que una neurona postsináptica libere endocannabinoides que se unen principalmente a los receptores CB1 en la neurona presináptica[48]. Esto provoca la inhibición del canal de calcio presináptico y, con ello, la liberación de neurotransmisores desde la neurona presináptica[48].

La utilización de cannabis con fines medicinales o recreacional puede llevar a la inhibición de neurotransmisores presinápticos, como la acetilcolina, que estimula los receptores colinérgicos, desempeñando un papel crucial en diversas funciones del organismo[48]. Por ello, los cannabinoides, principalmente el THC, puede generar eventos adversos anticolinérgicos[16], asociados a la inhibición de receptores muscarínicos (M_1, M_2, M_3, M_4, M_5) y nicotínicos (N_m y N_n)[47]. En este sentido, este efecto es la posible explicación de la presentación de taquicardia, cefalea causada por vasodilatación, inotropismo positivo, vómito (por disminución del vaciamiento gástrico), boca seca, midriasis, aumento de la presión intraocular y retención urinaria[49].

Por otro lado, el cannabis puede modificar la actividad de otros neurotransmisores, caso de la noradrenalina, la cual, al ser inhibida, provocará un efecto anti-adrenérgico en los receptores: α_1, β_2, β_3, β_4[49]. Dicho efecto puede verse reflejado en aumento de las secreciones nasales y bronquiales, disminución de secreción salival, broncoconstricción y relajación de los esfínteres, asociado por ejemplo a diarrea[4].

Adicionalmente, algunas de las propiedades psicotrópicas de los cannabinoides se explican por modificaciones del SNC[48]. Algunas áreas y efectos incluyen: “Hippocampus: deterioro de la memoria a corto plazo; neocórtex: deterioro del juicio y la sensación; ganglios basales: tiempo de reacción y movimiento alterados; hipotálamo: aumento del apetito; núcleo accumbens: euforia; amígdala: pánico y paranoia; cerebelo: ataxia; tronco encefálico: anti-emesis y médula espinal: analgesia”[48].

Por tanto, la utilización de cannabinoides, como ocurre con cualquier sustancia, conlleva efectos adversos que pueden variar desde leves a letales, dependiendo de los métodos de administración y los riesgos asociados[48]. Sin embargo, en esta revisión no se identificaron eventos adversos graves o letales. En este contexto, los efectos adversos más frecuentes de los cannabinoides se evidencian más con el uso con fines recreacionales, tanto en el corto como en el largo plazo[48].

- **Corto plazo:** Euforia, ansiedad, distorsión visual-temporal, amplificación sensorial, taquicardia, hipotensión postural, conjuntivitis, hambre y sequedad de garganta, boca y ojos[48].

- **Largo plazo:** Trastorno por uso de cannabis (incluyendo dependencia), desarrollo cerebral alterado y deterioro cognitivo en adolescentes. Además, el consumo crónico se ha asociado con bronquitis crónica, síndrome de dificultad respiratoria aguda, cáncer de pulmón; mayor riesgo de infarto de miocardio, accidente cerebrovascular y eventos tromboembólicos, y exacerbación de trastornos del estado de ánimo (ansiedad, depresión) y trastornos psicóticos (esquizofrenia)[48].

Los eventos adversos asociados a la utilización de cannabis, identificados en la presente revisión, se fundamentan en resultados de revisiones sistemáticas, estudios clínicos, estudios analíticos, reportes

de casos y revisiones narrativas. Sin embargo, se requiere seguir realizando estudios de seguridad y farmacovigilancia enfocados en cannabis medicinal, ya que los eventos adversos de su uso recreacional pueden diferir del uso medicinal[7]. También analizar la seguridad en poblaciones sanas y que no utilicen otros medicamentos, debido a que ambos factores se consideran confusores de eventos adversos[40]. Por tanto, es clave el apoyo y motivación para diseñar y realizar investigaciones, orientadas a generar información de los resultados generados por la utilización de este tipo de productos, especialmente en poblaciones vulnerables.

De esta manera, se facilitará la definición de políticas públicas que equilibren el acceso a sus posibles ventajas terapéuticas con la necesidad de minimizar los eventos adversos. La generación y visibilidad de información relacionada con los riesgos de la utilización de cannabis es clave para garantizar la mejor toma decisiones terapéuticas, contribuyendo a proteger la salud y el bienestar de la sociedad.

En definitiva, el uso del cannabis con fines recreativos y medicinales ha sido objeto de un creciente interés y debate en la sociedad contemporánea. Si bien esta planta tiene propiedades terapéuticas que pueden ofrecer diferentes beneficios al paciente con relación a un contexto médico, también conlleva riesgos significativos, en especial con el aumento del uso de este tipo de productos. Por ejemplo, en el caso de los Estados Unidos de América, un 10% de los usuarios de cannabis la utilizan para tratar algún tipo de afección médica[50]; por su parte, el 14% de canadienses usaron cannabis para tratar alguna afección médica en 2022[1].

Limitaciones. Los resultados obtenidos de esta revisión pueden presentar algunas limitaciones; por tanto, los resultados deben interpretarse y utilizarse con precaución. En este contexto, la principal limitación es la restricción de la búsqueda a una sola base de datos, asociado al riesgo de no identificar completamente los eventos adversos que se presentan tras el uso del cannabis. No obstante, esta situación se puede minimizar con la inclusión de publicaciones identificadas como relevantes en las referencias de los artículos incluidos. Además, en algunos estudios no se detalló las dosis o concentración de CBD o THC utilizada por las personas incluidas en el respectivo estudio.

Conclusiones

La utilización de cannabis con fines medicinales se puede acompañar de la presentación de eventos adversos, por ello, su utilización clínica se debe fundamentar en un análisis detallado de relación riesgo/beneficio, por parte del personal médico, idealmente con la contribución de otros profesionales de la salud. Los sistemas con más afectación son, el sistema nervioso central (esquizofrenia, pérdida de concentración, somnolencia cefalea, confusión, mareos, pensamientos disociativos y alucinaciones) el sistema cardiovascular (hipertensión arterial), respiratorio (broncoespasmo) y órganos de los sentidos (ojos, nariz, oídos y piel).

Se requiere más información y evidencia de los resultados alcanzados con el uso de cannabis en los problemas de salud en los que se les utiliza; lo que requiere del diseño y realización de estudios focalizados en generar una mayor comprensión de los riesgos y beneficios asociados con el uso del cannabis.

Bibliografía

1. Skypala IJ, Jeimy S, Brucker H, et al. Cannabis-related allergies: An international overview and consensus recommendations. Allergy 2022; 77: 2038–2052. DOI: 10.1111/all.15237

2. Arnold JC, McCartney D, Suraev A, et al. The safety and efficacy of low oral doses of cannabidiol: An evaluation of the evidence. Clin Transl Sci 2023; 16: 10–30. DOI: 10.1111/cts.13425

3. Marques Azzini GO, Marques Azzini VO, Santos GS, et al. Cannabidiol for musculoskeletal regenerative medicine. Exp Biol Med (Maywood) 2023; 248: 445–455. DOI: 10.1177/15353702231162086

4. Gottschling S, Ayonrinde O, Bhaskar A, et al. Safety Considerations in Cannabinoid-Based Medicine. Int J Gen Med 2020; 13: 1317–1333. DOI: 10.2147/IJGM.S275049

5. Choi NG, DiNitto DM, Marti CN, et al. Sex differences in cannabis forms and exposure reasons in cannabis-related poison control center cases aged 50. Clin Toxicol (Phila) 2021; 59: 822–831. DOI: 10.1080/15563650.2020.1869756

6. Subramaniam VN, Menezes AR, DeSchutter A, et al. The Cardiovascular Effects of Marijuana: Are the Potential Adverse Effects Worth the High? Mo Med 2019; 116: 146–153.

7. Arnold JC. A primer on medicinal cannabis safety and potential adverse effects. Aust J Gen Pract 2021; 50: 345–350. DOI: 10.31128/AJGP-02-21-5845

8. Lombardi E, Gunter J, Tanner E. Ohio physician attitudes toward medical Cannabis and Ohio's medical marijuana program. J Cannabis Res 2020;2(1):16. DOI: 10.1186/s42238-020-00025-1.

9. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;n71.

10. Bar-Sela G, Zalman D, Semenysty V, et al. The Effects of Dosage-Controlled Cannabis Capsules on Cancer-Related Cachexia and Anorexia Syndrome in Advanced Cancer Patients: Pilot Study. Integr Cancer Ther 2019; 18: 1534735419881498. DOI: 10.1177/1534735419881498

11. Crescioli G, Lombardi N, Bettiol A, et al. Adverse events following cannabis for medical use in Tuscany: An analysis of the Italian Phytovigilance database. Br J Clin Pharmacol 2020; 86: 106–120. DOI: 10.1111/bcp.14140

12. Raghunathan NJ, Brens J, Vemuri S, et al. In the weeds: a retrospective study of patient interest in and experience with cannabis at a cancer center. Support Care Cancer 2022; 30: 7491–7497. DOI: 10.1007/s00520-022-07170-8

13. Richards JR. Mechanisms for the Risk of Acute Coronary Syndrome and Arrhythmia Associated With Phytogenic and Synthetic Cannabinoid Use. J Cardiovasc Pharmacol Ther 2020; 25: 508–522. DOI: 10.1177/1074248420935743

14. Rickner SS, Cao D, Kleinschmidt K, et al. A little 'dab' will do ya' in: a case report of neuro-and cardiotoxicity following use of cannabis concentrates. Clin Toxicol (Phila) 2017; 55: 1011–1013. DOI: 10.1080/15563650.2017.1334914

15. Boukerche F, Khalil MZ, Shirk DV, et al. Serotonin Syndrome Misdiagnosed in a Patient Who Used a Cannabis Dab Pen. Prim Care Companion CNS Disord 2022; 24: 21cr03008. https://doi.org/10.4088/PCC.21cr03008

16. Mangot AG. Bad trip due to anticholinergic effect of cannabis. Gen Hosp Psychiatry 2013; 35: 682.e5–6. DOI: 10.1016/j.genhosppsych.2013.06.010

17. Bao C, Bao S. Neonate Death Due to Marijuana Toxicity to the Liver and Adrenals. Am J Case Rep 2019; 20: 1874–1878. DOI: 10.12659/AJCR.919545

18. Liskow B, Liss JL, Parker CW. Allergy to marihuana. Ann Intern Med 1971; 75: 571–573. DOI: 10.7326/0003-4819-75-4-571

19. Corso B, Bacle A, Demay E, et al. Place of therapeutic cannabis in France and safety data: A literature review. Ann Pharm Fr 2023; 81: 583–595. DOI: 10.1016/j.pharma.2023.02.002

20. Beck PJ, Reddy A. Cannabis Pen-Induced Psychosisin a First-Time Adolescent User. Psychopharmacol Bull 2023;53(3):61-5.

21. Nadeem Z, Wu C, Burke S, Parker S. Serotonin syndrome and cannabis: A case report. Australas Psychiatry 2024;32(1):100-1. DOI: 10.1177/10398562231219858

22. Krebs M-O, Kebir O, Jay TM. Exposure to cannabinoids can lead to persistent cognitive and psychiatric disorders. Eur J Pain 2019; 23: 1225–1233. DOI: 10.1002/ejp.1377

23. Andréasson S, Allebeck P, Engström A, et al. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet 1987; 2: 1483–1486. DOI: 10.1016/s0140-6736(87)92620-1

24. Arendt M, Rosenberg R, Foldager L, et al. Cannabis-induced psychosis, and subsequent schizophrenia-spectrum disorders: follow-up study of 535 incident cases. Br J Psychiatry 2005; 187: 510–515. DOI: 10.1192/bjp.187.6.510

25. Z Goldberger C, Dervaux A, Gourion D, et al. Variable individual sensitivity to cannabis in patients with schizophrenia. Int J Neuropsychopharmacol 2010; 13: 1145–1154. DOI: 10.1017/S1461145710000647

26. Sultan SR, Millar SA, O'Sullivan SE, et al. A Systematic Review and Meta-Analysis of the In Vivo Haemodynamic Effects of Δ8-Tetrahydrocannabinol. Pharmaceuticals (Basel) 2018; 11: 13. DOI: 10.3390/ph11010013

27. Beaumont H, Jensen J, Carlsson A, et al. Effect of delta9-tetrahydrocannabinol, a cannabinoid receptor agonist, on the triggering of transient lower oesophageal sphincter relaxations in dogs and humans. Br J Pharmacol 2009; 156: 153–162. DOI: 10.1111/j.1476-5381.2008.00010.x

28. Thiele EA, Marsh ED, French JA, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 2018; 391: 1085–1096. DOI: 10.1016/S0140-6736(18)30136-3

29. Wade DT, Makela PM, House H, et al. Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis. Mult Scler 2006; 12: 639–645. DOI: 10.1177/1352458505070618

30. Lorenzetti V, Solowij N, Yücel M. The Role of Cannabinoids in Neuroanatomic Alterations in Cannabis Users. Biol Psychiatry 2016; 79: e17-31. DOI: 10.1016/j.biopsych.2015.11.013

31. Gruber SA, Sagar KA. Marijuana on the Mind? The Impact of Marijuana on Cognition, Brain Structure, and Brain Function, and Related Public Policy Implications. Policy Insights Behav. Brain Sci 2017; 4: 104–111. DOI: 10.1177/2372732216684851

32. Karniol IG, Carlini EA. Comparative studies in man and in laboratory animals on 8 - and 9 -trans-tetrahydrocannabinol. Pharmacology 1973; 9: 115–126. DOI: 10.1159/000136375

33. Karniol IG, Shirakawa I, Takahashi RN, et al. Effects of delta9-tetrahydrocannabinol and cannabinol in man. Pharmacology 1975; 13: 502–512. DOI: 10.1159/000136944

34. Zimmer BD, Bickel P, Dittrich A. Changes of simple somatic parameters by delta-9-trans-tetrahydrocannabinol (delta-9-THC) in a double-blind-study. Short communication. Arzneimittelforschung 1976; 26: 1614–1616.

35. Haney M. Opioid Antagonism of Cannabinoid Effects: Differences between Marijuana Smokers and Nonmarijuana Smokers. Neuropsychopharmacol 2007; 32: 1391–1403. DOI: 10.1038/sj.npp.1301243

36. Klooker TK, Leliefeld KEM, Van Den Wijngaard RM, et al. The cannabinoid receptor agonist delta-9-tetrahydrocannabinol does not affect visceral sensitivity to rectal distension in healthy volunteers and IBS patients. Neurogastroenterol Motil 2011; 23: 30–35, e2. DOI: 10.1111/j.1365-2982.2010.01587.x

37. Gracie K, Hancox RJ. Cannabis use disorder and the lungs. Addiction 2021; 116: 182–190. DOI: 10.1111/add.15075

38. Lorenz DR, Uno H, Wolinsky SM, et al. Effect of marijuana smoking on pulmonary disease in HIV-infected and uninfected men: a longitudinal cohort study. EClinical Medicine 2019; 7: 55–64. DOI: 10.1016/j.eclinm.2019.01.003

39. Owen KP, Sutter ME, Albertson TE. Marijuana: respiratory tract effects. Clin Rev Allergy Immunol 2014; 46: 65–81. DOI: 10.1007/s12016-013-8374-y

40. Aviram J, Samuelly-Leichtag G. Efficacy of Cannabis-Based Medicines for Pain Management: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Pain Physician 2017; 20: E755–E796.

41. Monte AA, Shelton SK, Mills E, et al. Acute Illness Associated With Cannabis Use, by Route of Exposure: An Observational Study. Ann Intern Med 2019; 170: 531–537. DOI: 10.7326/M18-2809

42. WHO. The importance of pharmacovigilance. World Health Organization & WHO Collaborating Centre for International Drug Monitoring. Geneve: 2002.

43. Lopera V, Rodríguez A, Amariles P. Clinical Relevance of Drug Interactions with Cannabis: A Systematic Review. J Clin Med 2022; 11: 1154. DOI: 10.3390/jcm11051154

44. Singh C, Rao K, Yadav N, et al. Current Cannabidiol Safety: A Review. Curr Drug Saf 2023;18(4):465-473. DOI: 10.2174/1574886317666220902100511

45. World Population Prospects - Population Division - United Nations. [Internet]. Population.un.org. Recuperado el 3 de noviembre de 2024, de https://population.un.org/wpp2019/

46. Zhou B, Perel P, Mensah GA, Ezzati M. Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. Nat Rev Cardiol 2021;18(11):785-802. DOI: 10.1038/s41569-021-00559-8

47. Solmi M, Toffol MD, Kim JY, et al. Balancing risks and benefits of cannabis use: umbrella review of meta-analyses of randomised controlled trials and observational studies. BMJ 2023; 382: e072348. DOI: 10.1136/bmj-2022-072348

48. Sheikh NK, Dua A. Cannabinoids. 2023 Feb 27. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–.

49. Langnas E, Maze M. Clinical Use of Adrenergic Receptor Ligands in Acute Care Settings. Handb Exp Pharmacol. 2024;285:617-637. DOI: 10.1007/164_2023_705

50. Levinsohn EA, Hill KP. Clinical uses of cannabis and cannabinoids in the United States. J Neurol Sci 2020; 411: 116717. DOI: 10.1016/j.jns.2020.116717

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Artículos revisión

Curcumin and its derivatives as potential antiviral candidates against monkeypox (mpox): a review of computational studies

La curcumina y sus derivados como posibles candidatos antivirales contra la viruela del mono (mpox): una revisión de estudios computacionales

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Resumen

Introducción: La viruela del mono (mpox) es una enfermedad infecciosa causada por el virus mpox que es motivo de preocupación sanitaria mundial porque el brote, a mayo de 2023, ha afectado a más de 80.000 personas en cientos de países. Actualmente, no existe un tratamiento específico, incluidos los antivirales, para los pacientes con mpox. Continúa la exploración de compuestos activos para mpox, uno de los cuales es la curcumina y sus derivados. La curcumina es un compuesto polifenólico que se encuentra predominantemente en la cúrcuma y que se ha documentado que tiene efectos antivirales. Por lo tanto, este estudio tiene como objetivo explorar estudios que investiguen el potencial de la curcumina y sus derivados como candidatos antivirales para atacar a la mpox.

Método: Se buscó literatura publicada desde su inicio hasta 2024 en ScienceDirect, PubMed, Scopus y Google Scholar. Las palabras clave utilizadas en esta búsqueda incluyeron cúrcuma, curcumina, tetrahidrocurcumina, demetoxicurcumina, bisdemetoxicurcumina, cúrcuma, viruela del mono y mpox.

Resultados: Los resultados de la búsqueda bibliográfica encontraron cinco estudios computacionales que involucran el compuesto curcumina y sus derivados, incluida la tetrahidroxicurcumina y la demetoxicurcumina. Todos los estudios demostraron que la curcumina y sus derivados tienen una mejor afinidad de unión con proteínas de mpox en comparación con el control de varios antivirales. La curcumina y sus derivados tienen un gran potencial para inhibir la replicación del virus mpox y modular el sistema inmunológico.

Conclusiones: Esta revisión concluye que la curcumina y sus derivados tienen potencial como candidatos antivirales para el mpox. Sin embargo, los estudios relacionados siguen siendo limitados y se limitan a estudios computacionales. Se necesitan más estudios preclínicos, experimentales y clínicos para confirmar su eficacia y mecanismos de acción.

Palabras clave: Antiviral; Curcumina; Viruela del mono; Mpox; Cúrcuma

Abstract

Introduction: Monkeypox (mpox) is an infectious disease caused by the mpox virus that is of global health concern because the outbreak, as of May 2023, has affected more than 80,000 people in hundreds of countries. Currently, there is no specific treatment, including antivirals, for mpox patients. Exploration of active compounds for mpox continues, one of which is curcumin and its derivatives. Curcumin is a polyphenol compound predominantly found in turmeric which has been documented to have antiviral effects. Therefore, this study aims to explore studies investigating the potential of curcumin and its derivatives as antiviral candidates in targeting mpox.

Method: Literature published from inception to 2024 in ScienceDirect, PubMed, Scopus, and Google Scholar was searched. Keywords used in this search included curcuma, curcumin, tetrahydrocurcumin, demethoxycurcumin, bisdemethoxycurcumin, turmeric, monkeypox, and mpox.

Results: The literature search results found five computational studies involving the compound curcumin and its derivatives, including tetrahydroxycurcumin and demethoxycurcumin. All studies showed that curcumin and its derivatives have better binding affinity with mpox proteins compared to control of several antivirals. Curcumin and its derivatives have strong potential in inhibiting mpox virus replication and modulating the immune system.

Conclusions: This review concludes that curcumin and its derivatives have potential as antiviral candidates for mpox. However, related studies remain limited and confined to computational studies. Further preclinical experimental and clinical studies are needed to confirm their effectiveness and mechanisms of action.

Keywords: Antiviral; Curcumin; Monkeypox; Mpox; Turmeric

Highlights

Curcumin and its derivatives have documented antiviral effects through mechanisms such as viral replication inhibition and immune system modulation, but their potential against monkeypox (mpox) remains largely unexplored.

This review highlights curcumin and its derivatives as promising antiviral candidates against mpox based on computational evidence, with superior binding affinities to the virus proteins compared to existing antiviral drugs.

The findings support the potential of curcumin and its derivatives as antiviral candidates for mpox, encouraging further preclinical and clinical studies to validate their efficacy and mechanisms for therapeutic development.

Introduction

Mpox, formerly called monkeypox, is a viral infection caused by a type of zoonotic virus from the genus *Orthopoxvirus* that has a similar clinical presentation to smallpox[1]. Mpox was first discovered in 1958 and the first human case of mpox infection was identified in the Democratic Republic of Congo in 1970. This infection is transmitted through transmission from infected animals or human to human through body fluids, lesions, droplets, and sexual contact[2-4]. The clinical presentation of mpox infection is systemic symptoms caused by the viremia phase that occurs before the skin rash, including fever, myalgia, sore throat, and lymphadenopathy. Following systemic symptoms, a skin rash appears on the face, oral mucosa and lips, perioral, genital, perianal, and anorectal[5-8]. As of May 2023, data from 111 countries reported 87,704 confirmed cases of mpox with 140 deaths globally[9]. As a result, the World Health Organization (WHO) declared the mpox outbreak a global health emergency of concern[10].

As of the writing of this study, there is no specific treatment for patients with mpox, including specific antivirals; however, supportive care can be given because the clinical progression of mpox is usually mild and self-limiting[11]. Because mpox and smallpox share similarities, antiviral drugs may be beneficial for monkeypox, such as tecovirimat, cidofovir, and brincidofovir[12,13]. However, studies on the effectiveness of these antivirals remain very limited. Tecovirimat is considered promising and safe, but controlled clinical trials are not yet available[14]. Meanwhile, cidofovir is reported to be highly nephrotoxic, and brincidofovir has no significant effectiveness in mpox patients[15]. Therefore, antivirus exploration continues to be conducted. One method that is currently being widely used to analyze drug candidates is computational techniques or *in silico* methods, which can overcome the problems of cost, time, and large resources[16].

Curcumin, one of the most important compounds derived from turmeric (*Curcuma longa*), is a traditional herbal medicine that is widely used and studied. To date, curcumin and its derivatives have been reported to be effective for various diseases[17-21]. Furthermore, studies reported the antiviral effects of curcumin and its derivatives which are promising for treating a number of infectious diseases caused by both RNA and DNA viruses[22-24]. Furthermore, curcumin is considered effective in inhibiting poxvirus infections[25].

Evidence suggests that curcumin, in general, is effective against viruses by mechanisms in which viral replication and/or cellular signaling pathways related to viral replication, such as phosphatidylinositol 3-kinase (PI3K)/AKT and nuclear factor-kappa B (NF-κB), are inhibited[26]. Therefore, they suggest that curcumin and its derivatives may have good efficacy as antiviral candidates for mpox infection.

Exploration of curcumin against mpox is still rare and limited to computational methods. In addition, to the best of our knowledge, as of this writing, no study has summarized and reviewed studies on the potential of curcumin and its derivatives on mpox. Therefore, we attempted to review and summarize the published studies. This study is expected to be used as a reference for future studies related to the development of curcumin and its derivatives as an antiviral for mpox.

Methods

This study is a literature review on the exploration of the potential of curcumin and its derivatives as an antiviral against mpox. A literature search for computational studies published in ScienceDirect, PubMed, Scopus, and Google Scholar was performed. In the literature search, we used the following keyword combinations: "curcuma", "curcumin", "tetrahydrocurcumin", "demethoxycurcumin", "bisdemethoxycurcumin", "turmeric", "monkeypox", and "mpox". All studies discussing the analysis of the effects of curcumin on mpox published from inception to 2024 were considered for inclusion.

We included all articles if they met the inclusion criteria such as computational studies discussing the potential of curcumin and its derivatives for mpox, written in English, peer-reviewed, and full-text. Review articles, editorials, commentaries, letters to editors, short communications, written in languages other than English, and unavailable full-text were exclusion criteria in the study selection. No restriction on the year of publication was applied. Data extraction was performed using a table containing references/authors, curcumin and/or its derivatives compounds, mpox targets, controls (if any), and results. Finally, all results from the included computational studies were reviewed qualitatively.

Results

We finally included five computational studies that analyzed the effects of curcumin and its derivatives on mpox. Compounds analyzed in eligible studies include curcumin[27-31], tetrahydroxycurcumin[28], and demethoxycurcumin[31]. Four studies compared the potency of curcumin and/or its derivatives with the antiviral controls acyclovir[27], tecovirimat[28,29], and cidofovir[30]. A summary of the included studies is presented in Table 1.

Table 1. Summary of effects of curcumin and its derivatives on mpox targets.

Reference	Compounds	Mpox Targets	Control	Results
Akash et al.[27]	Curcumin	4QWO	Acyclovir	Curcumin had a binding energy of -7.7 kcal/mol to -8.9 kcal/mol with 4QWO. Acyclovir demonstrated a binding energy of -6.4 kcal/mol.
Alagarsamy et al.[28]	Curcumin, tetrahydroxy-curcumin	VarTMPK	Tecovirimat	Tetrahydroxy-curcumin had the strongest binding energy, which is -9.7 kcal/mol, and curcumin showed a binding energy of -7.2 kcal/mol. Tecovirimat had a binding energy of -7.2 kcal/mol.
Banik et al.[29]	Curcumin	4QWO	Tecovirimat	Curcumin showed the lowest binding energy with 4QWO which was -37.43 kcal/mol. Tecovirimat had a binding energy of -20.41 kcal/mol.
Maurya et al.[30]	Curcumin	4E90, 4QWO, 8HG1, 8CEQ	Cidofovir	Curcumin showed a strong binding affinity with 8HG1, -8.5 kcal/mol, compared to cidofovir with a binding affinity of -7.2 kcal/mol.
Rout et al.[31]	Curcumin, demethoxy-curcumin	F13	Not available	Demethoxy-curcumin showed the strongest binding energy with F13, which was -64.86±1.30 kJ/mol, while curcumin was -48.53±1.80 kJ/mol.

Discussion

Computational methods were performed to determine the binding affinity between curcumin and its derivatives with mpox-related molecular targets, which in this study, included A42R profilin-like protein (4QWO), thymidylate kinase from Variola virus (VarTMPK), envelope protein (F13), DNA polymerase holoenzyme (8HG1), methyltransferase VP39 (8CEQ), and envelope protein E8 (4E9O). The smaller or

lower the binding energy/affinity, the higher the potential of the compound to be a drug candidate. There is agreement that a docking score of less than -6.0 kcal/mol is considered as standard drug[32].

Curcumin is the most abundant component found in turmeric. Curcumin docked with 4QWO showed stronger and better binding affinity (-7.7 to -8.9 kcal/mol) compared to acyclovir (-6.4 kcal/mol)[27]. A similar study exploring several potential compounds for mpox also reported that curcumin had the strongest binding affinity (-37.43 kcal/mol) with 4QWO compared to other compounds such as gedunin, piperine, and coumadin which had binding affinity values of -34.89, -34.58, and -34.14 kcal/mol, respectively. Surprisingly, tecovirimat, which is considered the most promising for use as an antiviral in mpox patients, had a binding energy of -20.41 kcal/mol[29]. In line with previous studies, the findings of this study reported that curcumin has a strong binding affinity when docked with, in order of the strongest, 8HG1, 8CEQ, 4QWO, and 4E9O with docking scores of -8.5, -8.4, -7.9, and -7.3 kcal/mol, respectively. Interestingly, cidofovir as a control showed a binding affinity of -7.2 kcal/mol when docked with 8HG1[30]. The findings concluded that curcumin showed strong binding affinity with the mpox virus protein compared to the antiviral control and other compounds.

There are two studies that tested not only curcumin, but also curcumin derivatives. Alagarsamy and colleagues docked various compounds with the VarTMPK protein and found that tetrahydroxycurcumin, a curcumin derivative, had the lowest binding energy (-9.7 kcal/mol). In this study, curcumin showed a binding energy of -7.2 kcal/mol. Interestingly, the control in this study, tecovirimat, had a binding energy of -7.2 kcal/mol[28]. Another study also reported that demethoxycurcumin, a curcumin derivative, docked with F13 and showed the strongest binding energy (-64.86±1.30 kJ/mol or -15.50±0.31 kcal/mol) followed by curcumin (-48.53±1.80 kJ/mol or -11.60±0.43 kcal/mol)[31]. It can be concluded that both curcumin and its derivatives showed strong binding energy when docked with mpox protein.

The mechanism of action by which antiviral effect of curcumin on mpox remains unclear. However, evidence proposed a mechanism of action of curcumin against mpox, where curcumin blocks the process of attachment and entry of the virus into the host cell. In addition, curcumin inhibits the transcription and translation processes required for viral genome replication.

Three included studies[27,29,30] using the target 4QWO, the A42R profilin-like protein of mpox that plays a critical role in virus replication and assembly, corroborate the statement where curcumin inhibiting the profilin-like protein resulted in inhibition of mpox virus replication. Additionally, one study[30] docked curcumin with DNA polymerase holoenzyme, an enzyme that plays a role in DNA synthesis during viral replication, and methyltransferase VP39, an enzyme that plays a role in the stabilization of viral mRNA and the efficiency of protein translation during mpox infection, resulting in strong binding affinity. The study, again, found that curcumin was more effective at inhibiting DNA polymerase holoenzyme than the antiviral cidofovir, which is currently considered effective against mpox[33]. Based on these findings, curcumin is believed to be effective in inhibiting mpox virus replication and the process of viral infection; as has been described for other viruses[27,34].

Furthermore, curcumin, in general, has the capacity to modulate host cell signaling pathways, including the PI3K/AKT pathway, NF-κB, Jun activation domain-binding protein 1 (Jab-1), and virus-related inflammatory processes[22]. This evidence is supported by one of the included computational study results, in which curcumin regulated the inflammatory pathway associated with mpox infection, including mitogen-activated protein kinase (MAPK) signaling, tumor necrosis factor (TNF), NF-κB, prostaglandin-endoperoxide synthase 2 (PTGS2), and Toll-like receptor 4 (TLR4), as indicated by strong binding affinity[30]. The MAPK signaling pathway is a promising therapeutic target in combating mpox because MAPK signaling plays a critical role in the response to mpox[35]. In addition, a study also stated that therapy targeting immune responses, such as NF-κB, cytokines, and chemokines, demonstrates inhibited mpox virus replication and systemic inflammation[36]. Overall, these proposed mechanisms suggest the potential of curcumin as an antiviral candidate for the treatment of mpox, not only by inhibiting viral function, but also by supporting modulation of the immune system.

Several computational studies have reported that curcumin and its derivatives, tetrahydroxycurcumin and demethoxycurcumin, have good potential as active compounds that can inhibit mpox virus. The limitations of this review are the lack of studies testing the potential of curcumin and its derivatives

against mpox virus and limited to computational studies. Additionally, the exact mechanism of curcumin and its derivatives in inhibiting the mpox virus remains unclear; therefore, preclinical *in vivo* and *in vitro* studies are needed to prove the effectiveness of curcumin and its derivatives against the mpox virus along with the underlying mechanisms. Furthermore, no clinical studies testing the efficacy of curcumin in humans with mpox infection have been conducted; thus, the findings of this literature review may serve as a consideration for further exploration and clinical testing as an antiviral against mpox.

Conclusion

This study underlines the antiviral effects of curcumin and its derivatives, tetrahydroxycurcumin and demethoxycurcumin, against mpox virus. However, no preclinical or clinical trials have been reported so far. Therefore, this study provides an overview and proposal of curcumin and its derivatives as candidates for active compounds that have the potential to be antiviral for mpox in further preclinical and clinical investigations. In addition, computational studies to explore other active compounds to determine potential candidates as antivirals for mpox may be needed.

References

1. Kaler J, Hussain A, Flores G, Kheiri S, Desrosiers D. Monkeypox: A Comprehensive Review of Transmission, Pathogenesis, and Manifestation. Cureus. 2022;14(7):e26531. Doi: 10.7759/cureus.26531

2. Anwar F, Haider F, Khan S, Ahmad I, Ahmed N, Imran M, et al. Clinical Manifestation, Transmission, Pathogenesis, and Diagnosis of Monkeypox Virus: A Comprehensive Review. Life. 2023;13(2):522. Doi: 10.3390/life13020522

3. Thakur M, Das P, Sobti RC, Kaur T. Human monkeypox: epidemiology, transmission, pathogenesis, immunology, diagnosis and therapeutics. Mol Cell Biochem. 2023;478(9):2097–110. Doi: 10.1007/s11010-022-04657-0

4. Zahmatyar M, Fazlollahi A, Motamedi A, Zolfi M, Seyedi F, Nejadghaderi SA, et al. Human monkeypox: history, presentations, transmission, epidemiology, diagnosis, treatment, and prevention. Front Med. 2023;10:1157670. Doi: 10.3389/fmed.2023.1157670

5. Bragazzi NL, Kong JD, Mahroum N, Tsigalou C, Khamisy-Farah R, Converti M, et al. Epidemiological trends and clinical features of the ongoing monkeypox epidemic: A preliminary pooled data analysis and literature review. Journal of Medical Virology. 2023;95(1):e27931. doi: 10.1002/jmv.27931

6. Mitjà O, Ogoina D, Titanji BK, Galvan C, Muyembe JJ, Marks M, et al. Monkeypox. The Lancet. 2023;401(10370):60–74. doi: 10.1016/S0140-6736(22)02075-X

7. Ridho FM, Iqbal M, Laksono EP, Leal RSS. Oral and Perioral Manifestations in Monkeypox Patients: A Systematic Review of Case Reports and Case Series. Jurnal Kedokteran Diponegoro (Diponegoro Medical Journal). 2024;13(5):240–53. Doi: 10.14710/dmj.v13i5.42539

8. Sari M, Hairunisa N. A Review of the Monkeypox Outbreak in Indonesia in 2022. Jurnal Kedokteran Diponegoro (Diponegoro Medical Journal). 2022;11(5):268–74. Doi: 10.14710/dmj.v11i5.35895

9. Khan G, Perveen N. The 2022 monkeypox outbreak 1 year on: The 5 Ws. Reviews in Medical Virology. 2024;34(1):e2489. Doi: 10.1002/rmv.2489

10. Shomuyiwa DO, Manirambona E. Monkeypox virus declared as a global health emergency: What next for Africa's preparedness? Travel Medicine and Infectious Disease. 2023;53:102577. Doi: 10.1016/j.tmaid.2023.102577

11. Rizk JG, Lippi G, Henry BM, Forthal DN, Rizk Y. Prevention and Treatment of Monkeypox. Drugs. 2022;82(9):957–63. Doi: 10.1007/s40265-022-01742-y

12. Cheema AY, Ogedegbe OJ, Munir M, Alugba G, Ojo TK. Monkeypox: A Review of Clinical Features, Diagnosis, and Treatment. Cureus. 2022;14(7):e26756. Doi: 10.7759/cureus.26756

13. Sudarmaji N, Kifli N, Hermansyah A, Yeoh SF, Goh BH, Ming LC. Prevention and Treatment of Monkeypox: A Systematic Review of Preclinical Studies. Viruses. 2022;14(11):2496. Doi: 10.3390/v14112496

14. Shamim MA, Padhi BK, Satapathy P, Veeramachaneni SD, Chatterjee C, Tripathy S, et al. The use of antivirals in the treatment of human monkeypox outbreaks: a systematic review. International Journal of Infectious Diseases. 2023;127:150–61. Doi: 10.1016/j.ijid.2022.11.040

15. Singhal T, Kabra SK, Lodha R. Monkeypox: A Review. Indian J Pediatr. 2022;89(10):955–60. Doi: 10.1007/s12098-022-04348-0

16. Shaker B, Ahmad S, Lee J, Jung C, Na D. In silico methods and tools for drug discovery. Computers in Biology and Medicine. 2021;137:104851. Doi: 10.1016/j.compbiomed.2021.104851

17. Patel SS, Acharya A, Ray RS, Agrawal R, Raghuwanshi R, Jain P. Cellular and molecular mechanisms of curcumin in prevention and treatment of disease. Critical Reviews in Food Science and Nutrition. 2020;60(6):887–939. Doi: 10.1080/10408398.2018.1552244

18. Peng Y, Ao M, Dong B, Jiang Y, Yu L, Chen Z, et al. Anti-Inflammatory Effects of Curcumin in the Inflammatory Diseases: Status, Limitations and Countermeasures. DDDT. 2021;15:4503–25. Doi: 10.2147/DDDT.S327378

19. Rahmani A, Alsahli M, Aly S, Khan M, Aldebasi Y. Role of Curcumin in Disease Prevention and Treatment. Adv Biomed Res. 2018;7(1):38. Doi: 10.4103/abr.abr_147_16

20. Ridho FM, Syachputra AJ, Fahrudin P, Nurhuda A, Nurliana N, Latuamury NS. In vitro and in vivo effects of curcumin on oral cancer: a systematic review. Current Biomedicine. 2024;2(2):101–15. Doi: 10.29244/currbiomed.2.2.101-115

21. Ridho FM, Syachputra AJ, Nur'aini AD, Ulfah K, Faqih M, Nurhuda A. Pre-clinical and clinical efficacy of curcumin as an anti-inflammatory agent for periodontitis. A systematic review. Revista Científica Odontológica. 2024;12(4):e222. Doi: 10.21142/2523-2754-1204-2024-222

22. Ardebili A, Pouriayevali MH, Aleshikh S, Zahani M, Ajorloo M, Izanloo A, et al. Antiviral Therapeutic Potential of Curcumin: An Update. Molecules. 2021;26(22):6994. Doi: 10.3390/molecules26226994

23. Jennings MR, Parks RJ. Curcumin as an Antiviral Agent. Viruses. 2020;12(11):1242. Doi: 10.3390/v12111242

24. Sharma A, Sharma T, Bhaskar R, Ola M, Sharma A, Sharma PC. Promising Potential of Curcumin and Related Compounds for AntiviralDrug Discovery. MC. 2024;20(6):597–612. Doi: 10.2174/0115734064277371240325105016

25. Srivastava BBL, Ripanda AS, Mwanga HM. Ethnomedicinal, Phytochemistry and Antiviral Potential of Turmeric (Curcuma longa). Compounds. 2022;2(3):200–21. Doi: 10.3390/compounds2030017

26. Mathew D, Hsu WL. Antiviral potential of curcumin. Journal of Functional Foods. 2018;40:692–9. Doi: 10.1016/j.jff.2017.12.017

27. Akash S, Hossain A, Hossain MdS, Rahman MdM, Ahmed MZ, Ali N, et al. Anti-viral drug discovery against monkeypox and smallpox infection by natural curcumin derivatives: A Computational drug design approach. Front Cell Infect Microbiol. 2023;13:1157627. Doi: 10.3389/fcimb.2023.1157627

28. Alagarsamy V, Shyam Sundar P, Raja Solomon V, Narendhar B, Sulthana MT, Rohitha K, et al. Pharmacophore modelling-based drug repurposing approaches for monkeypox therapeutics. Journal of Biomolecular Structure and Dynamics. 2023;41(20):10678–89. Doi: 10.1080/07391102.2023.2188428

29. Banik A, Ahmed SR, Shahid SB, Ahmed T, Tamanna HK, Marma H. Therapeutic Promises of Plant Metabolites against Monkeypox Virus: An In Silico Study. Lin SC, editor. Advances in Virology. 2023;2023:1–16. Doi: 10.1155/2023/9919776

30. Maurya VK, Kumar S, Maurya S, Ansari S, Paweska JT, Abdel-Moneim AS, et al. Structure-based drug designing for potential antiviral activity of selected natural product against Monkeypox (Mpox) virus and its host targets. VirusDis. 2024;35(4):589–608. Doi: 10.1007/s13337-024-00900-y

31. Rout M, Mishra S, Dey S, Singh MK, Dehury B, Pati S. Exploiting the potential of natural polyphenols as antivirals against monkeypox envelope protein F13 using machine learning and all-atoms MD simulations. Computers in Biology and Medicine. 2023;162:107116. Doi: 10.1016/j.compbiomed.2023.107116

32. Kumer A, Chakma U, Rana MM, Chandro A, Akash S, Elseehy MM, et al. Investigation of the New Inhibitors by Sulfadiazine and Modified Derivatives of α-D-glucopyranoside for White Spot Syndrome Virus Disease of Shrimp by In Silico: Quantum Calculations, Molecular Docking, ADMET and Molecular Dynamics Study. Molecules. 2022;27(12):3694. Doi: 10.3390/molecules27123694

33. Xu Y, Wu Y, Wu X, Zhang Y, Yang Y, Li D, et al. Structural basis of human mpox viral DNA replication inhibition by brincidofovir and cidofovir. International Journal of Biological Macromolecules. 2024;270:132231. Doi: 10.1016/j.ijbiomac.2024.132231

34. Hussain Y, Alam W, Ullah H, Dacrema M, Daglia M, Khan H, et al. Antimicrobial Potential of Curcumin: Therapeutic Potential and Challenges to Clinical Applications. Antibiotics. 2022;11(3):322. Doi: 10.3390/antibiotics11030322

35. Lu J, Xing H, Wang C, Tang M, Wu C, Ye F, et al. Mpox (formerly monkeypox): pathogenesis, prevention and treatment. Sig Transduct Target Ther. 2023;8(1):458. Doi: 10.1038/s41392-023-01675-2

36. Parnian R, Heydarifard F, Mousavi FS, Heydarifard Z, Zandi M. Innate Immune Response to MPOX Infection: Mechanisms and Immune Escape. J Innate Immun. 2024;16(1):413–24. Doi: 10.1159/000540815

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Artículos de revisión

Gradual dose reduction versus abrupt deprescription of antipsychotic in patients with dementia: A systematic review

Reducción gradual de dosis frente a deprescripción abrupta de antipsicóticos en pacientes con demencia: una revisión sistemática

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Conflict of interests

The authors of this document declare that they have no relevant conflicts of interest, which may have an effect on the study design, analysis, or presentation of results.
Gradual dose reduction versus abrupt deprescription of antipsychotic in patients with dementia: A systematic review

Resumen

Objetivo: Sintetizar la evidencia disponible sobre la reducción gradual de la dosis de antipsicóticos o su deprescripción abrupta en personas mayores con demencia.

Métodos: Revisión sistemática de estudios de intervención. Se consultaron las bases de datos *PubMed, Embase, Web of Science-Core Collection, Cochrane Library, Scopus, MEDLINE (Ovid)* y *PsycINFO*. Se incluyeron estudios (ensayos aleatorios o cuasiexperimentales) que evaluaban la efectividad de estrategias de deprescripción de antipsicóticos de reducción de dosis o abstinencia completa. Dos revisores independientes, triangulando por un tercer revisor realizaron el proceso de selección, extracción y análisis de datos, y evaluación del riesgo de sesgo.

Resultados: Se incluyeron 8 ensayos clínicos en la revisión sistemática, dos de los cuales fueron cuasi experimentales. Más del 60% de los participantes procedían de residencias de personas ancianas. Existe evidencia de varias estrategias para deprescribir antipsicóticos. Cinco estudios utilizaron un calendario de retiro abrupto y tres estudios utilizaron una reducción gradual de la dosis. La deprescripción mediante esquemas de retiro abrupto y gradual no mostró diferencias significativas en el manejo de los síntomas conductuales, y mostraron tasas significativamente más altas de recaída y/o eventos adversos.

Conclusión: La deprescripción de antipsicóticos es factible en personas con demencia, y se asocia a beneficios en términos de supervivencia y con posibles mejoras en el manejo y la recaída de los síntomas conductuales y psicológicos de la demencia. Parece razonable que la reducción gradual de la medicación antipsicótica se evalúe después de 12 semanas de tratamiento o cuando los síntomas conductuales estén bajo control.

Palabras clave: Demencia; Antipsicóticos; Prescripción inadecuada; Deprescribir; Síntomas conductuales

Abstract

Objective: To synthesize the available evidence on antipsychotic gradual dose reduction or abrupt deprescription in the older people population with dementia.

Methods: A systematic review of intervention studies. PubMed, Embase, Web of Science-Core Collection, Cochrane Library, Scopus, MEDLINE(Ovid), and *PsycINFO* databases were consulted. Articles were eligible for inclusion if they were intervention studies (randomized or quasi-experimental trials) evaluating the effectiveness of dose reduction or complete withdrawal antipsychotic deprescribing strategies in people with dementia. The screening process, data extraction, data analysis and bias risk assessment were performed by two independent reviewers and any discrepancies were triangulated with a third reviewer.

Results: Eight clinical trials were ultimately included in the systematic review, two of which were quasi experimental. Over 60% of participants came from nursing or care homes. There is evidence of several strategies for deprescribing antipsychotics. Five studies used an abrupt withdrawal schedule and three studies used a gradual dose reduction. Deprescription through abrupt and gradual withdrawal schedules showed no significant differences in the management of behavioral symptoms, although abrupt withdrawals showed significantly higher rates of relapse and/or adverse events.

Conclusion: Deprescribing antipsychotics is feasible in those people with dementia, and it is associated with benefits in terms of survival, and with potential improved outcomes in the management and relapse of behavioral and psychological symptoms of dementia. It seems reasonable that tapering off antipsychotic medication should be assessed after 12 weeks of treatment or when behavioural symptoms are under control.

Keywords: Dementia; Antipsychotic Agents; Inappropriate prescribing; Behavioral Symptoms.

Highlight

This systematic review shows that deprescription of antipsychotic medicines in patients with dementia is feasible.

The results of the review, together with the principle of prudence, show that gradual deprescription is a better alternative than abrupt deprescription.

Further research is needed to examine the effectiveness of other deprescribing strategies in terms of different variables.

Introduction

Around the world, populations are ageing, and this demographic transition will affect almost all aspects of society; today, there are more than 1 billion people aged 60 years or older. One condition, in particular, could well challenge World Health Organization (WHO) and the United Nations (UN) ambitions: dementia[1-3].

Dementia is a major cause of dependence and disability among older people worldwide. It is estimated that around 57 million (2019) people suffer from some type of dementia globally and is expected to increase to 153 million in 2050[1,4]. Alzheimer's disease (AD) is the most common form of dementia and may contribute to 60-70 % of cases[3,4] . The global cost of dementia is estimated to be US$ 818 billion, 16 % of which is associated with direct health care costs[3] . Dementia is currently the seventh leading cause of death and one of the major causes of disability and women are disproportionately affected by dementia, both directly and indirectly. Women experience higher disability-adjusted life years and mortality due to dementia, but they also provide 70% of care hours for people living with dementia[5].

Dementia is characterised by deterioration in memory and in at least one other higher cognitive function that is severe enough to cause significant limitations in social or occupational functioning and is not explained by delirium or another axis I disorder[6] .These limitations present themselves as cognitive and non-cognitive or behavioral and psychological symptoms of dementia (BPSD). BPSD are defined as disturbances in perception, thought content, mood or behaviour that frequently occur in patients with dementia[7], and account for greater functional disruptions along with higher family burden[8].

Given the high prevalence of neuropsychiatric symptoms (NPS), the commonly used treatment is based on psychotropic medications. Atypical antipsychotics have greater recommendations in clinical guidelines for the management of agitation, aggression, and psychosis[9] .While these have been proven effective[10,11], the use of antipsychotics is associated with increased risk of mortality, stroke and other adverse events such as falls, sedation and cognitive decline[12]. This increase in adverse events confirms that antipsychotics (in generic form) should not be used routinely to treat patients with dementia with aggression or psychosis, unless there is serious distress or risk of physical harm to those who live and work with them[13,14]. A meta-analysis published by Schneider et al. (2005) estimated a similarly increased risk in mortality (OR = 1.54, 95 % CI 1.06 to 2.23, p= 0.02) for atypical neuroleptics[15].

The available evidence, including clinical consensus, indicates that given the potential adverse effects, the first line of treatment for behavioral symptoms in dementia should be a non-pharmacological intervention. In addition, withdrawal of the medication must be contemplated. Prolonged use is indicated in patients with a history of severe episodes of psychosis or concomitant schizophrenia[15].

According to Reeve et al.[17], deprescribing can be defined as the process of ceasing inappropriate medication, supervised by a healthcare professional, with the aim of managing polypharmacy and improving clinical outcomes. Strategies described to promote deprescribing practices include comprehensive medication reviews, educational interventions and auditing of prescribing practices. These interventions have shown a number of benefits such as reducing polypharmacy, potential drug-to-drug interactions (pDDI) and the costs related to the use of medications. Thus, the objective of this article is to conduct a systematic review to synthesize the evidence about the withdrawal of antipsychotics in people with dementia that should be tapered or abrupt.

Methods

A systematic review was conducted following the *Preferred Reporting Items for Systematic Reviews and Meta-analysis* (PRISMA) guideline[18]. For this purpose, we designed a literature search strategy with both controlled terms (Medical Subject Heading) and free-text terms and adapted them to each of the databases used: *PubMed, Embase, Web of Science-Core Collection, Cochrane Library, Scopus, MEDLINE (Ovid)*, and *PsycINFO* (Appendix A).

Inclusion/exclusion criteria

We included intervention studies (randomized or quasi-experimental trials), published between year 2000 and 2023 in Spanish or English, evaluating the effectiveness of antipsychotic deprescribing strategies (dose reduction or complete withdrawal) in people with dementia. The outcome measures under consideration were mortality, relapses of behavioral symptoms, quality of life and cardiovascular events. We excluded articles that examined deprescription of both antipsychotics and benzodiazepines together.

Data extraction and synthesis of results After removing duplicates, we screened by title and abstract all references identified in the different databases consulted to verify they potentially met the inclusion criteria. The full text of the selected articles was then reviewed and evaluated (AOC and AOL or EMR) by two independent reviewers. Any disagreement between reviewers throughout the screening and selection process was resolved by consensus by another reviewer (AOL or EMR). The Rayyan QCRI™ program was used for this purpose.

A data extraction form was designed and piloted to gather information on the methodology of the study, characteristics of the target population, interventions developed, main outcomes, follow-up time and study design. This work was performed independently by the three authors to reduce the risk of potential biases or errors.

Risk of bias

For assessment of risk of bias in the clinical trials, we used the check list prepared by Higgins et al.[19] for the Cochrane Collaboration, covering six domains. A parallel independent assessment was carried out by the two authors and discrepancies were resolved by the third investigator. In the case of quasi-experimental studies, an ad hoc modification of the tool was made for the items linked to random generation and sequence generation, since these criteria are not applicable in the included studies. The evaluation has focused on assessing aspects related to selection bias and its minimization strategies through the selection and number of participants included, the control of confounding variables and the types of analysis performed.

The authors carried out a qualitative synthesis of results from the included studies and classified the articles according to type of intervention. Given the considerable heterogeneity observed among studies, in terms of type of intervention and outcome measures, a quantitative synthesis was not considered appropriate.

Results

The search returned a total of 2248 references, of which 1158 duplicate references were removed, leaving a total of 1090 references (Figure 1). After the first reading of titles and abstract, a total of 127 articles were selected for full-text review. Finally, eight articles were included in this revision. Four reviews were used only to identify relevant articles from their reference lists and for background information or discussion[20-23].

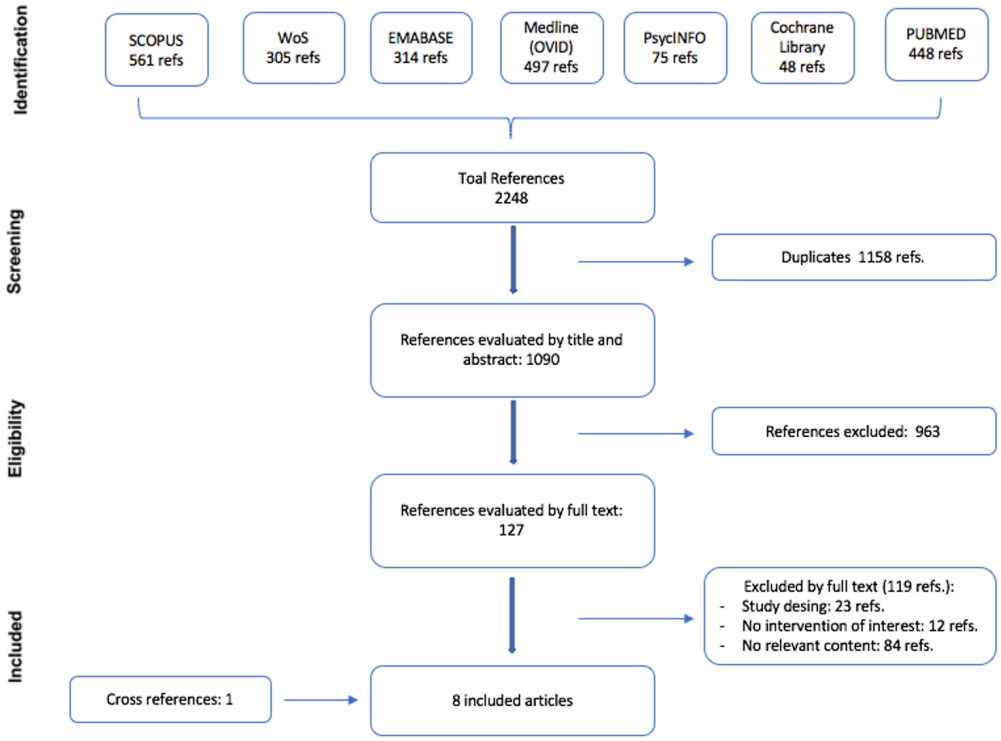

Figure 1: Study Flow diagram.

With regard to the characteristics of the included studies (a total of 789 patients included), it is worth noting that six of them were clinical trials[24-29] and two were a quasi-experimental design[30,31] . With regard to the study site, two were conducted in the United Kingdom, the others were conducted in the United States (n= 2), Norway (n= 1), Australia (n= 1), Spain (n= 1) and Canada (n= 1) (Table 1). 25% of the studies had sample sizes involving less than 40 participants, most of them (62.5%) living in nursing or residential care homes for the older people, 12.5% were users of geriatric day hospitals, and 25% had non-institutional nursing care. As for the intervention, three studies used an abrupt withdrawal schedule[25-27], four used a gradual dose reduction as stipulated in a protocol[24,28,29,31] and one compared abrupt withdrawal with gradual reduction[30] (Table 1).

Table 1. Characteristics and outcomes of studies included.

Author	Methods	Participants	Description of the Intervention and follow up	Participants description	Outcomes
Intervention: abrupt withdrawal schedule					
Ballard, 2004[25]	Double-blind randomized clinical trial	Senior residents with BPSD from United Kingdom residences.	Abrupt discontinuation of treatment (12-week follow-up). CG was treated with placebo and IG, received active treatment with antipsychotic.	100 patients (IG= 46 and CG= 54) were studied, who were randomized to 2 groups. The CG the mean age was 83.6 ± 9.3 years for the IG and 83.1 ± 7.1 years for the CG.	The IG had a mean score NPI = 16.0 and the CG NPI = 14.0. When the APS was interrupted abruptly, the IG (mean NPI score <14 (p= 0.46)) shows statistically non-significant changes in agitation (p= 0.89), mood (p= 0.85) and psychosis (p= 0.41). In patients with low doses of APS and NPI <14, the change in agitation reaches statistical significance (p= 0.018).
Ruths, 2008[26]	Randomized clinical trial with 4-week follow-up	Older people with dementia in residences (≥3 months) being treated with antipsychotic for BPSD.	Abrupt discontinuation of antipsychotics for 4 weeks. Following: (≥3 months). CG maintained with antipsychotic treatment for four-week intervention. IG was antipsychotic discontinuation.	55 patients (IG= 27 and CG= 28) were included, mean age 84.1 ± 7.1 years, and 43 women.	In IG, 22 participants were maintained without requiring antipsychotic treatment. The BPSD (evaluated with NPI) remained stable or even improved in 42 patients (IG = 18 and CG = 24; p=0.18). Patients with greater behavioral impairment required higher doses of antipsychotics at baseline compared to those with better NPI scores (p=0.42).
Ballard, 2009[27]	Randomized clinical trial	Institutionalized older people with a diagnosis of dementia and BPSD (≥3 months) in residences in the UK.	Abrupt discontinuation of antipsychotics.104 weeks follow-up. CG was maintained with placebo and IG treated with their antipsychotic treatment for 12 months.	165 patients were randomized (CG= 83 and IG= 82), Baseline SIB and NPI scores for IG were 73.8 ± 20.7 and 15.8 ± 11.3 and for CG 71.1 ± 22.7 and 17.4 ± 14.6 for CG. 128 (78 %) started the study, 64 of them in each group.	12-month mortality: The cumulative probability of survival during the 12 months was 89.7 % (95 % CI: 71.3 %-96.5 %) and 97.1% (95 %CI: 80.9-99.6 %), for CG and IG respectively. Among those who received at least one dose, survival (ITTm) was 70.3% (95 %CI 57.5-79.9 %) and 76.6% (95 %CI 64.2 % -85.2 %), while it was 74.7% (95 %CI 63.9–82.7 %) and 79.3 % (95 %CI 68.8–86.6 %) according to randomization (ITT). 54-month mortality: CG showed higher mortality than IG (p=0.03, HR 0.58 [95 %CI: 0.35-0.95]; ITT according to randomization p=0.02, HR 0.58 [95 %CI 0.36-0.92]), the difference in mortality was more pronounced after the first year.
Devanand, 2011[28]	Randomized clinical trial in two phases.	Alzheimer's outpatients with agitation, aggression, or psychosis	Two phases (with follow-up of 44 weeks): Phase A: after a washout period of one week washing of antipsychotics, a flexible dose of haloperidol was prescribed (according to response and EA). Phase B: those who responded favorably were randomized to carry out the intervention: discontinuation or continue with haloperidol. CG, there was a 2-week double-blind sequential placebo substitution tapering period: patients on 4 mg daily at end-Phase A switched to 2 mg daily for 1 week, 1 mg daily for the next week and then switched completely to placebo; patients on 2 or 3 mg daily switched to 1 mg daily for 2 weeks and then switched to placebo, and patients who received 0.5 mg or 1 mg were switched directly to placebo without a tapering period.	78 patients with a mean age of 75±8.0 years were studied, 57 % of which were women.	Phase A: target symptoms (agitation and psychosis) and BPRS score decreased (p <0.001), but extrapyramidal symptoms increased (p <0.01). At the end, 22 people responded to the treatment, of which 20 completed the follow-up Phase B: the proportion of patients was higher (40 % vs 80 %; p=0.07) and less time to relapse in the discontinuation group compared to the haloperidol group (p=0.04). Neither baseline nor residual severity of target symptoms predicted relapse in phase B.
Devanand, 2012[29]	Randomized clinical trial in two phases	Patients from veteran medical centers, memory clinics and geriatric clinics in the USA with Alzheimer's who present agitation, aggressiveness or psychosis	Two phases (48-week follow-up): Phase A (16 weeks): after one week of washout, a flexible dose of risperidone was prescribed (according to response and adverse events). Phase B (32 weeks): those who responded were randomized to: (Group 1) continue risperidone. (Group 2) risperidone 16 weeks and placebo 16 weeks) and (Group 3) placebo. Phase A: equal treatment. Phase B: CG received placebo for 32 weeks.	180 patients initiated the study with a mean age of 79.6 ± 7.6 and 59 % women. 80 % had psychosis and 81 % agitation-aggressiveness.	Phase A: The severity of psychosis and agitation symptoms was reduced (p <0.001), although extrapyramidal symptoms increased (p=0.009). General physical symptoms decreased, and physical self-maintenance worsened (p <0.001). The relapse or recurrence rate of BPSD was higher among those on placebo than with risperidone (60 % [24 of 40 patients in G3] v / s 33% [23 of 70 patients in G1 and G2], p=0.004 Phase B: of the 112 patients who responded, 110 were randomized. The group that received placebo (Group 3), compared to those that continued with risperidone (mean dose 0.97 ± 0.74 mg) (Group 1 and 2) showed a higher risk of relapse (HR 1.94; 95 % CI 1.09-3.45; p=0.02). During the next 16 weeks, the group that stopped risperidone and switched to placebo (Group 2) showed a higher risk of relapse HR 4.88; 95 % CI: 1.08-21.98 (p=0.02), than the group that continued with risperidone (Group 1). No significant differences were found in adverse events between the 3 groups.

Author	Methods	Participants	Description of the Intervention and follow up	Participants description	Outcomes
Intervention: gradual dose reduction					
Brodaty, 2018[31]	Longitudinal study Prospective cohort)	Older adults living in 23 nursing homes and receiving antipsychotic treatment (> 3 months)	Two components (follow-up of 52 weeks): 1) Training for health workers. 2) Deprescription protocol based on dose reduction to 50% every 2 weeks and complete withdrawal after 2 weeks in minimum dose.	A total of 139 residents met the inclusion criteria, with 93 residents completing the follow-up. The mean age of 84.3 ± 7.3 years, 65.6 % were women and 66.9 % completed the follow-up.	The most common cognitive symptom was agitation/ aggressiveness (89%); risperidone was the most widely used antipsychotic (n=62.4 %). Withdrawal from antipsychotics was achieved in 86.2 %, 79.1%, 81.7 % of patients at 3, 6 and 12 months, respectively. There was no significant change in the NPI-NH score.
Bravo, 2019[30]	Quasi-experimental trial without a control group	Older people with dementia and treated with ≥1 antipsychotics from a care center in Spain	Reduction and / or suspension of antipsychotic treatment, according to symptoms with a follow-up of 52 weeks. Dose reduction to 50 % every 2 weeks and completely withdrawn after two weeks in minimal dose.	The study began with 38 residents, of whom 3 withdrew due to deterioration. The mean age was 82.31 ± 5.81 and 60 % were women.	The patients had severe Barthel dependence = 33.29 ± 28.62 and a previous NPI-NH score of 12.91 ± 12.80. At 6 months of evaluation, there was no statistically significant difference in the NPI-NH score = 13.76 ± 16.68 (p=0.124). At the end of the follow-up period, 2 residents required a return to the prescription of antipsychotics.
Van Reekum, 2002[24]	Randomized double-blind clinical trial	Patients with dementia under treatment with antipsychotics (> 6 months) from 2 nursing homes and a geriatric ward in Canada.	Antipsychotic dose reduction of 50 % in the first week and half of the remaining dose in the second week (26-week follow-up). CG, received placebo during pretrial phase and dose reduction phase.	A total of 34 residents (IG= 16 and CG= 17) were studied, the mean age of the IG was 84.4 ± 4.6 and 82.9 ± 6.9 years for CG.	Daily doses (chlorpromazine equivalents) were 24.9 mg for IG and 34.3 mg for CG. 23.5 % of the IG subjects were withdrawn from the study early due to behavioral worsening, compared with 18.8 % of the CG (RR=1.33; 95 %CI 0.25-7.17). When evaluating the intervention, the CG showed a tendency to worsen behavioral problems (p=0.06), self-harm (p=0.08), especially in the persistence initiation subscale (p=0.05). In IG, the antipsychotic dose was higher among those who worsened than in those who were stable (p=0.06).

APS: antipsychotics IG: intervention group; CG: control group; SIB: Severe Impairment battery; BPSD: Behavioural and Psychological Symptoms of Dementia; NPI: Neuropsychiatric Inventory; NPI- NH: Neuropsychiatric Inventory - Nursing Home Version; RR: Relative Risk; 95% CI: 95% confidence Interval; ITT: Intention to treat: ITTm: Intention to treat-modified; HR: Hazard ratio; BPRS: Brief Psychiatric Rating Scale.

Risk of Bias

The results of the risk of bias assessment are shown in figures appendix A1, A2 and A3. The risk of bias in the included clinical randomized trials is low in general. It should be stressed that the blinding of participants and personnel was clearly described in all the trials. The domain regarding selective reporting scored to be at high risk of bias in one trial[24]. Regarding quasi-experimental studies, Bravo[30] scored at high risk of bias in three of the domains.

Abrupt withdrawal schedule

Regarding the effectiveness of the interventions (Table 1), only Ballard (2009)[27] assessed survival, using Cox regression. Findings revealed that patients who followed an abrupt withdrawal from antipsychotic medication showed a statistically significant lower risk of mortality at 24 months (HR=0.58; 95 % CI: 0.36-0.92) than patients who continued with treatment.

Two cross-over clinical trials examined relapse rates following abrupt withdrawal from haloperidol[28] and risperidone,[29] with follow-ups of 24 and 48 weeks respectively, including 44[28] and 110[29] patients, who remained stable (responders) the last month. Devanand[28] observed a higher rate of relapse of BPSD (p= 0.07) and a shorter time to a relapse (p= 0.04) in the group that received placebo compared to the group that continued to receive antipsychotic medication. Later, in 2012, Devanand[29] showed that after 16 weeks of follow-up, the relapse rate of BPSD was higher in patients who received placebo than in those who continued with risperidone (HR= 1.94, 95 % CI: 1.09-3.45). This result was maintained for 32 weeks (HR= 4.88, 95 % CI: 1.08-21.98).

Ballard[25] evaluated the abrupt discontinuation of antipsychotic medication, performing cluster analyses according to the Neuropsychiatric Inventory (NPI) score. He found that patients with NPI score <14 (mild) who were discontinued from antipsychotics showed no significant difference in the baseline NPI (p=0.46) (intragroup difference). In contrast, patients with NPI >14 (moderate to severe) showed a significant difference in the management and relapse of BPSD variables compared with the control group (p= 0.018) (intergroup difference). Ruths' review showed that, regarding the abrupt withdrawal of antipsychotics (risperidone, haloperidol, or olanzapine), there are no differences in relation to improvement or harm in BPSD (p= 0.18)[26]. It is important to underline that patients who received previously high doses showed greater levels of behavioral deterioration after withdrawal (p= 0.042).

Gradual dose reduction

Three studies evaluated the effectiveness of the gradual withdrawal of antipsychotics, by tapering to half of the daily dose in the first week and removing the dose over the following two weeks. Bravo (2019)[30] included 35 patients, and at 6 months achieved total withdrawal in 80% of patients and dose reduction in the rest of patients; whereas Brodaty[31] achieved withdrawal from antipsychotics in 79.1 % and 81.7 % (95 % CI: 72.4 %-89 %) of patients at 6 and 12 months, respectively. Both studies showed no statistically significant differences in BPSD (p> 0.005), compared to baseline values. It should be noted that Brodaty[31] found statistically lower rates of relapse of BPSD among patients who were withdrawn from antipsychotics compared to patients who continued with treatment (p= 0.005). Finally, the Van Reekum study[24] involving 34 patients, found that those who were withdrawn from medication had a non-significant increased likelihood of exacerbation of behavioral symptoms (RR 1.33; 95 % CI: 0.25-7.14). Bravo[30] used a strategy of reduction and/or discontinuation of antipsychotic medication, and at 6 months of follow-up no significant differences were found in the Neuropsychiatric Inventory-Nursing Home version (NPI-NH) score.

Discussion

This review shows that deprescribing antipsychotics is feasible among people with dementia, but further research is needed to reconfirm these findings[32,33] . The review also shows that deprescribing is associated with benefits in terms of survival, and with potential improved outcomes in the management and relapse of BPSD[18]. These results are similar to those described in the review by Van Leeuwen

(2018)[33] which worked on a similar objective to the present review, but presents different inclusion criteria.

When reviewing the selected articles and comparing the clinical results, no major difference is observed in the management of BPSD through abrupt or gradual withdrawal schemes. In contrast, abrupt withdrawals showed significantly higher rates of relapse and/or adverse events[28,29]. In this regard, a systematic review that included four clinical trials comparing abrupt versus gradual antipsychotic discontinuation in patients with schizophrenia[34] found no significant differences in clinical outcomes (extrapyramidal signs, adverse events, etc.). In summary, there are no differences between reducing or continuing antipsychotic doses in terms of quality of life and functionality. However, with dose reduction there was a higher risk for relapses and dropouts, and potentially for rehospitalisations[35].

Thus, it is concluded that it is preferable to withdraw gradually, because of the principle of prudence[36]. On the other hand, the systematic review conducted by Sheehan (2017)[36], including 21 studies, failed to draw firm conclusions on the best way to approach deprescription in patients with intellectual disability, since it was found that patients did not tolerate discontinuation and required that the antipsychotic medication be re-prescribed.[37]

These findings are consistent with the review conducted by Page (2016)[20], which included a total of 132 articles (34,143 participants) that evaluated deprescribing one or more medications in older people. In non-randomized studies, deprescribing was shown to significantly reduce mortality (OR 0.32; 95 % CI: 0.17-0.60). However, deprescribing did not significantly modify mortality in the randomized studies (OR 0.82; 95 % CI: 0.61-1.11).

Two factors found in the literature are associated with higher probabilities of successful deprescribing. One is a lower dose of antipsychotics[30,34] , since in patients requiring low doses (even without reaching therapeutic threshold), the need for the use of antipsychotic medication may not be justified. It is important to pay special attention to the narrow dose-time relationship; according to evidence some doses of antipsychotics greater than 62-74 mg chlorpromazine equivalents are associated with a lower probability of achieving sustained deprescription over time[21] .The other factor is related with the fact that patients with lower levels of functional impairment (NPI lower than 14) show better outcomes in the management of the BPSD (though with no statistical significance in relation to total NPI) and lower rates of relapse.[23] This could indicate that measurements of BPSD (using the NPI rating scale) could be interfered by other symptoms or contexts[38,39] and this issue has important clinical relevance. This result has also been observed in patients with a diagnosis of schizophrenia[35].

It should be noted that evidence and clinical practice guidelines recommend antipsychotic treatment with risperidone, which is the neuroleptic that offers the biggest benefits in the management of BPSD[40,41] . On the other hand, typical antipsychotics, olanzapine injection (atypical antipsychotic) or haloperidol (as a second line), are recommended in emergency situations where there is severe psychomotor agitation[39,41] . In addition, typical antipsychotics show a higher incidence of known adverse events than their more modern congeners (atypical)[40] .

The potential benefits of the use of antipsychotics may be diminished when the treatment is longer than required[16,23]. In this sense, there is a greater likelihood of adverse events linked to the use of antipsychotic medication (extrapyramidal signs, metabolic disorders, cardiovascular risk, death) after four months of treatment, and an increased risk of mortality after 12 months of treatment. Based on the above and taking into account the results yielded by this review, it seems reasonable that tapering off antipsychotic medication should be assessed after 12 weeks of treatment or once BPSD are under control[16,39]. This assessment should take into account the risk of occurrence of adverse events and the loss of effectiveness of treatment.[7] Follow-up of the patient is required after medication withdrawal. In this regard, it is important to take a holistic and integrated approach to assessment, considering not only patients' BPSD but also their family or supportive situation, etc.[39-41]. The evidence suggests that deprescription is necessary, and when it is guided by ad-hoc professionals, it shows better results[42]. Ballard (2002)[43] showed that liaison psychiatry turns out to be a powerful strategy when it comes to reducing the inappropriate use of antipsychotics in people with dementia.

This review has several limitations that should be considered when interpreting the results. First, the low number of subjects included in the different studies, followed by the fact that most studies involved institutionalized patients, 5 trials involving nursing homes out of 8 included. These aspects may limit the external validity of the results, since pharmacotherapeutic monitoring and control may differ at community levels. The heterogeneity in terms of proximity and accessibility to health services of some residences should be taken into account. Other factors and contexts that could lead to different results should also be take into consideration. These issues make the extrapolation of the results to the community setting somewhat limited.

Finally, the authors consider that an optimal deprescription of antipsychotics, in accordance with the de-implementation of low-value clinical activities[44-46] must include training that allows clinical teams to evaluate and compare performance between professionals and health centers, based on a body of evidence, under a multisectoral approach, and taking into account the principle of patient autonomy[47,48]. It is also worth noting that a systematic review found that physicians frequently overestimated benefit and underestimated harm when evaluating treatments[47]. Further research is needed in this area, with studies involving a larger number of participants, other contexts and different sociodemographic characteristics.

References

1. The Lancet Public Health. Will dementia hamper healthy ageing? *Lancet Public Health*. 2022;7(2):e93. doi: 10.1016/S2468-2667(22)00009-3.

2. Nichols E, Steinmetz JD, Vollset SE, et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Heal. 2022;7(2):e105–25. doi: 10.1016/S2468-2667(21)00249-8.

3. Cahill S. WHO's global action plan on the public health response to dementia: some challenges and opportunities. *Aging Ment Health*. 2020;24(2):197-199. doi: 10.1080/13607863.2018.1544213.

4. Wimo A, Jönsson L, Bond J, Prince M, Winblad B. Alzheimer Disease International. The worldwide economic impact of dementia 2010. *Alzheimers Dement*. 2013;9(1):1-11.e3. doi: 10.1016/j.jalz.2012.11.006.

5. World Health Organization [Web page]. Dementia. 2023. Consulting: june 15 of 2024. [https://www.who.int/news-room/fact-sheets/detail/dementia]

6. Prince MJ, de Rodríguez JL, Noriega L, López A, Acosta D, Albanese E, et al. 10/66 Dementia research group. The 10/66 Dementia Research Group's fully operationalised DSM-IV dementia computerized diagnostic algorithm, compared with the 10/66 dementia algorithm and a clinician diagnosis: a population validation study. *BMC Public Health*. 2008;8:219. doi: 10.1186/1471-2458-8-219.

7. Ma H, Lu X, Zhou A, et al. Clinical Practice Guidelines for the Management of Behavioral and Psychological Symptoms of Dementia: A Systematic Review with AGREE II. *Front Neurol*. 2022;13:799723. doi: 10.3389/fneur.2022.799723.

8. García-Alberca JM, Lara Muñoz JP, Berthier Torres M. Sintomatología neuropsiquiátrica y conductual en la enfermedad de Alzheimer. *Actas Esp Psiquiatr*. 2010;38(4):212-22. doi: 10.1016/s0210-4806(10)70045-0.

9. Kirkham J, Sherman C, Velkers C, et al. Antipsychotic Use in Dementia. *Can J Psychiatry*. 2017;62(3):170-181. doi: 10.1177/0706743716673321.

10. Tampi RR, Tampi DJ, Balachandran S. Antipsychotics, Antidepressants, Anticonvulsants, Melatonin, and Benzodiazepines for Behavioral and Psychological Symptoms of Dementia: a Systematic Review of Meta-analyses. *Curr Treat Options Psych*. 2017;4(1):55-79.

11. Ballard C, Lana MM, Theodoulou M, et al. A randomised, blinded, placebo-controlled trial in dementia patients continuing or stopping neuroleptics (the DART-AD trial). *PLoS medicine*. 2008;5(4):e76. doi: 10.1371/journal.pmed.0050076.

12. Rogowska M, Thornton M, Creese B, et al.. Implications of Adverse Outcomes Associated with Antipsychotics in Older Patients with Dementia: A 2011-2022 Update. *Drugs Aging.* 2023;40(1):21-32. doi: 10.1007/s40266-022-00992-5. Epub 2022 Dec 14. PMID: 36513918; PMCID: PMC9747539.

13. Ballard CG, Waite J, Birks J. Atypical antipsychotics for aggression and psychosis in Alzheimer's disease. *Cochrane Database Syst Rev.* 2006, Issue 1. Art. No.: CD003476. Doi: 10.1002/14651858.CD003476.pub2

14. Watt JA, Thompson W, Marple R, Brown D, Liu B. Managing neuropsychiatric symptoms in patients with dementia. *BMJ*. 2022;376:e069187. doi: 10.1136/bmj-2021-069187.

15. Schneider LS, Dagerman KS, Insel P. Risk of Death with Atypical Antipsychotic Drug Treatment for Dementia. *JAMA*. 2005;294(15):1934-43.

16. Zuidema SU, Johansson A, Selbaek G, et al. A consensus guideline for antipsychotic drug use for dementia in care homes. Bridging the gap between scientific evidence and clinical practice. *Int Psychogeriatr*. 2015;27(11):1849-59. doi: 10.1017/S1041610215000745.

17. Reeve E, Gnjidic D, Long J, Hilmer S. A systematic review of the emerging definition of "deprescribing" with network analysis: Implications for future research and clinical practice. *Br J Clin Pharmacol*. 2015;80(6):1254-68. doi: 10.1111/bcp.12732

18. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* 2021;372:n71. doi:10.1136/bmj.n71

19. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomized trials. *BMJ.* 2011;343:d5928. doi: 10.1136/bmj.d5928.

20. Page AT, Clifford RM, Potter K, Schwartz D, Etherton-Beer CD. The feasibility and effect of deprescribing in older adults on mortality and health: a systematic review and meta-analysis. *Br J Clin Pharmacol.* 2016;82(3):583-623. doi: 10.1111/bcp.12975.

21. Escudero TJ, Fernández VE, Peris MJ. Papel de los antipsicóticos en el tratamiento de los síntomas psicológicos y conductuales de los pacientes con demencia. Optimizando su prescripción. Valencia; 2021. [Consulted 13 April 2023]. Available from: https://www.fundacionfemeba.org.ar/blog/farmacologia-7/post/papel-de-los-antipsicoticos-en-el-tratamiento-de-los-sintomas-psicologicos-y-conductuales-de-los-pacientes-con-demencia-optimizando-su-prescripcion-49654

22. CADIME. Polimedicación y deprescripción: recomendaciones prácticas. *Bol Ter Andal*. 2021;36(1). [Consulted 19 February 2023] Available from: https://www.cadime.es/images/documentos_archivos_web/BTA/2021/CADIME_BTA_2021_36_01.pdf.

23. CADIME. Antipsicóticos para el tratamiento de síntomas del comportamiento en las demencias. *Bol Ter Andal.* 2018;33(3). [Consulted 17 January 2023] Available from: https://cadime.es/images/documentos_archivos_web/BTA/2018/CADIME_BTA_33_03.pdf

24. van Reekum R, Clarke D, Conn D, et al. A randomized, placebo-controlled trial of the discontinuation of long-term antipsychotics in dementia. *Int Psychogeriatr*. 2002;14(2):197-210. doi: 10.1017/s1041610202008396.

25. Ballard C, Thomas A, Fossey J, et al. A 3-month, randomized, placebo-controlled, neuroleptic discontinuation study in 100 people with dementia: the neuropsychiatric inventory median cutoff is a predictor of clinical outcome. *J Clin Psychiatry*. 2004;65(1):114-9. doi: 10.4088/jcp.v65n0120.

26. Ruths S, Straand J, Nygaard HA, Aarsland D. Stopping antipsychotic drug therapy in demented nursing home patients: a randomized, placebo-controlled study. The Bergen District Nursing Home Study (BEDNURS). *Int J Geriatr Psychiatry*. 2008;23(9):889-95. doi: 10.1002/gps.1998.

27. Ballard C, Hanney ML, Theodoulou M, et al. The dementia antipsychotic withdrawal trial (DART-AD): long-term follow-up of a randomised placebo-controlled trial. *Lancet Neurol*. 2009;8(2):151-7. doi: 10.1016/S1474-4422(08)70295-3.

28. Devanand DP, Pelton GH, Cunqueiro K, Sackeim HA, Marder K. A 6-month, randomized, double-blind, placebo-controlled pilot discontinuation trial following response to haloperidol treatment of psychosis and agitation in Alzheimer's disease. *Int J Geriatr Psychiatry*. 2011;26(9):937-43. doi: 10.1002/gps.2630.

29. Devanand DP, Pelton GH, Andrews HF, et al. Relapse Risk after Discontinuation of Risperidone in Alzheimer's Disease. *N Engl J Med*. 2012;367(16):1497-507. doi: 10.1056/NEJMoa1114058.

30. Bravo-José P, Einstein CA, Sáez-Lleó CI, Peris-Martí JF. Deprescrpción de antipsicóticos en pacientes institucionalizados con demencia. *Farm Hosp*. 2019;43(4):140–5.

31. Brodaty H, Aerts L, Harrison F, et al. Antipsychotic Deprescription for Older Adults in Long-term Care: The HALT Study. *J Am Med Dir Assoc*. 2018;19(7):592-600.e7. doi: 10.1016/j.jamda.2018.05.002.

32. Moth AE, Hølmkjær P, Holm A, Rozing MP, Overbeck G. What Makes Deprescription of Psychotropic Drugs in Nursing Home Residents with Dementia so Challenging? A Qualitative Systematic Review of Barriers and Facilitators. *Drugs Aging*. 2021;38(8):671-685. doi: 10.1007/s40266-021-00875-1.

33. van Leeuwen E, Petrovic M, van Driel ML, et al. Discontinuation of Long-Term Antipsychotic Drug Use for Behavioral and Psychological Symptoms in Older Adults Aged 65 Years and Older With Dementia. *J Am Med Dir Assoc*. 2018;19(11):1009-1014. doi: 10.1016/j.jamda.2018.06.023.

34. Azermai M, Petrovic M, Engelborghs S, et al. The effects of abrupt antipsychotic discontinuation in cognitively impaired older persons: A pilot study. *Aging Ment Health*. 2013;17(1):125-32. doi: 10.1080/13607863.2012.717255.

35. Rodolico A, Siafis S, Bighelli I, et al. Antipsychotic dose reduction compared to dose continuation for people with schizophrenia. *Cochrane Database Syst Rev.* 2022;11(11):CD014384. doi: 10.1002/14651858.CD014384.pub2.

36. Takeuchi H, Remington G. Immediate versus wait-and-gradual discontinuation in antipsychotic switching: A meta-analysis. *J Psychopharmacol*. 2020;34(8):914-919. doi: 10.1177/0269881120922964.

37. Sheehan R, Hassiotis A. Reduction or discontinuation of antipsychotics for challenging behaviour in adults with intellectual disability: a systematic review. *Lancet Psychiatry*. 2017;4(3):238-256. doi: 10.1016/S2215-0366(16)30191-2.

38. Gao RL, Lim KS, Luthra AS. Discontinuation of antipsychotics treatment for elderly patients within a specialized behavioural unit: a retrospective review. *Int J Clin Pharm*. 2021;43(1):212-219. doi: 10.1007/s11096-020-01135-9.

39. Begemann MJH, Thompson IA, Veling W, et al. To continue or not to continue? Antipsychotic medication maintenance versus dose-reduction/discontinuation in first episode psychosis: HAMLETT, a pragmatic multicenter single-blind randomized controlled trial. *Trials*. 2020;21(1):147. doi: 10.1186/s13063-019-3822-5.

40. Calsolaro V, Femminella GD, Rogani S, et al. Behavioral and Psychological Symptoms in Dementia (BPSD) and the Use of Antipsychotics. *Pharmaceuticals* (Basel). 2021;14(3):246. doi: 10.3390/ph14030246.

41. National Institute for Health and Care Excellence (NICE) 2018. Dementia: Assessment, management and support for people living with dementia and their carers. NICE Guidelines (NG 97).

42. Huang YY, Teng T, Shen XN, et al. Pharmacological treatments for psychotic symptoms in dementia: A systematic review with pairwise and network meta-analysis. *Ageing Res Rev*. 2022;75:101568. doi: 10.1016/j.arr.2022.101568.

43. Ballard, C., Powell, I., James, I., et al. Reichelt, K. Can psychiatric liaison reduce neuroleptic use and reduce health service utilization for dementia patients residing in care facilities. *Int. J. Geriat. Psychiatry,* 2022;17: 140-145. doi.org/10.1002/gps.543

44. Sanfélix-Gimeno G, Cervera-Casino P, Peiró S, López-Valcárcel BG, Blázquez A, Barbera T. Effectiveness of safety warnings in atypical antipsychotic drugs: an interrupted time-series analysis in Spain. *Drug Saf*. 2009;32(11):1075-87. doi: 10.2165/11316520-000000000-00000.

45. Kales HC, Maust DT. Good Intentions, But What About Unintended Consequences? *Drug Saf*. 2017;40(8):647-649. doi: 10.1007/s40264-017-0554-x.

46. Colla CH, Mainor AJ, Hargreaves C, Sequist T, Morden N. Interventions Aimed at Reducing Use of Low-Value Health Services: A Systematic Review. *Med Care Res Rev*. 2017;74(5):507-550. doi: 10.1177/1077558716656970.

47. Colquhoun HL, Squires JE, Kolehmainen N, Fraser C, Grimshaw JM. Methods for designing interventions to change healthcare professionals' behavior: a systematic review. *Implement Sci*. 2017;12(1):30. doi: 10.1186/s13012-017-0560-5.

48. Cohen-Mansfield J. The Rights of Persons with Dementia and Their Meanings. *J Am Med Dir Assoc*. 2021;22(7):1381-1385. doi: 10.1016/j.jamda.2021.03.007.

Annexes

Figure Annex-A1: Results of the risk of bias assessment

	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Clinical randomised trials							
Ballard 2004[25]	?	?	+	+	+	+	+
Ballard 2009[27]	+	+	+	+	+	+	+
Devanand 2011[28]	?	?	+	?	+	?	+
Devanand 2012[29]	+	+	+	+	+	?	+
Ruths 2008[26]	+	?	+	?	?	+	?
Van Reekum 2002[24]	+	?	+	?	+	-	+
	Selection bias	**Confounders**	**Blinding of participants and personnel**	**Blinding of outcome assessment**	**Incomplete outcome data**	**Selective reporting**	**Other bias**
Quasi Experimental Studies							
Bravo 2019[30]*	-	-	?	?	?	?	-
Brodaty 2018[31]†	?	+	**N. A**	?	+	+	?

*Pretest-posttest design; †Repeated-measures, longitudinal, single-arm study; ‡Control group design not equivalent.

Key			
+ Low risk of bias	- High risk of bias	N. A. Not applicable	? Unclear risk of bias

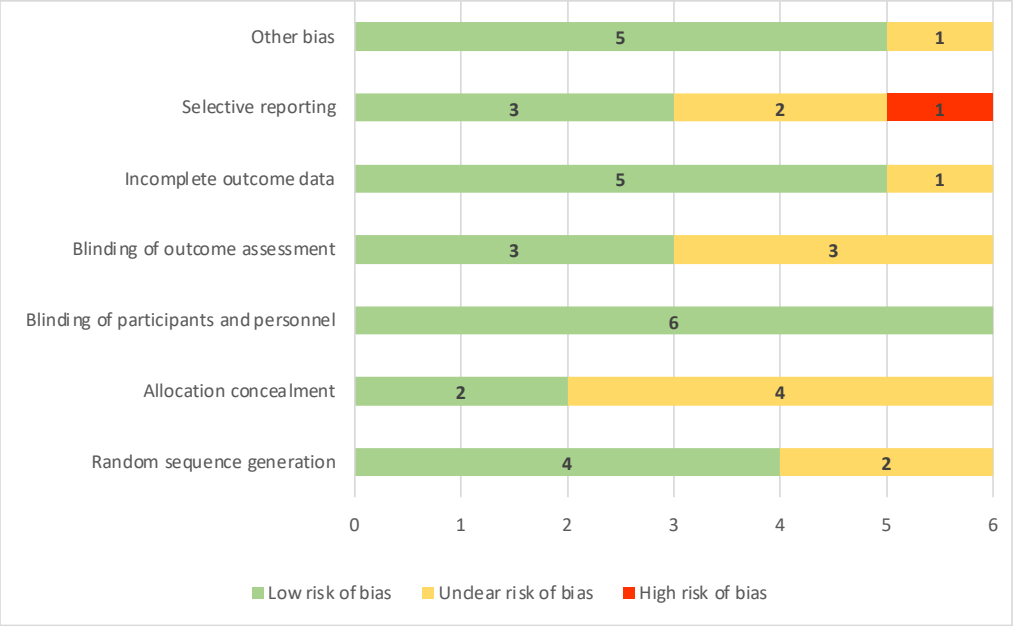

Figure Annex-A2: Result of the risk of bias assessment: clinical randomized trial

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Artículos de revisión

Aplicación de la tecnología de impresión en 3D para la formulación farmacéutica de flavonoides

Application of 3D printing technology for the pharmaceutical formulation of flavonoids

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Resumen

Introducción: La impresión en 3D es una tecnología vanguardista que puede emplearse para formular compuestos farmacéuticos de baja solubilidad, como lo son los flavonoides, que son compuestos de origen vegetal a los cuales se les ha atribuido diversos efectos farmacológicos. Esta revisión describe los estudios en los que se ha aplicado esta tecnología para formular flavonoides en distintas formas farmacéuticas.

Método: Se realizó una revisión bibliográfica buscando en la base de datos Google Scholar y el motor de búsqueda Pubmed hasta el mes de enero del año 2025 empleando las palabras clave "3D printing", "flavonoids" y "formulation". Se seleccionaron artículos originales publicados en idioma inglés.

Resultados: Se encontraron 5 artículos. En ellos, se formularon los flavonoides puerarin, catequina, apigenina y quercetina. La puerarin, un flavonoide con efectos cardiovasculares se formuló en tabletas flotantes gástricas que extendieron su tiempo de permanencia en el estómago y mejoraron su liberación en comparación con tabletas convencionales. Para la catequina y apigenina, (flavonoides con propiedades antioxidantes y anticancerígenas, respectivamente) se diseñaron películas orales mucoadhesivas que permiten una absorción local rápida para el tratamiento de úlceras orales y leucoplasia, respectivamente. La quercetina, con actividad antimicrobiana contra Mycobacterium tuberculosis, se formuló en parches dérmicos que lograron liberar el fármaco de forma sostenida durante 70 h en estudios *in vitro* y mantuvieron niveles plasmáticos estables por hasta 18 días en modelos animales.

Conclusión: La impresión en 3D se ha empleado para formular exitosamente flavonoides en diferentes formas farmacéuticas, lo cual ha mejorado notablemente sus características biofarmacéuticas. Sin embargo, se trata de investigación básica, por lo que es importante llevar a cabo ensayos preclínicos de manera formal para que estos productos beneficien a pacientes reales, incluyendo pacientes veterinarios.

Palabras clave: Flavonoides; Impresión tridimensional; Manufactura de Fármacos.

Abstract

Introduction: 3D printing is an advanced technology that can be used to formulate pharmaceutical compounds with low solubility, such as flavonoids—plant-derived compounds to which various pharmacological effects have been attributed. This review describes studies in which this technology has been applied to formulate flavonoids into different pharmaceutical forms.

Methodology: A bibliographic review was conducted by searching databases such as Google Scholar and PubMed search engine up to January 2025, using the keywords "3D printing," "flavonoids," and "formulation." Original articles published in English were selected.

Results: Five articles were found. In these studies, the flavonoids puerarin, catechin, apigenin, and quercetin were formulated. Puerarin, a flavonoid with cardiovascular effects, was used in gastric floating tablets that extended its stomach retention time and improved its release compared to conventional tablets. For catechin and apigenin (flavonoids with antioxidant and anticancer properties, respectively), oral mucoadhesive films were designed, allowing rapid local absorption for the treatment of oral ulcers and leukoplakia, respectively. Quercetin, known for its antimicrobial activity against *Mycobacterium tuberculosis*, was formulated into dermal patches that achieved sustained drug release for 70 hours in *in vitro* studies and maintained stable plasma levels for up to 18 days in animal models.

Conclusion: 3D printing has been successfully used to formulate flavonoids into different pharmaceutical forms, significantly improving their biopharmaceutical properties. However, this is basic research, therefore, it is important to conduct formal preclinical trials to benefit real patients, including veterinary patients.

Keywords: Flavonoids; Three-Dimensional Printing; Drug Compounding.

Puntos clave

Los flavonoides son compuestos fitoquímicos de amplio interés farmacéutico, pero, debido a sus características fisicoquímicas, pueden ser difíciles de formular, por lo que el empleo de la tecnología de impresión en 3D puede ser útil.

Esta revisión recopila estudios recientes sobre el uso de dicha tecnología para el desarrollo de formas farmacéuticas que contienen flavonoides y describe los métodos empleados y la evaluación de los productos elaborados.

La tecnología de impresión en 3D es una herramienta versátil capaz de generar diferentes formas farmacéuticas que mejoran la solubilidad y biodisponibilidad de los flavonoides, además de ofrecer gran margen de personalización. Sin embargo, se cuenta con pocos estudios y, sólo se han evaluado unos cuantos flavonoides.

Introducción

Los flavonoides son el grupo de metabolitos secundarios más diversos producidos por plantas[1]; en el año 1930 el, científico húngaro Albert Szent-Gyorgyino descubrió en la naranja el primer flavonoide, la rutina (inicialmente nombrado como "vitamina P")[2], y desde entonces, se tienen identificados más de 10,000 flavonoides diferentes[3]. Estructuralmente, los flavonoides son polifenoles que cuentan con tres anillos (C6-C3-C6; A, B y C), incluyendo un anillo heterociclo central. De acuerdo con el grado de oxidación del heterociclo central, se clasifican en diferentes grupos: flavonoles, flavonas, isoflavonoides, flavanonas, antocianinas flavanoles y chalconas[4].

Los flavonoides se encuentran ampliamente distribuidos en el reino vegetal y son abundantes en flores, hojas, frutos y semillas. Tienen diferentes funciones, incluyendo la de dar color a los vegetales para atraer a polinizadores y proteger a la planta de la radiación ultravioleta, atrapar radicales libres para proteger al vegetal del estrés oxidativo, y proteger a la planta de herbívoros, hongos y bacterias, entre otras[4]. Es posible encontrarlos en una gran variedad de plantas y productos vegetales comestibles comunes[5] así como en diversas plantas medicinales, siendo los compuestos responsables de sus actividades terapéuticas[6]. Su diversidad estructural es proporcional a la diversidad de actividades biológicas que poseen, pues existe evidencia científica de muchos efectos terapéuticos que ejercen los flavonoides, incluyendo actividad antioxidante, anticancerígena, antiinflamatoria, antibacteriana, cardioprotectora, e inmunomoduladora[7], entre muchas otras, por lo cual son compuestos prometedores para ser formulados en productos medicinales.

Sin embargo, una de las grandes limitantes es que los flavonoides son compuestos poco solubles en agua, y por lo tanto su absorción y biodisponibilidad es baja cuando se administran por vía entérica[8]. Esto es una gran desventaja ya que las tabletas son la forma farmacéutica más utilizada debido a su gran practicidad, estabilidad y aceptación por parte de los pacientes[9], razón por la cual ya se han hecho esfuerzos en investigación en química y tecnología farmacéutica para aumentar la biodisponibilidad de estos compuestos cuando se administran por vía oral[8] o en otras formas farmacéuticas como aquellas que involucran una administración por vía tópica[10].

Uno de los avances tecnológicos más vanguardistas es el de la de impresión en 3D, la cual consiste en un proceso de adición sucesiva de un material, capa por capa, para generar un objeto tridimensional con formas complejas de manera precisa a partir de un diseño generado por computadora. Esta tecnología tiene gran potencial en diferentes áreas e industrias, y es de gran interés en el campo de la medicina, pues ya incluso se ha empleado para la construcción personalizada de implantes y tejidos con fármacos incorporados para su liberación local[11]. Además, una de las aplicaciones médicas más estudiadas de esta tecnología es la impresión en 3D de formas farmacéuticas, siendo muy útil para elaborar tabletas con excelentes propiedades mecánicas y fisicoquímicas, con perfiles de liberación personalizables[12] y con características innovadoras, como las tabletas flotantes gástricas que son útiles para mejorar la biodisponibilidad de fármacos poco solubles[13]. Asimismo, también es posible elaborar otras formas farmacéuticas como películas adhesivas bucales de liberación controlada[14].

Esta tecnología es joven y aún hace falta mucha investigación para estandarizar procesos y aplicarlos a nivel industrial, sin embargo, ya se están obteniendo frutos; en 2015 la FDA aprobó el primer medicamento impreso en 3D, el Spritam®[15].

La presente revisión narrativa tiene como objetivo recopilar, organizar y analizar los avances científicos sobre la aplicación de la tecnología de impresión en 3D para la formulación farmacéutica de flavonoides.

Métodos

Se realizó una revisión bibliográfica narrativa basada en lo encontrado en la literatura científica. Para ello, se seleccionaron artículos científicos en idioma inglés buscando en bases de datos como Google Scholar y el motor de búsqueda Pubmed hasta el mes de enero del año 2025 empleando las palabras clave "3D printing", "flavonoids" y "formulation". Se incluyeron todos los estudios originales en idioma inglés que estuvieran enfocados en el uso de la tecnología de impresión en 3D aplicada a la formulación de flavonoides puros para la obtención de cualquier forma farmacéutica. Se excluyeron aquellos artículos que emplearon esta tecnología para la formulación de extractos complejos en los que no sólo había flavonoides, así como los que se enfocaron en otros usos médicos como la ingeniería de tejidos o aplicaciones diferentes como agropecuarios, alimenticios o procedimientos analíticos y de síntesis química.

Se encontraron 6 artículos, los cuales se clasificaron de acuerdo con la forma farmacéutica que estudiaron: tabletas (2 artículos), películas mucoadhesivas orales (2 artículos), parches (1 artículo) y cápsulas (1 artículo). Asimismo, se emplearon algunos artículos sobre los fundamentos de las técnicas generales de impresión en 3D y los excipientes empleados para complementar la discusión y el análisis de los estudios.

Resultados y discusión

Fundamentos y aplicaciones de la tecnología de impresión en 3D para la elaboración general de formas farmacéuticas

La tecnología de impresión en 3D para la formulación farmacéutica ofrece muchas ventajas con respecto a las manufacturas convencionales, ya que permite la personalización total del producto elaborado. Técnicamente, consiste en proceso aditivo en el que un material previamente elaborado que contiene el fármaco se deposita en capas secuenciales para dar lugar a una forma tridimensional diseñada por computadora. Gracias a ello, se pueden obtener diferentes perfiles de liberación al elaborar un producto con materiales de diferentes tamaños de poro o con capas de distinto grosor o solubilidad. Por ello, esta tecnología es ideal para obtener productos farmacéuticos especiales para poblaciones o personas específicas. La gran desventaja es que el proceso es lento, por lo que no es viable para llevarlo a cabo a gran escala. Además, la elaboración del material a imprimir es compleja y costosa, y requiere de personal altamente capacitado, por lo que no es una tecnología costo-efectiva para la industria farmacéutica[15].

Existen diferentes técnicas para llevar a cabo el proceso de impresión en 3D. Una de las más importantes para la obtención de formas farmacéuticas es el método basado en extrusión, el cual se puede llevar a cabo a temperatura ambiente o en caliente. Este método requiere de la elaboración previa de una pasta semisólida del fármaco y los excipientes para depositar capa por capa a temperatura ambiente[16]. La viscosidad adecuada de la pasta para una impresión óptima se obtiene empleando excipientes como microcelulosa (MC), hidroxipropilcelulosa (HPC) o hidroxipropilmetilcelulosa (HPMC), entre otros, con los cuales se generan estructuras porosas internas complejas que consiguen perfiles de liberación controlada[17].

La impresión en 3D basada en extrusión permite la elaboración de tabletas flotantes gástricas de liberación inmediata o controlada. Estas tabletas, como el nombre lo indica, son capaces de mantenerse flotando en los fluidos del tracto gastrointestinal, reteniendo la tableta por más tiempo. Con ello, aumenta la biodisponibilidad de fármacos de baja solubilidad y, si son de liberación controlada, pueden mejorar la adherencia terapéutica del paciente, ya que requerirán de menos administraciones al día[17].

Además, la impresión en 3D basada en extrusión también permite la elaboración de otras formas farmacéuticas y productos medicinales tales como implantes, parches transdérmicos[18] o incluso películas mucoadhesivas orales con excelentes características fisicoquímicas para la absorción local de fármacos[19].

Asimismo, la impresión en 3D por extrusión también puede llevarse a cabo en caliente para obtener diferentes formas farmacéuticas o productos medicinales, lo cual presenta las ventajas de requerir menos pasos y no requiere de secado del producto fabricado, pero presenta la desventaja de que no es posible emplear este método con compuestos termolábiles[20]. Uno de estos métodos es el modelado de deposición fundida (FDM), el cual es el método de impresión en 3D más empleado. Este método utiliza filamentos termoplásticos cargados de fármaco como material de partida, los cuales son extruidos por encima de su punto de fusión y son depositados secuencialmente en una placa, creando el objeto tridimensional como en cualquier método de impresión en 3D. Los filamentos empleados como material de partida en el FDM pueden elaborarse mediante un proceso conocido como extrusión de fusión en caliente (HME), el cual es un proceso continuo en el que se aplica calor y presión a un material o mezcla de materiales para fundirlos o ablandarlos, los cuales se extruyen a través de un orificio para producir nuevos productos de forma, contenido y densidad uniformes y homogéneas, como lo son los filamentos cargados de fármacos[21]. Cabe dejar en claro que a pesar de que la HME es útil para elaborar formas farmacéuticas[20], y que incluso ya se han obtenido formulaciones farmacéuticas de flavonoides con el objetivo de aumentar su biodisponibilidad[22,23], la HME por sí sola no es una técnica de impresión en 3D, sino un procedimiento diferente, pero que se ha empleado con frecuencia para la obtención del material de partida que se emplea en la impresión por FDM en 3D[20,24].

Actualidad de la tecnología de impresión en 3D para la formulación farmacéutica de flavonoides

En nuestro conocimiento, esta es la primera revisión que aborda el tema de la aplicación de la tecnología de impresión en 3D para la formulación farmacéutica de flavonoides. Asimismo, en nuestro conocimiento, sólo se cuenta con 6 estudios originales al respecto. Los flavonoides que han sido estudiados con estos enfoques son sólo 4; puerarin, catequina, apigenina y quercetina. El resumen de los estudios sobre la aplicación de la tecnología de impresión en 3D para la formulación medicinal de flavonoides se encuentran en la Tabla 1.

Tabla 1. Características de los estudios incluidos

Flavonoide	Forma farmacéutica elaborada	Técnica de impresión en 3D empleada	Propósito del producto elaborado	Referencia
Puerarin	Tabletas flotantes gástricas Tabletas con excipiente acarreador	HME	Sin especificar	(16) (25)
Catequina	Películas mucoadhesivas orales	HME	Tratamiento de úlceras orales	(26)
Apigenina	Películas mucoadhesivas orales Cápsulas	HME FDM	Tratamiento de leucoplasia Múltiples	(27) (28)
Quercetina	Parches dérmicos	HME acoplado a FDM	Mitigar la tuberculosis pulmonar destructiva	(29)

Tabletas flotantes gástricas de puerarin

Las enfermedades cardiovasculares son una de las principales causas de incapacitación y muerte alrededor del mundo. A pesar de que existe farmacoterapia efectiva para tratar estas enfermedades, aún existen lagunas importantes en estos tratamientos[30], por lo cual es necesario seguir buscando y desarrollando opciones farmacoterapéuticas.

El compuesto puerarin es un isoflavonoide *C*-glicosilado que se encuentra como componente principal de las raíces de *Pueraria lobata*; una planta medicinal muy utilizada en China para diferentes pade-

cimientos, sus principales actividades terapéuticas están relacionadas a efectos a nivel cardiovascular[31]. Este flavonoide está presente en las tabletas de Yufeng Ningxin, que es un preparado herbolario patentado y utilizado en China para aliviar la presión arterial y mejorar el flujo sanguíneo[32]. Sin embargo, estos preparados presentan el inconveniente de tener baja biodisponibilidad[16].

Como se mencionó anteriormente, las tabletas flotantes gástricas pueden mejorar la biodisponibilidad de fármacos poco solubles. Sin embargo, estas formas farmacéuticas presentan algunos inconvenientes, incluyendo un retraso no intencional en la liberación del fármaco, altas proporciones de excipientes en la tableta, excipientes no biocompatibles y procedimientos complicados. La tecnología de impresión en 3D puede superar estos obstáculos[13]. Este enfoque ya se ha empleado para formular puerarin.

En un estudio, se empleó la impresión en 3D basada en extrusión para preparar tabletas flotantes gástricas de puerarin de liberación sostenida con patrón interno anular concéntrico. Para ello, se empleó una solución de etanol al 75 % como aglutinante para formar la pasta de puerarin al 24 %, HPC (11 %), celulosa microcristalina (16 %), HPMC (11 %) y lactosa como diluyente. Esta pasta se empleó para imprimir las tabletas. Las tabletas obtenidas presentaron buenas propiedades físicas *in vitro*, con una flotabilidad de 10-12 h y un buen perfil de disolución. Posteriormente, se seleccionaron dos voluntarios sanos de edad media de 24 años y se les administraron tabletas radiomarcadas con tecnecio para monitorear su flotabilidad *in vivo*. Las tabletas impresas en 3D se mantuvieron flotando en el estómago por 6 h mientras que tabletas de puerarin con los mismos excipientes, pero elaboradas con un método de tableteo por compresión convencional se mantuvieron en el estómago sólo por 2 h. Si bien no se evaluó la biodisponibilidad, se sabe que las tabletas flotantes prolongan la retención de la forma farmacéutica en el tracto gastrointestinal y mejoran este parámetro farmacocinético[16].

Otra estrategia para aumentar la solubilidad de fármacos poco solubles formulados en tabletas es mediante el uso de vehículos, los cuales son compuestos solubles en agua que mejoran la solubilidad y difusión en el medio del fármaco. Para ello, el fármaco de interés se dispersa en el excipiente mediante diferentes métodos; uno de los más estudiados y efectivos consiste en fusionar el fármaco con polietilenglicol (PEG)[33,34].

Esta estrategia se puede llevar a cabo mediante impresión en 3D por HME y ya se ha empleado para formular tabletas de puerarin de liberación inmediata de puerarin utilizando polietilenglicol (PEG 4000) como único excipiente, el cual fungió como acarreador. Para elaborar la pasta, se mezclaron 50 mg de puerarin con una cantidad variable de PEG 4000, se mezcló manualmente con mortero y pistilo por 10 min y posteriormente se calentó a 70 °C en una parrilla con agitación magnética hasta obtener una pasta semisólida homogénea, la cual se empleó para imprimir las tabletas. Las tabletas obtenidas tenían una estructura interna de anillo concéntrico y de resorte serpentino análogo. Apoyándose de calorimetría diferencial de barrido y test de disolución, se encontró que la puerarin se dispersó efectivamente en todo el PEG 4000 y que la proporción adecuada de puerarin : PEG 4000 fue de 1:5. Asimismo, se observó que las tasas de liberación del fármaco aumentaron conforme se aumentó el ancho de relleno y el ancho del contorno de la tableta, probablemente debido a que al aumentar el área superficial de la tableta aumenta también la permeación del medio, la disolución de la matriz y la difusión del fármaco, logrando una liberación total del fármaco de 7,5 min como mínimo. Este estudio demuestra que la impresión en 3D por HME empleando PEG 4000 como excipiente acarreador es un buen procedimiento para aumentar la solubilidad de peurarina. Sin embargo, en el estudio no se llevaron a cabo ensayos biológicos, por lo que aún hace falta más investigación al respecto[25].

Películas mucoadhesivas orales de catequina y apigenina

Como se mencionó anteriormente, la tecnología de impresión en 3D se puede emplear para elaborar películas mucoadhesivas orales para la absorción local de fármacos, lo cual da a lugar a un inicio terapéutico rápido a partir de dosis más pequeñas comparado con las formulaciones entéricas ya que evitan la degradación gástrica y el metabolismo del primer paso[19]. Este enfoque se ha aplicado para formular los flavonoides catequina y apigenina.

La catequina es un flavan-3-ol presente en hojas de té, vino tinto, habas, uvas negras, albaricoques, fresas y chocolate, entre otras fuentes. A este flavonoide se le han atribuido varias actividades biológicas, como antidiabética, antihiperlipidémica, antihipertensiva, anticoagulante y antiulcerosa, entre otras[35].

En un estudio, se realizaron formulaciones de hidrogel cargadas con catequina a base de HPMC para elaborar películas orales mucoadhesivas obtenidas con tecnología de impresión en 3D basada en extrusión. Para ello, 50 mg de catequina se disolvieron en 3 ml de una mezcla de etanol/agua (2/1 v/v), se calentó a 80 ºC y se adicionaron 0,04 ml de Tween 80 y glicerol y diferentes concentraciones de HPMC (100 – 500 mg). Las formulaciones con 100 mg de HPMC eran poco viscosas y se fugaron por la boquilla de la impresora, mientras que las de 500 mg eran tan viscosas que eran difíciles de extruir, por lo que sólo se estudiaron las preparaciones de HPMC de 200 – 400 mg. Posteriormente, se observó *in vitro* que a mayor concentración de HPMC (400 mg) se obtenía un mayor retraso en la disolución del fármaco, dando lugar a una cinética de liberación controlada. Asimismo, se evaluaron dos métodos de secado para elaborar las películas; secado con aire y liofilizado. Las películas preparadas por secado al aire no presentaron microestructuras porosas mientras que las liofilizadas sí; a mayor HPMC menor tamaño de poro y menor tiempo de disolución. Además, las películas elaboradas por liofilizado son más fáciles de manejar y presentan mejores características visuales, por lo cual son ventajosas. El objetivo de estas formulaciones de catequina era tratar úlceras orales, e incluso se lograron obtener películas de diferentes formas y tamaños y un gran potencial de personalización terapéutica. Sin embargo, sólo se evaluaron en estudios *in vitro*[26], por lo cual, aunque muy prometedoras, aún hace falta evaluar la funcionalidad de estas formulaciones en sistemas biológicos.

Por otro lado, la apigenina es una flavona muy abundante presente en plantas de las familias *Asteraceae*, *Artemisia*, *Achillea*, *Matricaria* y *Tanacetum*, entre otras. Este flavonoide tiene diferentes actividades terapéuticas, tales como antidiabética, antidepresiva antioxidante, antimicrobiana y anticancerígena. Además, se ha demostrado que tiene potencial quimiopreventivo en carcinogénesis bucal[36], la cual es una de sus propiedades terapéuticas de mayor interés actualmente[37].

En un estudio, se empleó la tecnología de impresión en 3D basada en extrusión para elaborar películas orales mucoadhesivas de apigenina con el objetivo de tratar leucoplasia y prevenir la carcinogénesis oral. Para ello, se elaboraron diferentes formulaciones, encontrando que la más adecuada contenía 2,5 mg de apigenina, 4,7 ml de etanol, 4,7 ml de agua 200 mg de carbopol, 200 mg de poloxamer y 200 mg de HPMC. Esta película se aplicó en lenguas de ratas que habían sido expuestas a 4-nitroquinolona 1-óxido para inducir carcinogénesis. La aplicación de la película se realizó dos veces a la semana durante 22 días. La tasa de supervivencia de los animales que recibieron este tratamiento fue del 100 %, mientras que la del grupo control fue de 83,3 %. Posteriormente, mediante una técnica inmunohistoquímica, se observó que los marcadores tumorales Ki-67(MIB-1) y 8-hidroxi-2-desoxiguanosina (8-OHdG) se redujeron significativamente comparados con los del grupo control, pero no los del marcador inflamatorio factor nuclear kappa B (NF-κB). Además, se determinó que hubo una reducción del 50 % en la incidencia de tumores en los ratones sometidos a este tratamiento comparado con un 100 % del grupo control. Los autores discuten que estos resultados, aunque prometedores, podrían mejorarse si la frecuencia de aplicación de la película se aumenta en futuras investigaciones[27].

Cápsulas de apigenina

Como se mencionó anteriormente, la apigenina es un flavonoide al cual se le atribuyen diversas actividades biológicas. En un estudio, se obtuvieron cápsulas mediante FDM con filamentos de alcohol polivinílico. Estas se rellenaron con dispersiones de apigenina en una matriz de quitosano, el cual le confirió propiedades mucoadhesivas y para lograr una liberación sostenida. La relación apigenina:quitosano 1:1.5 fue la de mayor rendimiento (94,2 %) y de mayor eficiencia de encapsulación (99,5 %), por lo que fue la que se estudió a profundidad. Este producto logró efectivamente una liberación sostenida (86,73 % en 24 h) y presentó mayor actividad antioxidante (92.32 %) comparado con apigenina sola (61,6%) en la prueba 2,2-difenil-1-picrilhidracilo (DPPH). También fue efectiva contra bacterias Grampositivas (*Staphylococcus aureus*, 19 mm de inhibición) y hongos (*Candida albicans*, 22 mm), aunque no tanto para *Escherichia coli* (13 mm). Finalmente, se demostró que el producto presenta actividad

citotóxica contra la línea celular de cáncer pulmonar A549, presentando un IC_{50} más bajo (3,77 µg/mL) en comparación con apigenina pura (5,39 µg/mL). Los autores discuten que el quitosano no sólo mejora la solubilidad y liberación del flavonoide, sino que también aporta a la actividad biológica, por ejemplo, alterando membranas celulares bacterianas, haciendo de este un producto prometedor para posteriores pruebas *in vivo*[28].

Parches dérmicos de quercetina

Mycobacterium tuberculosis es una bacteria patógena agente causal de la tuberculosis, la cual puede presentarse en diferentes variantes, incluyendo la tuberculosis pulmonar. A pesar de que existe tratamiento, algunos de los fármacos empelados están asociados a hepatotoxicidad[38] y existen muchos casos de resistencia bacteriana por lo que en muchas ocasiones, es necesario un procedimiento quirúrgico[39]. Por esa razón, es necesario buscar nuevos fármacos antimicobacterianos. Además, muchos fármacos antimicobacterianos administrados por vía oral presentan problemas de biodisponibilidad, por lo que también es necesario buscar formas farmacéuticas alternativas como lo son las formulaciones de liberación transdérmica para absorción sistémica[40].

Por otro lado, la quercetina es uno de los flavonoides más estudiados. Se trata de un flavonol de baja solubilidad y biodisponibilidad que se encuentra ampliamente distribuido en vegetales comestibles comunes como cebollas, manzanas, cocoa, mora azul, mango, té, entre otros[41]. Este compuesto presenta varias actividades biológicas, incluyendo actividad antioxidante, anticancerígena, antiinflamatoria, antiviral, anticancerígena, entre otras[42]. Una de sus actividades biológicas más prometedoras es la antimicrobiana, específicamente contra diferentes cepas de *M. tuberculosis*[43,44], y ya incluso se ha observado que la quercetina formulada con polivinilpirrolidona (PVP) por vía intravenosa y combinada con quimioterapia puede reducir la tuberculosis pulmonar en pacientes reales[45].

El PVP es un polímero hidrofílico empleado como excipiente para preparar una matriz polimérica en la que el fármaco está disperso en un arreglo amorfo y estable. A estos preparados se les conoce como dispersiones sólidas amorfas y su objetivo es aumentar la solubilidad y biodisponibilidad de fármacos poco solubles. Además, los preparados con arreglos amorfos de los fármacos tienen mayor nivel de energía que los arreglos cristalinos, por lo cual son más solubles también[46]. Otros polímeros importantes son los polimetacrilatos Eudragit®, que son determinantes para establecer el perfil de liberación del producto; para preparados de liberación sostenida obtenidos mediante HME suele emplearse el Eudragit® RS[47]

Una de las técnicas más estudiadas con la que se pueden realizar dispersiones de fármaco en PVP y Eudragit® es mediante HME[48], la cual, como se mencionó anteriormente, puede acoplarse con FDM para obtener formas farmacéuticas[20,24].

Este enfoque ya se ha empleado para la formulación de parches de quercetina-PVP de liberación sostenida con el propósito de mitigar la tuberculosis pulmonar destructiva. Para ello, se realizaron diferentes formulaciones y se determinó que la óptima consistió en 1 % p/p de quercetina, 49 % p/p de PVP 40, 38 % p/p del polímero Eudragit® RS PO y 12 % p/p del plastificante trietil citrato (TEC). Esta mezcla se sometió a un proceso de HME (40 rpm y temperatura de 120°C) para obtener filamentos, los cuales se emplearon para elaborar diferentes parches mediante el uso de la tecnología de impresión en 3D por FDM. Cada parche tardó 20 minutos en imprimirse. Estas formulaciones presentaron buenas características fisicoquímicas. Asimismo, mediante diferentes técnicas analíticas se confirmó la conversión de la quercetina cristalina a su forma amorfa, lo cual explica por qué los parches presentaron un buen perfil de disolución *in vitro*; el porcentaje de liberación de quercetina en estado estacionario (liberación sostenida) se mantuvo por más de 70 h a pH 7,4. Posteriormente, los productos se evaluaron *in vivo*. Para ello, se aplicó el parche a tres ratas Sprague Dawley adultos. Se aplicó el parche a cada rata una sola vez y se fueron tomando muestras sanguíneas a diferentes intervalos de tiempo. Como resultado, se encontró que el parche efectivamente logró sostener concentraciones plasmáticas de quercetina por hasta 18 días sin fluctuación alguna[29].

Perspectivas a futuro

En general, la tecnología de impresión en 3D es ampliamente estudiada para la elaboración de tabletas que mejoren la biodisponibilidad de fármacos poco solubles[49], como sería el caso de los flavonoides. A pesar de ello, sólo existen dos estudios con este enfoque, ambos enfocados en la puerarin[16,25]. Sin embargo, aunque las formas farmacéuticas sólidas orales como las tabletas son los medicamentos más frecuentemente empleados, es importante reconocer que en la práctica, la tecnología de impresión en 3D para la elaboración de tabletas presenta una serie de desventajas, incluyendo dificultades en el escalado y, en muchas ocasiones, alto costo y tiempos muy largos de producción[13] Por ello, también es importante reconocer el empleo de esta tecnología para la obtención de otras formas farmacéuticas que, por sus características, presentan menos limitaciones de biodisponibilidad, como lo son las películas mucoadhesivas orales[50] y los parches[51].

A pesar de las dificultades de su escalado a nivel industrial, la impresión en 3D se puede emplear para elaborar productos farmacéuticos que tendrían mucho impacto a baja escala, como lo son los fármacos huérfanos, empelados para tratar enfermedades raras e infrecuentes[52]. Anteriormente, ya se han identificado flavonoides con potencial para tratar enfermedades raras[53,54] y se ha empleado la tecnología de impresión en 3D para mejorar los perfiles de disolución de fármacos huérfanos liposolubles[55]. Además, en 2017, las compañías Aprecia Pharmaceuticals, la empresa que produce Spritam®, firmó un acuerdo de colaboración con Cycle Pharmaceuticals para desarrollar medicamentos huérfanos empleando la tecnología de impresión en 3D[15], por lo que es probable que se adopte este enfoque en los próximos años para formular este tipo de fármacos.

Otro enfoque provechoso puede ser la medicina veterinaria debido a que los productos farmacéuticos disponibles para esta población son limitados, por lo que se suelen emplear medicamentos diseñados para humanos[56,57]. Sin embargo, es evidente que las dosis y las características fisicoquímicas de esos medicamentos no son compatibles con esta población, por lo cual con la tecnología de impresión en 3D se pueden elaborar formas farmacéuticas adecuadas para animales como lo son tabletas masticables[56] o películas orodispensables con contenido de fármaco adecuado[57]. Además, en ocasiones, el paciente veterinario no sólo requiere de ajuste de dosis y de forma farmacéutica, sino de un fármaco específico para uso veterinario[58]. En ese sentido, ya se ha planteado el uso de flavonoides para tratar enfermedades que afectan a animales como cerdos y aves de corral[59], por lo que la complementariedad de estas disciplinas pueden ser explotadas con este enfoque en un futuro.

La población pediátrica también podría verse beneficiada por esta tecnología, ya que debido a las diferencias fisiológicas que tienen con el adulto, los fármacos no presentan las mismas características farmacocinéticas en ellos. Existen pocas formulaciones de uso pediátrico y en ocasiones, es necesario ajustar las dosis de los medicamentos diseñados para adultos cuando se aplican a esta población[60]. Por ello, se ha planteado que con la tecnología de impresión en 3D se pueden obtener formas farmacéuticas con dosis y perfiles de liberación adecuados para la población pediátrica. Además, con esta tecnología es posible mejorar las características organolépticas de los medicamentos, por ejemplo, con diferentes texturas, o incluso colores y formas llamativas imposibles de obtener con técnicas convencionales, mejorando la aceptabilidad del medicamento[61].

Por último, es necesario resaltar que a pesar de que la tecnología de impresión en 3D aplicada a la formulación farmacéutica de flavonoides es una línea de investigación prometedora, aún es incipiente y hace falta llevar a cabo más estudios con otros flavonoides y otras formas farmacéuticas que ya se han desarrollado con esta tecnología como lo son cápsulas[62], supositorios[63], y gomas masticables[64], entre otras. Además, hacen falta más pruebas clínicas para demostrar la eficacia de las formas farmacéuticas elaboradas.

Conclusiones

La tecnología de impresión en 3D aplicada a la formulación de flavonoides se alza como una herramienta prometedora ya que todos los productos farmacéuticos de flavonoides que hasta ahora se han obtenido a partir de ella han demostrado tener calidad y potenciales aplicaciones farmacológicas. Se

trata de una herramienta versátil capaz de generar diferentes formas farmacéuticas que mejoran la solubilidad y biodisponibilidad de los flavonoides, además de ofrecer gran margen de personalización. Sin embargo, estas metodologías todavía son incipientes, pues se cuenta con muy pocos estudios todavía. Para futuras investigaciones, es necesario estudiar la formulación de otros flavonoides y otras formas farmacéuticas, así como mayores estudios a nivel clínico para establecer su eficacia en humanos.

Bibliografía

1. Nabavi SM, Šamec D, Tomczyk M, Milella L, Russo D, Habtemariam S, et al. Flavonoid biosynthetic pathways in plants: Versatile targets for metabolic engineering. Biotechnol Adv. 2020; 38: 107316. Doi: 10.1016/j.biotechadv.2018.11.005.

2. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 2020; 8(2): 603–608. Doi: 10.22271/chemi.2020.v8.i2i.8834.

3. Kozłowska A, Szostak-Węgierek D. Flavonoids – Food Sources, Health Benefits, and Mechanisms Involved. 2018; 65(2):79–85.

4. Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chem. 2022; 383: 132531. Doi: 10.1016/j.foodchem.2022.132531.

5. Terahara N. Flavonoids in Foods: A Review. Natural Products Communications. 2015; 10(3): 1934578X1501000334. Doi: 10.1177/1934578X15010003.

6. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, et al. Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. Biomed Res Int. 2022; 2022(1): 5445291. Doi: 10.1155/2022/5445291.

7. Jucá MM, Cysne Filho FMS, de Almeida JC, Mesquita D da S, Barriga JR de M, Dias KCF, et al. Flavonoids: biological activities and therapeutic potential. Nat Prod Res. 2018; 34(5): 692–705. Doi: 10.1080/14786419.2018.1493588.

8. Zhao J, Yang J, Xie Y. Improvement strategies for the oral bioavailability of poorly water-soluble flavonoids: An overview. Int J Pharm. 2019; 570: 118642. Doi: 10.1016/j.ijpharm.2019.118642.

9. Gaikwad SS, Kshirsagar SJ. Review on Tablet in Tablet techniques. Beni-Suef Univ J Basic Appl Sci. 2020; 9(1): 1–7. Doi: 10.1186/s43088-019-0027-7.

10. Nagula RL, Wairkar S. Recent advances in topical delivery of flavonoids: A review. J Control Release. 2019; 296: 190–201. Doi: 10.1016/j.jconrel.2019.01.029.

11. Yan Q, Dong H, Su J, Han J, Song B, Wei Q, et al. A Review of 3D Printing Technology for Medical Applications. Engineering. 2018; 4(5): 729–742. Doi: 10.1016/j.eng.2018.07.021.

12. Prasad LK, Smyth H. 3D Printing technologies for drug delivery: a review. Drug Development and Industrial Pharmacy. 2015; 42(7):1019–1031. Doi: 10.3109/03639045.2015.1120743.

13. Wook Huh H, Na YG, Kang HC, Kim M, Han M, Mai Anh Pham T, et al. Novel self-floating tablet for enhanced oral bioavailability of metformin based on cellulose. Int J Pharm. 2021; 592: 120113. Doi: 10.1016/j.ijpharm.2020.120113.

14. Jovanović M, Petrović M, Cvijić S, Tomić N, Stojanović D, Ibrić S, et al. 3D Printed Buccal Films for Prolonged-Release of Propranolol Hydrochloride: Development, Characterization and Bioavailability Prediction. Pharmaceutics. 2021; 13(12): 2143. Doi: 10.3390/pharmaceutics13122143.

15. Mohammed AA, Algahtani MS, Ahmad MZ, Ahmad J, Kotta S. 3D Printing in medicine: Technology overview and drug delivery applications. Ann 3D Print Med. 2021; 4: 100037. Doi: 10.1016/j.stlm.2021.100037.

16. Li P, Zhang S, Sun W, Cui M, Wen H, Li Q, et al. Flexibility of 3D Extruded Printing for a Novel Controlled-Release Puerarin Gastric Floating Tablet: Design of Internal Structure. AAPS PharmSciTech. 2019; 20(6): 236. Doi: 10.1208/s12249-019-1455-3.

17. Li Q, Guan X, Cui M, Zhu Z, Chen K, Wen H, et al. Preparation and investigation of novel gastro-floating tablets with 3D extrusion-based printing. Int J Pharm. 2018; 535(1–2): 325–332. Doi: 10.1016/j.ijpharm.2017.10.037.

18. Seoane-Viaño I, Januskaite P, Alvarez-Lorenzo C, Basit AW, Goyanes A. Semi-solid extrusion 3D printing in drug delivery and biomedicine: Personalised solutions for healthcare challenges. J Control Release. 2021; 332. Doi: 10.1016/j.jconrel.2021.02.027.

19. Karavasili C, Eleftheriadis GK, Gioumouxouzis C, Andriotis EG, Fatouros DG. Mucosal drug delivery and 3D printing technologies: A focus on special patient populations. Adv Drug Deliv Rev. 2021;176: 113858. Doi: 10.1016/j.addr.2021.113858.

20. Dumpa N, Butreddy A, Wang H, Komanduri N, Bandari S, Repka MA. 3D printing in personalized drug delivery: An overview of hot-melt extrusion-based fused deposition modeling. Int J Pharm. 2021; 600: 120501. Doi: 10.1016/j.ijpharm.2021.120501.

21. Tan DK, Maniruzzaman M, Nokhodchi A. Advanced Pharmaceutical Applications of Hot-Melt Extrusion Coupled with Fused Deposition Modelling (FDM) 3D Printing for Personalised Drug Delivery. Pharm. 2018; 10(4): 203. Doi: doi.org/10.3390/pharmaceutics10040203.

22. Shi X, Fan N, Zhang G, Sun J, He Z, Li J. Quercetin amorphous solid dispersions prepared by hot melt extrusion with enhanced solubility and intestinal absorption. Pharm Dev Technol. 2020; 25(4): 472–481. Doi: 10.1080/10837450.2019.1709502.

23. Adnan M, Azad MOK, Ju HS, Son JM, Park CH, Shin MH, et al. Development of biopolymer-mediated nanocomposites using hot-melt extrusion to enhance the bio-accessibility and antioxidant capacity of kenaf seed flour. Appl Nanosci. 2020; 10(4): 1305–1317. Doi: 10.1007/s13204-019-01205-z.

24. Deshkar S, Rathi M, Zambad S, Gandhi K. Hot Melt Extrusion and its Application in 3D Printing of Pharmaceuticals. Curr Drug Deliv. 2021; 18(4): 387–407. Doi: 10.2174/1567201817999201110193655.

25. Li P, Jia H, Zhang S, Yang Y, Sun H, Wang H, et al. Thermal Extrusion 3D Printing for the Fabrication of Puerarin Immediate-Release Tablets. AAPS PharmSciTech. 2020; 21(1): 20. Doi: 10.1208/s12249-019-1538-1.

26. Tagami T, Yoshimura N, Goto E, Noda T, Ozeki T. Fabrication of muco-adhesive oral films by the 3D printing of hydroxypropyl methylcellulose-based catechin-loaded formulations. Biol Pharm Bull. 2019; 42(11): 1898–1905. Doi: 10.1248/bpb.b19-00481.

27. Takashima H, Tagami T, Kato S, Pae H, Ozeki T, Shibuya Y. Three-Dimensional Printing of an Apigenin-Loaded Mucoadhesive Film for Tailored Therapy to Oral Leukoplakia and the Chemopreventive Effect on a Rat Model of Oral Carcinogenesis. Pharmaceutics. 2022; 14(8): 1575. Doi: 10.3390/pharmaceutics14081575.

28. Alali AS, Muqtader Ahmed M, Fatima F, Anwer MK, Ibnauf M, Aboudzadeh MA. Chitosan-based spray-dried solid dispersions of apigenin in a 3D printable drug delivery system. J Appl Polym Sci. 2025; 142(1):e56310. Doi: 10.1002/app.56310.

29. Chaudhari VS, Malakar TK, Murty US, Banerjee S. Extruded filaments derived 3D printed medicated skin patch to mitigate destructive pulmonary tuberculosis: design to delivery. Expert Opin Drug Deliv. 2021; 18(2): 301–313. Doi: 10.1080/17425247.2021.1845648.

30. Flora GD, Nayak MK. A Brief Review of Cardiovascular Diseases, Associated Risk Factors and Current Treatment Regimes. Curr Pharm Des. 2019; 25(38): 4063–4084. Doi: 10.2174/1381612825666190925163827.

31. Jiang Z, Cui X, Qu P, Shang C, Xiang M, Wang J. Roles and mechanisms of puerarin on cardiovascular disease: A review. Biomed Pharmacother. 2022; 147: 112655. Doi: 10.1016/j.biopha.2022.112655.

32. Huang M, Long L, Deng M, Yu Z, Qu H, Tan L, et al. Effectiveness and safety of Yufeng Ningxin for the treatment of essential hypertension: A protocol for systematic review and meta-analysis. Medicine (Baltimore). 2021; 100(9): e24858. Doi: 10.1097/MD.0000000000024858.

33. Joshi HN, Tejwani RW, Davidovich M, Sahasrabudhe VP, Jemal M, Bathala MS, et al. Bioavailability enhancement of a poorly water-soluble drug by solid dispersion in polyethylene glycol-polysorbate 80 mixture. Int J Pharm. 2004; 269:251–258. Doi: 10.1016/j.ijpharm.2003.09.002.

34. Sheen PC, Khetarpal VK, Cariola CM, Rowlings CE. Formulation studies of a poorly water-soluble drug in solid dispersions to improve bioavailability. Int J Pharm. 1995; 118(2): 221–227. Doi: 10.1016/0378-5173(94)00366-D.

35. Gadkari PV, Balaraman M. Catechins: Sources, extraction and encapsulation: A review. Food Bioprod Process. 2015; 93: 122–138. Doi: 10.1016/j.fbp.2013.12.004.

36. Salehi B, Venditti A, Sharifi-Rad M, Kręgiel D, Sharifi-Rad J, Durazzo A, et al. The therapeutic potential of Apigenin. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute. 2019; 20(6): 1305. Doi: 10.3390/ijms20061305.

37. Jang JY, Sung B, Kim ND. Role of Induced Programmed Cell Death in the Chemopreventive Potential of Apigenin. Int J Mol Sci. 2022; 23(7): 3757. Doi: 10.3390/ijms23073757.

38. Lyon SM, Rossman MD. Pulmonary Tuberculosis. 2017; 5(1): 10-1128. Doi: 10.1128/microbiolspec.tnmi7-0032-2016.

39. Giller DB, Giller BD, Giller GV, Shcherbakova GV, Bizhanov AB, Enilenis II, et al. Treatment of pulmonary tuberculosis: past and present. Eur J Cardio-Thoracic Surg. 2018; 53(5): 967–972. Doi: 10.1093/ejcts/ezx447.

40. Hussain A, Altamimi MA, Alshehri S, Imam SS, Shakeel F, Singh SK. Novel approach for transdermal delivery of rifampicin to induce synergistic antimycobacterial effects against cutaneous and systemic tuberculosis using a cationic nanoemulsion gel. Int J Nanomedicine. 2020; 15: 1073–1094. Doi: 10.2147/IJN.S236277.

41. Kandemir K, Tomas M, McClements DJ, Capanoglu E. Recent advances on the improvement of quercetin bioavailability. Trends Food Sci Technol. 2022; 119: 192–200. Doi: 10.1016/j.tifs.2021.11.032.

42. Wang W, Sun C, Mao L, Ma P, Liu F, Yang J, et al. The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. Trends Food Sci Technol. 2016; 56: 21–38. Doi: 10.1016/j.tifs.2016.07.004.

43. Butov DO, Zaitseva SI, Pitenko MM, Stepanenko GL, Butova TS. Morphological changes in experimental tuberculosis resulting from treatment with quercetin and polyvinylpyrrolidone. Int J Mycobacteriology. 2015; 4(4): 296–301. Doi: 10.1016/j.ijmyco.2015.08.003.

44. Sasikumar K, Ghosh AR, Dusthackeer A. Antimycobacterial potentials of quercetin and rutin against Mycobacterium tuberculosis H37Rv. 3 Biotech. 2018; 8(10): 427. Doi: 10.1007/s13205-018-1450-5.

45. Butov D, Zaitseva S, Butova T, Stepanenko G, Pogorelova O, Zhelezniakova N. Efficacy and safety of quercetin and polyvinylpyrrolidone in treatment of patients with newly diagnosed destructive pulmonary tuberculosis in comparison with standard antimycobacterial therapy. Int J Mycobacteriology. 2016; 5(4): 446–453. Doi: 10.1016/j.ijmyco.2016.06.012.

46. Lehmkemper K, Kyerematen SO, Heinzerling O, Degenhardt M, Sadowski G. Long-Term Physical Stability of PVP- and PVPVA-Amorphous Solid Dispersions. Mol Pharm. 2017; 14(1): 157–171. Doi: 10.1021/acs.molpharmaceut.6b00763.

47. Dos Santos J, da Silva GS, Velho MC, Beck RCR. Eudragit®: A Versatile Family of Polymers for Hot Melt Extrusion and 3D Printing Processes in Pharmaceutics. Pharmaceutics. 2021; 13(9): 1424. Doi: 10.3390/pharmaceutics13091424.

48. Agrawal AM, Dudhedia MS, Zimny E. Hot Melt Extrusion: Development of an Amorphous Solid Dispersion for an Insoluble Drug from Mini-scale to Clinical Scale. AAPS PharmSciTech. 2016; 17(1): 133–147. Doi: 10.1208/s12249-015-0425-7.

49. Tracy T, Cheng S, Wu L, Liu X, Li X. Enhancing Oral Bioavailability Using 3D Printing Technology. Oral Bioavailab Drug Deliv. 2023; 657–676. Doi: 10.1002/9781119660699.ch34.

50. Shaikh R, Raj Singh T, Garland M, Woolfson A, Donnelly R. Mucoadhesive drug delivery systems. J Pharm Bioallied Sci. 2011; 3(1): 89–100. Doi: 10.4103/0975-7406.76478.

51. Bird D, Ravindra NM. Transdermal drug delivery and patches—An overview. Med Devices Sensors. 2020; 3(6): e10069. Doi: 10.1002/mds3.10069.

52. Vicente E, Pruneda L, Ardanaz E. Paradox of rarity: about the percentage of population affected by rare diseases. Gac Sanit. 2020; 34(6): 536–538. Doi: 10.1016/j.gaceta.2020.02.012.

53. Khan H, Ullah H, Tundis R, Belwal T, Devkota HP, Daglia M, et al. Dietary Flavonoids in the Management of Huntington's Disease: Mechanism and Clinical Perspective. eFood. 2020; 1(1): 38–52. Doi: 10.2991/efood.k.200203.001.

54. Deep A, Marwaha RK, Marwaha MG, Jyoti, Nandal R, Sharma AK. Flavopiridol as cyclin dependent kinase (CDK) inhibitor: a review. New J Chem. 2018; 42(23): 18500–18507. Doi: 10.1039/C8NJ04306J.

55. Saydam M, Takka S. Improving the dissolution of a water-insoluble orphan drug through a fused deposition modelling 3-Dimensional printing technology approach. Eur J Pharm Sci. 2020; 152: 105426. Doi: 10.1016/j.ejps.2020.105426.

56. Sjöholm E, Mathiyalagan R, Wang X, Sandler N. Compounding Tailored Veterinary Chewable Tablets Close to the Point-of-Care by Means of 3D Printing. Pharmaceutics. 2022; 14(7): 1339. Doi: 10.3390/pharmaceutics14071339.

57. Sjöholm E, Mathiyalagan R, Prakash DR, Lindfors L, Wang Q, Wang X, et al. 3D-Printed Veterinary Dosage Forms—A Comparative Study of Three Semi-Solid Extrusion 3D Printers. Pharmaceutics. 2020; 12(12): 1239. Doi: 10.3390/pharmaceutics12121239.

58. Scott KA, Qureshi MH, Cox PB, Marshall CM, Bellaire BC, Wilcox M, et al. A Structural Analysis of the FDA Green Book-Approved Veterinary Drugs and Roles in Human Medicine. 2020; 63(24): 15449-15482. Doi: 10.1021/acs.jmedchem.0c01502.

59. Karancsi Z, Balázs A, Gálfi P, Farkas O. Flavonoids - new perspectives in the veterinary medicine. Magy Állatorvosok Lapja. 2015; 137(11): 695–704.

60. Khan D, Kirby D, Bryson S, Shah M, Rahman Mohammed A. Paediatric specific dosage forms: Patient and formulation considerations. Int J Pharm. 2022; 616: 121501. Doi: 10.1016/j.ijpharm.2022.121501

61. McCloskey AP, Bracken L, Vasey N, Ehtezazi T. 3D printing – an alternative strategy for pediatric medicines. Expert Rev Clin Pharmacol. 2023; 16(7): 613–6. Doi: 10.1080/17512433.2023.2233416

62. Russi L, Del Gaudio C. 3D printed multicompartmental capsules for a progressive drug release. Ann 3D Print Med. 2021; 3: 100026. Doi: 10.1016/j.stlm.2021.100026.

63. Awad A, Hollis E, Goyanes A, Orlu M, Gaisford S, Basit AW. 3D printed multi-drug-loaded suppositories for acute severe ulcerative colitis. Int J Pharm X. 2023; 5: 100165. Doi: 10.1016/j.ijpx.2023.100165.

64. Zhu C, Tian Y, Zhang E, Gao X, Zhang H, Liu N, et al. Semisolid Extrusion 3D Printing of Propranolol Hydrochloride Gummy Chewable Tablets: an Innovative Approach to Prepare Personalized Medicine for Pediatrics. AAPS PharmSciTech. 2022; 23(5): 166. Doi: 10.1208/s12249-022-02304-x.