E-ISSN 2340-9894 · EDITA: FACULTAD DE FARMACIA DE LA UNIVERSIDAD DE GRANADA

Ars Pharmaceutica

Vol. 66(3) · Julio-septiembre 2025

Imagen: Free Stock Photo

Ars Pharmaceutica

Universidad de Granada

Revista fundada en 1960 | Periodicidad trimestral | Un volúmen anual compuesto de cuatro números

E-ISSN 2340-9894

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.

Ars Pharmaceutica está indexada actualmente en las siguientes bases de datos, directorios y repertorios: **EMERGING SOURCE CITATION INDEX (ESCI), EBSCO, EMBASE, DIALNET, DOAJ, GOOGLE ACADÉMICO, LATINDEX, REDIB, SCIELO, IBECS y MIAR**. Nuestra intención es seguir ampliando su presencia en otras bases de datos relevantes.

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.

Contacto de la Redacción
Editorial Office Contact Info

Ars Pharmaceutica.
Facultad de Farmacia.
Universidad de Granada.
Campus de Cartuja s/n.
18071 -Granada. España
email: ars@ugr.es

Edita
Scientific Editor

Facultad de Farmacia de la Universidad de Granada

Publica
Publisher

Facultad de Farmacia de la Universidad de Granada
Campus de Cartuja, s.n.
18071 - GranadaEspaña | Spain

Toda la información sobre la revista en
More info about the journal available on

http://revistaseug.ugr.es/index.php/ars

Equipo Editorial

E-ISSN 2340-9894

Vol. 66(3) · Julio-septiembre 2025 · pag. 260-398

Sumario
Contents

Artículos Originales / Original Articles

Artículos especiales / Special Articles

Artículos revisión / Review Articles

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.32021
Artículos originales

Seguridad de Inmunoglobulina G (Gammaraas®) en Pacientes atendidos en una Clínica de Alta Complejidad: Estudio Descriptivo

Safety of Immunoglobulin G (Gammaraas®) in Patients Treated at a High-Complexity Clinic: A Descriptive Study

Alfredo Hernández-Ruiz[1] 0000-0003-2359-09313
Jhojan Sebastián Herrera-Vargas[1] 0000-0002-6919-8751
Laura Victoria Vasco-Correa[1] 0009-0004-0967-4308
Mónica Rocío Hernández-Valdivieso[1] 0009-0009-5410-5696
María Cristina Angarita-Galvis[1] 0009-0009-9242-10406
[1]Grupo de Investigación Clínica Somer, Unidad de Investigación y Docencia, Rionegro, Colombia

Correspondencia

Jhojan Sebastián Herrera Vargas.
jherrera@clinicasomer.com

Recibido: 27.11.2024
Aceptado: 13.04.2025
Publicado: 20.06.2025

Financiación

Esta investigación fue financiada por Laboratorios Delta SA; sin embargo, se permitió expresar de forma libre el concepto científico de los investigadores.

Conflicto de intereses

Los autores declaran no tener conflicto de interés.

Agradecimientos

Especial agradecimiento a la profesional en Microbiología y Bioanalista Lina Ortiz, quién contribuyó con la elaboración del protocolo de investigación.

Resumen

Introducción: En los últimos años, se ha incrementado el uso de la inmunoglobulina intravenosa (IGIV) para tratar inmunodeficiencias y trastornos inmunomoduladores. A pesar de ser generalmente segura, puede causar eventos adversos leves a graves. Este estudio describió los aspectos de seguridad de Gammaraas® 5 % en pacientes de una clínica de alta complejidad entre 2020 y 2022.

Método: Se realizó un estudio observacional retrospectivo en una clínica de alta complejidad entre 2000 y 2022, de pacientes tratados con inmunoglobulina G intravenosa. Se analizaron las historias clínicas para recopilar información demográfica, indicación, dosis y eventos adversos. Los datos se presentaron como frecuencias absolutas y relativas para variables cualitativas y como medidas de tendencia central para las variables cuantitativas. Se siguieron los lineamientos éticos de la declaración de Helsinki, y el estudio fue aprobado por el comité de ética institucional.

Resultados: Durante el período estudiado, 49 pacientes recibieron la inmunoglobulina G intravenosa en estudio. Predominando el sexo masculino con 27 (55,1 %), distribuidos equitativamente entre adultos y pediátricos, con una mediana de edad de 17,2 años (IQR: 3,7 - 48,2), los cuales recibieron 98 infusiones. Cinco pacientes (10,2 %), presentaron reacciones adversas, la mayoría de estas clasificadas como leves. Las principales indicaciones fueron: Púrpura trombocitopénica idiopática, miopatía inflamatoria, síndrome de Guillain-Barré y el síndrome mucocutáneo linfonodular "Kawasaki". Dos pacientes presentaron reacciones severas las cuales finalmente fueron clasificadas como no relacionadas con la inmunoglobulina G intravenosa.

Conclusiones: La aplicación de Gammaraas® 5 % intravenosa, fue segura en el 90 % de los casos. Las reacciones documentadas fueron leves, lo cual está en concordancia con lo expresado en la literatura.

Palabras clave: Eventos adversos; inmunoglobulina intravenosa; farmacovigilancia; usos terapéuticos, estudios retrospectivos.

Abstract

Introduction: Recent years have seen an increase in the use of intravenous immunoglobulin (IVIG) for treating primary and secondary immunodeficiencies, as well as immune modulation. Despite being generally safe, IVIG can cause mild to severe adverse events. This study aimed to describe safety aspects of Gammaraas® 5 % in high-complexity clinic patients between 2020 and 2022.

Method: A retrospective observational study conducted at a high-complexity clinic between 2000 and 2022, involving patients treated with intravenous immunoglobulin G. Medical records were analyzed to collect demographic information, indication, medication dosage, and adverse events. Data were presented as absolute and relative frequencies for qualitative variables and measures of central tendency for quantitative variables. The ethical guidelines of the Helsinki Declaration were followed, and the study was approved by the institutional ethics committee.

Results: During the study period, 49 patients received the intravenous immunoglobulin G under the study. Males predominated with 27 (55.1 %), equally distributed between adults and pediatrics, with a median age of 17.2 years (IQR: 3.7-48.2), who received 98 infusions. Five patients (10.2 %) experienced adverse reactions, most of them classified as mild.The main indications were: idiopathic thrombocytopenic purpura, inflammatory myopathy, Guillain-Barre syndrome and "Kawasaki" lymphonodular mucocutaneous syndrome. Two patients experienced severe reactions wich were finally classified as not related to intravenous immunoglobulin G.

Conclusions: The intravenous administration of Gammaraas® 5 % was safe in 90 % of cases. The documented reactions were mild, which is consistent with the literature.

Keywords: Adverse effects; intravenous immunoglobulins; pharmacovigilance; therapeutic uses; retrospective studies.

Puntos clave

La inmunoglobulina G intravenosa es una terapia segura y eficaz para diversas afecciones inmunológicas, aunque pueden presentarse eventos adversos leves, generalmente dentro de la primera hora de infusión.

Este estudio aporta datos específicos sobre la seguridad de la inmunoglobulina G en pacientes de una clínica de alta complejidad en Colombia, documentando la baja frecuencia de eventos adversos severos.

Los resultados muestran que las reacciones adversas a la inmunoglobulina G (Gammaraas® 5 %) son en su mayoría leves, lo que indica que su uso es generalmente seguro. No obstante, se recomienda monitoreo continuo y la realización de estudios adicionales con un enfoque analítico.

Introducción

En los últimos años, se ha observado un notable incremento en la aplicación de Inmunoglobulina G Intravenosa (IGIV) para tratar diversos trastornos relacionados con la inmunodeficiencia primaria humoral, inmunodeficiencia secundaria y trasplante alogénico de médula ósea. Adicionalmente, estudios han sugerido que la IGIV humana se utiliza en procesos de inmunomodulación, tratando casos como la púrpura trombocitopénica idiopática, el síndrome de Guillain-Barré y la enfermedad de Kawasaki, entre otras[1,2].

Las preparaciones comerciales de inmunoglobulina G (IgG) humana se obtienen a partir de muestras de plasma de al menos 1.000 donadores sanos[3]. Aunque la Inmunoglobulina (Ig) se considera una terapia segura y bien tolerada los eventos adversos pueden aparecer en cualquier momento, generalmente dentro de la primera hora después del inicio de la infusión[1]. Este riesgo se atribuye a que la IgG humana, derivada del plasma con una concentración superior al 95 %, aún contiene trazas de IgA, IgM, IgE, así como citocinas y moléculas solubles como CD4, CD8 y HLA[4,5] que pueden generar reacciones cruzadas. Además, la literatura ha sugerido que estos eventos adversos pueden ser el resultado de la relativa "impureza" de los preparados comerciales[6].

Los eventos adversos tras las infusiones de IGIV pueden clasificarse como inmediatos (durante la infusión) o tardíos (tras la finalización de esta)[6]. La mayoría de estos son leves y de naturaleza transitoria, abarcando síntomas como cefalea, escalofríos, fiebre, dolor, astenia, dolor de espalda, náuseas, vómitos, dolor abdominal, diarrea, reacción en el lugar de la infusión, erupción cutánea, prurito, urticaria, hipertensión, hipotensión y taquicardia[7]. Los efectos adversos más graves, como la trombosis e infarto cerebral, infarto de miocardio, tromboembolismo pulmonar, síndrome de leucoencefalopatía posterior a la insuficiencia renal aguda, azotemia, meningitis aséptica, se informan raramente[7,8].

En la actualidad, el proceso de comercialización de medicamentos importados en Colombia requiere evaluación farmacológica a fin de solicitar el trámite sanitario ante el Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA)[9]; con lo que se busca caracterizar y optimizar su perfil de calidad, efectividad y seguridad, abarcando el ciclo de vida del medicamento y documentando el sistema de gestión de riesgos asociados[9].

El seguimiento a eventos de seguridad, a través de estudios no clínicos, clínicos u observacionales, donde se describa el seguimiento a los pacientes, el uso del medicamento en la práctica clínica habitual, las indicaciones de uso aprobadas o no en el registro sanitario y la detección de reacciones o eventos de seguridad, es esencial para comprender el comportamiento de los pacientes hospitalizados o no que reciben IGIV[10].

En el contexto colombiano, se evidencia una marcada escasez de información nacional sobre este tema, reflejada en la limitada disponibilidad de literatura y reportes pertinentes. Por lo tanto, este estudio tuvo como objetivo describir aspectos relacionados con la seguridad luego del inicio de la IGIV (Gammaraas® 5 %) en los pacientes atendidos en una clínica de alto nivel de complejidad entre los años 2020 y 2022.

Métodos

Diseño y población de estudio

Se llevó a cabo un estudio observacional retrospectivo a partir del análisis de datos de pacientes hospitalizados y ambulatorios.

Se consideraron pacientes adultos (>18 años) y pediátricos, que recibieron tratamiento con IGIV (Gammaraas® 5 %, Shanghai Xinxing Medicine Ltd Co.), por cualquier causa en una clínica de alta complejidad entre los años 2020 y 2022, según los protocolos institucionales.

Colección de datos

Se construyó una base de datos en Microsoft Excel® a partir de las historias clínicas y el registro de farmacia de todos los pacientes que recibieron IGIV por cualquier causa durante el período de estudio. Esta base de datos incluyó información general del paciente como, edad, sexo, peso, indicación para la administración del medicamento según la Clasificación Internacional de Enfermedades (CIE-10), dosis calculada para el medicamento, número de infusiones y signos y síntomas relacionados con posibles eventos adversos. Para estos últimos, se tuvo en cuenta las consideraciones y análisis que hizo el grupo tratante en su momento y si requirió revisión del evento por el grupo de farmacovigilancia institucional.

Análisis de datos

Los datos fueron analizados usando el programa Microsoft Excel® (Microsoft Office 365 versión 2401). Los resultados se presentaron como frecuencia absoluta y relativa expresada en porcentaje para las variables cualitativas. La edad de los participantes se resumió con medianas y rangos intercuartílicos (IQR). La seguridad del medicamento se determinó al clasificar los eventos adversos de acuerdo con la severidad (serios o no serios), causalidad (relacionado o no relacionado), gravedad (leve, moderado, grave o letal) y seriedad; según las guías de seguridad del paciente de la Organización Mundial de la Salud (OMS)[11].

Consideraciones éticas

La recolección de datos fue realizada de manera retrospectiva acatando los lineamientos de la Declaración de Helsinki de la 64ª Asamblea General de 2013. Según las normativas nacionales vigentes, esta investigación se considera sin riesgo ya que se basa solo en la revisión de historias clínicas y fue aprobado por comité de ética institucional según acta número 68 realizada el 14 de agosto del 2023.

Resultados

Características generales de la población

Durante el período comprendido entre 2020 y 2022, un total de 49 pacientes recibieron la IGIV en estudio (Tabla 1), predominando el sexo masculino con 27 (55,1 %), distribuidos equitativamente entre adultos (n= 24; 49,0 %) y pediátricos (n=25; 51,0 %); con una mediana de edad de 17,2 años (IQR: 3,7 - 48,2). Los cuales recibieron un total de 98 infusiones.

Las indicaciones más comunes para la administración de IGIV fueron la púrpura trombocitopénica (n=13; 26,5 %), la miopatía inflamatoria no clasificada en otra parte (n=5; 10,2 %), el síndrome de Guillain-Barre (n=5; 10,2 %) y el síndrome mucocutáneo linfonodular “Kawasaki” (10,2 %) (Tabla 1).

De los 49 pacientes, tres (6,1 %) recibieron > 6 infusiones de IGIV debido a indicaciones asociadas como leucemia linfocítica crónica, síndrome de Guillain-Barre y púrpura trombocitopénica idiopática con un total de doce, siete y seis infusiones, respectivamente. Destaca el caso de un paciente diagnosticado con leucemia linfocítica crónica, quién recibió un total de 12 infusiones de IGIV, además de una infusión adicional por linfoma no Hodgkin de células pequeñas (difuso), como se describe en la Tabla 1.

Las indicaciones más comunes para la administración de IGIV fueron la púrpura trombocitopénica (n=13; 26,5 %), la miopatía inflamatoria no clasificada en otra parte (n=5; 10,2 %), el síndrome de Guillain-Barre (n=5; 10,2 %) y el síndrome mucocutáneo linfonodular “Kawasaki” (10,2 %) (Tabla 1).

De los 49 pacientes, tres (6,1 %) recibieron > 6 infusiones de IGIV debido a indicaciones asociadas como leucemia linfocítica crónica, síndrome de Guillain-Barre y púrpura trombocitopénica idiopática con un total de doce, siete y seis infusiones, respectivamente. Destaca el caso de un paciente diagnosticado con leucemia linfocítica crónica, quién recibió un total de 12 infusiones de IGIV, además de una infusión adicional por linfoma no Hodgkin de células pequeñas (difuso), como se describe en la tabla 1.

Tabla 1. Características demográficas de la población

Variables	Número de pacientes n=49								
Población adulta n (%) Población pediátrica n (%)	24 (49,0 %) 25 (51,0 %)								
Edad (Mediana – RIC[d])	17,2 (3,7-48,2)								
Sexo n (%)	Masculino 27 (55,1)								
Indicación según CIE-10 para administración de IVIG	**Número de infusiones**								
	Pacientes n (%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**12**
Enfermedad hemolítica del recién nacido	1 (2,0)	x							
Hipogammaglobulinemia transitoria de la infancia	1 (2,0)	x							
Ictericia neonatal severa incompatibilidad ABO con hemólisis	1 (2,0)	x							
Ictericia neonatal, no especificada	5 (10,2)	x							
Inmunodeficiencia no especificada	2 (4,1)	x							
Leucemia linfocítica crónica	1 (2,0)								x
Linfoma no Hodgkin de células pequeñas (difuso)		x							
Miastenia gravis	1 (2,0)	x							
Miopatía inflamatoria, no clasificada en otra parte	5 (10,2) [a]	x	x						
Otras encefalitis, mielitis y encefalomielitis	1 (2,0)	x							
Otras inmunodeficiencias especificadas	1 (2,0)	x							
Otras polineuropatías inflamatorias	2 (4,1)			x					
Otras púrpuras no trombocitopénicas	1 (2,0)	x							
Púrpura trombocitopénica idiopática	13 (26,5) [b]	x	x			x	x		
Reacción de incompatibilidad al grupo ABO	1 (2,0)	x							
Síndrome de Guillain-Barré	5 (10,2) [c]		x			x		x	
Síndrome inflamatorio multisistémico asociado con Covid-19, no especificado	2 (4,1)	x							
Síndrome mucocutáneo linfonodular [Kawasaki]	5 (10,2)	x							
Trastorno del metabolismo de la bilirrubina, no especificado	1 (2,0)	x							
Total, General	49 (100)	98							

a Se describen cinco casos, de los cuales uno recibió dos infusiones y los casos restantes recibieron una única infusión.
b Se describen trece casos, de los cuales cuatro recibieron dos infusiones, uno recibió cinco infusiones, siete recibieron una única infusión y un caso recibió en total seis infusiones (4 infusiones en un mes y 2 infusiones al mes posterior).
c Se describen cinco casos de los cuales tres recibieron cinco infusiones. Los dos casos restantes recibieron 2 y 7 infusiones, respectivamente.
d RIC: Rango Intercuartílico.

Eventos de seguridad

De las 98 infusiones de IGIV administradas, 88 no presentaron ningún reporte de reacciones adversas, lo que representó una tasa de seguridad del 89,8 %. Sin embargo, 5/49 pacientes (10,2 %) presentaron eventos adversos. Uno (20,0 %) en un niño menor de dos años y cuatro (80,0 %) en adultos (Tabla 2).

En cuanto a los eventos adversos, tres (75,0 %) estuvieron directamente relacionados con la administración de IGIV y fueron clasificados como leves según su gravedad. Estos incluyeron edema he-

mifacial, cefalea y prurito. Notablemente, dos (66,6 %) de estos tres pacientes fueron diagnosticados con Síndrome de Guillain-Barré y recibieron múltiples infusiones de IGIV, concretamente dos y cinco infusiones, respectivamente (Tabla 2).

Tabla 2. Eventos adversos

Indicación según CIE-10/ Administración de IGIV	**Evento Adverso**				
	Edema hemifacial (n=1)	**Cefalea (n=1)**	**Prurito (n=1)**	**Choque Anafiláctico mediado por IgE anti-IgA (n=2)**	
				Paciente 1	**Paciente 2**
Miopatia inflamatoria, no clasificada en otra parte		X			
Otras inmunodeficiencias especificadas				X	
Síndrome de Guillain-Barré	X		X		
Ictericia neonatal, no especificada					X
Comorbilidades					
Hipertensión arterial sistémica, diabetes mellitus tipo I, Otra		X			
Sin comorbilidad				X	X
Asma, trastornos mentales			X		
Hipertensión arterial sistémica	X				
Servicio que administró la IGIV					
Hospitalización general adulto	X	X			
Unidad de trasplantes hematopoyéticos				X	
UCI Adulto			X		
UCI Neonatal					X
Hospitalización general adulto					
Número de infusiones	5	1	2	1	1
Dosis calculada (mg/kg)	312,5	2000,0	1170,0	400,0	472,3
Causalidad	Relacionado	Relacionado	Relacionado	No relacionado	No relacionado
Gravedad	Leve	Leve	Leve	Severo	Severo

Dos pacientes presentaron choque anafiláctico mediado por IgE anti-IgA, clasificado como eventos adversos severos. Uno correspondió a un paciente pediátrico de 2 años, el cual de forma concomitante recibía recambio plasmático. El otro caso correspondió a un adulto de 48 años, con leucemia que presentaba un proceso séptico concomitantemente; sin embargo, el grupo tratante consideró que estos eventos adversos no estuvieron relacionados con la aplicación de IGIV dado que tenían una explicación causal alterna (Tabla 2).

Comorbilidades

Se observaron comorbilidades como hipertensión arterial sistémica, diabetes mellitus tipo I, asma y trastornos mentales en los pacientes que experimentaron eventos adversos leves. Es importante destacar que, 44/49 pacientes (89,8 %) que no experimentaron eventos adversos no tenían comorbilidades asociadas (Tabla 2).

Dosificación

Se observó una amplia variabilidad entre los pacientes que presentaron eventos adversos, con dosis que oscilaron entre 400,0 y 2000,0 mg/kg. La dosis más alta se observó en un paciente con miopatía inflamatoria no clasificada en otra parte (2000 mg/kg), seguida de 1170 mg/kg para un caso de síndrome de Guillain-Barré (Tabla 2).

Servicios de administración de la IGIV

El servicio con mayor frecuencia de administración del medicamento fue hospitalización general adulto (n=33; 33,7 %), seguido por la Unidad de Cuidados Especiales (UCE) pediátrica (n=9; 13,3 %). Por otro lado, los servicios UCE adulto, Unidad de Cuidado Intensivo (UCI) adulto y Unidad Funcional para la Atención Integral del Cáncer de Adulto (UFCA) ambulatorio tuvieron el mismo porcentaje de aplicación del medicamento, alcanzando 10 aplicaciones (10,2 %) cada uno (Figura 1).

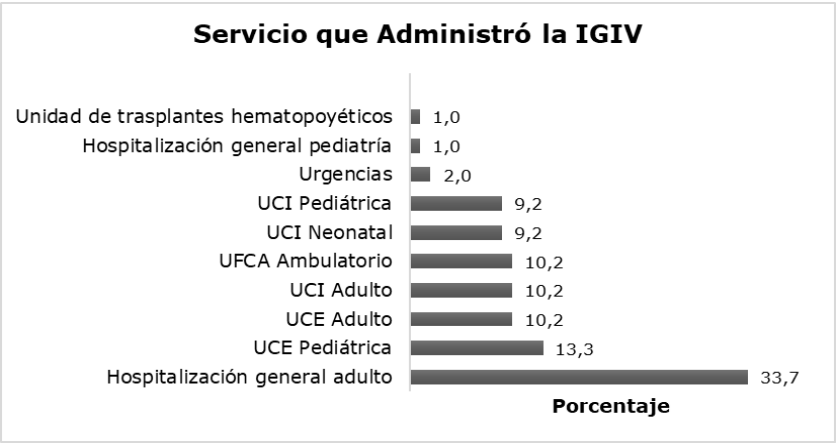

Figura 1. Servicio que administró la IGIV

Discusión

En este estudio, se evidenció una tasa de seguridad cercana al noventa por ciento, ya que 88/98 infusiones (89,8 %) no presentaron ningún reporte de reacciones adversas. Aunque varios estudios han confirmado la seguridad y tolerabilidad de la IGIV[1,6,12]. es importante reconocer la posibilidad de la ocurrencia de diferentes reacciones adversas.

En nuestro estudio, se registró una incidencia de eventos adversos del 10,2 % (n=5) entre todos los pacientes tratados con este medicamento. Esta cifra está dentro del rango reportado en la literatura científica, que varía desde el 1,0 % hasta más del 50,0 % de los pacientes[7,13]. Esta variabilidad depende de varios factores, como diferencias en las preparaciones de inmunoglobulina, variaciones en el diseño del estudio, tamaño de muestra, heterogeneidad de la población estudiada, dosis y durabilidad del tratamiento, así como la presencia de comorbilidades[1,7,8].

Los datos del presente estudio mostraron que el 75,0 % (tres de los eventos adversos) identificados, fueron clasificados como leves. Estos resultados se alinean con lo reportado en la literatura, donde los eventos adversos leves más comúnmente descritos incluyen dolor de cabeza, eventos dermatológicos y síntomas similares a los de la gripe[8]. Estos síntomas suelen manifestarse 30 minutos tras la administración de la IGIV y se ha sugerido que se relacionan con reacciones de hipersensibilidad causadas por la acumulación de moléculas de Ig lo que desencadena la activación del complemento o la reacción antígeno-anticuerpo[6].

Por otro lado, se observó que los eventos adversos leves relacionados con la administración de la IGIV fueron más propensos a ocurrir en pacientes adultos (n=4; 8,2 %) que en niños (n=1; 2,0 %). Este hallazgo coincide con un estudio realizado por Kato en 2021, en el cual se reportó una incidencia del 6,9 % en adultos y del 1,5 % en niños[7]. Los análisis de población clínica reportados en la literatura indican que tanto los niños como los ancianos podrían presentar una mayor susceptibilidad a los eventos adversos de los tratamientos en general[1]. Por lo tanto, estos hallazgos sugieren una posible influencia de la edad en la tolerabilidad y respuesta a la IGIV, lo cual puede atribuirse a que la absorción y metabolismo de los medicamentos son más variables y menos predecibles en este grupo poblacional[14]. Sin embargo, es importante resaltar que la influencia de la edad en los eventos adversos requiere investigaciones adicionales para comprender mejor los factores subyacentes a estas diferencias.

En cuanto a eventos adversos severos, en este estudio se identificaron dos casos de choque anafiláctico posiblemente mediados por IgE anti-IgA. Un caso se presentó en un bebé de dos años, en el cual de forma simultánea se realizó exanguinotransfusión cuando presentó el evento. El grupo tratante consideró como primera opción este procedimiento. El segundo caso reportado, se trató de un paciente con neoplasia hematológica en estado séptico, quien presentó estado de choque, el cual como primera opción se consideró el proceso infeccioso.

Guo, ha sugerido que la presencia de eventos adversos durante una única infusión puede ser relativamente baja en comparación al tratamiento con infusiones repetidas[8]. Sin embargo, nuestros hallazgos difieren de esta sugerencia, ya que se observó que de los cinco pacientes que experimentaron reacciones adversas, tres (60,0 %) recibieron una infusión. Este hallazgo resalta la importancia de una evaluación continua de la seguridad y la tolerabilidad de la terapia con IGIV en nuestro entorno clínico específico.

Con respecto a la identificación de eventos adversos por infusiones repetidas, en el presente estudio se identificaron dos pacientes que experimentaron eventos adversos relacionados con prurito y edema hemifacial después de recibir cinco y dos infusiones de IGIV, respectivamente y que fueron diagnosticados con síndrome de Guillain-Barré. Este hallazgo se alinea con estudios previos que han sugerido que la presencia de eventos adversos relacionados con la terapia de IgG puede atribuirse a la administración de infusiones repetidas. Por ejemplo, Seidling reportó que el 87,5 % de los pacientes experimentaron eventos adversos durante el tratamiento con infusiones repetidas de IGIV[15]. Además, investigaciones adicionales como la de Sherer y una encuesta realizada en Irán sugirieron que los pacientes que desarrollaron eventos adversos durante un curso anterior o aquellos que recibieron su primera infusión, pueden tener un mayor riesgo de experimentar eventos adversos posteriores al tratamiento[16,17].

Adicional a lo anterior, un estudio realizado por Kato, concluyó que tener una enfermedad neuromuscular es un factor que contribuye a los eventos adversos por IGIV[7]. Esto sugiere que los pacientes con enfermedades neuromusculares pueden ser más propensos a experimentar eventos adversos durante el tratamiento con IGIV, como en el caso de los pacientes con síndrome de Guillain-Barré.

Es importante resaltar, que durante el estudio un paciente sometido a 13 dosis idénticas de IGIV, indicadas para tratar leucemia linfocítica crónica y linfoma no Hodgkin de células pequeñas (difuso), administradas con un intervalo aproximado de un mes entre cada dosis, toleró bien el tratamiento y no presentó eventos adversos relacionados en ninguna de las infusiones. Estos hallazgos coinciden con lo reportado por Struff, quién encontró que las reacciones adversas son raras en pacientes con inmunodeficiencias cuando reciben la misma dosis previamente tolerada a intervalos regulares[18].

El análisis realizado reveló que los pacientes que experimentaron eventos adversos leves relacionados con la administración de IGIV también presentaban comorbilidades asociadas, especialmente relacio-

nadas con hipertensión arterial, Diabetes tipo I, asma y trastornos mentales. Esta observación sugiere una posible asociación entre ciertas condiciones médicas preexistentes y la susceptibilidad a eventos adversos durante el tratamiento con IGIV. Estos hallazgos están respaldados por la literatura previa, que ha informado que algunas comorbilidades pueden aumentar la probabilidad de eventos adversos en pacientes tratados con IGIV[19], lo que subraya la importancia de considerar cuidadosamente el perfil médico completo de los pacientes antes de inciar la terapia. Investigaciones futuras en este campo es fundamental para mejorar la comprensión de esta asociación, la cual es crucial para identificar y abordar de manera adecuada los riesgos asociados con la administración de IGIV en paciente con condiciones médicas subyacentes.

Nuestros resultados reflejaron una amplia variabilidad en las dosis de IGIV administradas a los pacientes, con dosis que oscilaron entre 400,0 y 2000,0 mg/kg. Estudios previos han sugerido que la aparición de eventos adversos es dosis-dependiente, ya que se ha reportado que dosis altas de IGIV (superiores a 0,8 g/kg) se han asociado con un mayor riesgo de eventos adversos[7,12]. En línea con esto, los hallazgos del presente estudio mostraron que 2/5 pacientes (40,0 %) que experimentaron eventos adversos recibieron dosis consideradas altas, específicamente 2000,0 mg/kg y 1170,0 mg/kg para tratar condiciones relacionadas con miopatia inflamatoria no clasificada en otra parte y síndrome de Guillain-Barré, respectivamente. De acuerdo con la literatura, esta asociación puede deberse al rápido incremento del nivel sérico de IgG[12]. Estos resultados resaltan la importancia de considerar cuidadosamente las dosis de IGIV administradas a los pacientes para minimizar el riesgo de eventos adversos y optimizar la seguridad de esta terapia.

Se pudo observar que la utilización de las salas de hospitalización general, fueron las más usadas por el personal de salud autorizado para el procedimiento; este hallazgo permite inferir que se podría optimizar el tratamiento con IGIV en salas de cuidado no crítico, lo cual contribuiría en la reducción de costos al sistema de salud, e incluso permitiría evaluar el manejo de terapia en casa para aquellas patologías que lo permitan, tal como fue descrito por Le Masson[20].

Conclusión

En nuestro estudio, se evidenció una tasa de seguridad cercana al 90,0 % lo que respalda la percepción general de que la IGIV es un tratamiento seguro y bien tolerado. Aunque se observó una incidencia del 10,2 % de reacciones adversas, los eventos relacionados al tratamiento fueron leves. Estos pacientes tenían comorbilidades, como hipertensión arterial, diabetes tipo I, asma y trastornos mentales. Adicionalmente, no se encontró una relación directa entre los eventos adversos severos identificados, como choque anafiláctico mediado por IgE anti-IgA, y la administración de IGIV. Este estudio contribuye al conocimiento sobre la seguridad de la IGIV y pueden guiar la práctica clínica para una mejor gestión de los pacientes que reciben esta terapia.

Limitaciones y Perspectivas Futuras

El estudio tiene varias limitaciones inherentes a su diseño retrospectivo, incluyendo la dependencia de la disponibilidad y calidad de los registros médicos, el sesgo de riesgo de información y la incapacidad para establecer relaciones causales debido a la naturaleza observacional del estudio y al número de casos evaluados. Una limitación adicional, es que este estudio se realizó en un solo centro; lo que podría limitar la generalización de los resultados a otras instituciones hospitalarias. El estudio se fortaleció al incluir a todos los pacientes que recibieron tratamiento con IGIV durante el período de 2020 a 2022. Otra limitación importante, fue la falta de disponibilidad de información sobre la velocidad de infusión (ml/min) de la IGIV en todos los casos descritos.

Los hallazgos del presente estudio abren la posibilidad de explorar estrategias para optimizar aún más la seguridad de la terapia con IGIV en la población colombiana, incluyendo la identificación más precisa de las poblaciones de mayor riesgo y el desarrollo de un enfoque de tratamientos más personalizados. Además, se destacan oportunidades para investigaciones adicionales que aborden la relación

entre la administración de IGIV y la incidencia de eventos adversos, como choque anafiláctico mediado por IgE anti-IgA, con el fin de mejorar la comprensión y el manejo de estas complicaciones.

La optimización de la seguridad en la terapia con IGIV podría conducir a una mejora en la calidad de vida de los pacientes y una reducción en los costos asociados con el tratamiento de eventos adversos, lo que beneficiaría significativamente al sistema de salud colombiano.

Bibliografía

1. Alsina L, Mohr A, Montañés M, Oliver X, Martín E, Pons J, et al. Surveillance study on the tolerability and safety of Flebogamma® DIF (10 % and 5 % intravenous immunoglobulin) in adult and pediatric patients. Pharmacol Res Perspect. 2017 Oct 25;5(5). DOI: 10.1002/prp2.345

2. Hartung HP, Mouthon L, Ahmed R, Jordan S, Laupland KB, Jolles S. Clinical applications of intravenous immunoglobulins (IVIg) – beyond immunodeficiencies and neurology. Clin Exp Immunol. 2009 Oct 30;158(Supplement_1):23–33. DOI: 10.1111/j.1365-2249.2009.04024.x

3. Espinosa Rosales FJ, Bergés García A, Coronado Zarco IA, Dávila Gutiérrez G, Faugier Fuentes E, García Campos JA, et al. Consenso Mexicano para la prescripción de inmunoglobulina G como tratamiento de reemplazo e inmunomodulación. Acta Pediátrica de México. 2018;39(2):134. DOI:10.18233/apm-39no2pp134-1711574

4. Hernández-Martínez C, Espinosa-Rosales FJ, Espinosa-Padilla SE, Hernández-Martínez AR, Blancas-Galicia L. Conceptos básicos de las inmunodeficiencias primarias. Rev Alerg Mex. 2016;63(2):180–9.

5. Katz J, Parikh K. Intravenous Immunoglobulin. Medscape . [Updated, Apr 21, 2023]. Disponible en: https://emedicine.medscape.com/article/210367-overview?form=fpf

6. Palabrica FRR, Kwong SL, Padua FR. Adverse events of intravenous immunoglobulin infusions: a ten-year retrospective study. Asia Pac Allergy. 2013 ;3(4):249–56. DOI: 10.5415/apallergy.2013.3.4.249

7. Kato H, Hayashi M, Ohashi W, Yamaguchi T, Tanaka S, Kozono A, et al. A Retrospective Observational Study of Adverse Reactions Associated With Intravenous Immunoglobulin Infusion. Front Immunol. 2021;12. DOI: 10.3389/fimmu.2021.740517

8. Guo Y, Tian X, Wang X, Xiao Z. Adverse Effects of Immunoglobulin Therapy. Front Immunol. 2018;9. DOI: 10.3389/fimmu.2018.01299

9. Ministerio de la Protección Social. Resolución 1403 de 2007 Por el cual se determina el modelo de Gestión del Servicio Farmacéutico, se adopta el manual de Condiciones Esenciales Procedimientos y se dictan otras disposiciones. [Internet]. 2007 [cited 2024 Feb 10]. p. 1–74. Available from: https://www.sanidadfuerzasmilitares.mil.co/transparencia-acceso-informacion-publica/2-normatividad/2-2-busqueda-normas/2-2-2-sistema-busquedas-normas-propio-1/normograma-digsa/subdireccion-salud-digsa/grupo-aseguramiento-salud-proas/normas-externas-aplicadas-al-regimen/resolucion-1403-2007-se-determina-modelo

10. Condino-Neto A, Costa-Carvalho BT, Grumach AS, King A, Bezrodnik L, Oleastro M, et al. Guidelines for the use of human immunoglobulin therapy in patients with primary immunodeficiencies in Latin America. Allergol Immunopathol (Madr). 2014;42(3):245–60. DOI: 10.1016/j.aller.2012.09.006

11. World Health Organization technical report series. International drug monitoring. The role of the hospital. Geneva; 1969.

12. Yori S, Belleri F, Testard J, Fierro Vidal A, Rousseau M. Intravenous immunoglobulin G use and pharmacovigilance in a tertiary care children's hospital. Arch Argent Pediatr. 2021;119(3):192–7. DOI: 10.5546/aap.2021.eng.192

13. Waheed W, Ayer GA, Jadoo CL, Badger GJ, Aboukhatwa M, Brannagan TH, et al. Safety of intravenous immune globulin in an outpatient setting for patients with neuromuscular disease. Muscle Nerve. 2019;60(5):528–37. DOI: 10.1002/mus.26678

14. Alomar MJ. Factors affecting the development of adverse drug reactions (Review article). Saudi Pharmaceutical Journal. 2014;22(2):83–94. DOI: 10.1016/j.jsps.2013.02.003

15. Seidling V, Hoffmann J, Enk A, Hadaschik E. Analysis of High-dose Intravenous Immunoglobulin Therapy in 16 Patients with Refractory Autoimmune Blistering Skin Disease: High Efficacy and No Serious Adverse Events. Acta Dermato Venereologica. 2013;93(3):346–9. DOI: 10.2340/00015555-1471

16. Sherer Y, Levy Y, Langevitz P, Rauova L, Fabrizzi F, Shoenfeld Y. Adverse Effects of Intravenous Immunoglobulin Therapy in 56 Patients with Autoimmune Diseases. Pharmacology. 2001;62(3):133–7. DOI: 10.1159/000056085

17. Dashti-Khavidak S, Aghamohammadi A, Farshadi F, Movahedi M, Parvaneh N, Pouladi N, et al. Adverse reactions of prophylactic intravenous immunoglobulin; a 13-year experience with 3004 infusions in Iranian patients with primary immunodeficiency diseases. J Investig Allergol Clin Immunol. 2009;19(2):139–45.

18. Struff WG, Klasser M, Eckert V, Dietrich RLJ. Safety monitoring of a polyvalent immunoglobulin preparation: documentation of 15,548 administrations. Int Journal of Clinical Pharmacology and Therapeutics. 2005 Sep 1;43(09):420–8. DOI: 10.5414/cpp43420

19. Orbach H, Katz U, Sherer Y, Shoenfeld Y. Intravenous immunoglobulin: adverse effects and safe administration. Clin Rev Allergy Immunol. 2005 Dec; 29(3):173-84. doi: 10.1385/CRIAI:29:3:173. DOI: 10.1385/CRIAI:29:3:173

20. Le Masson G, Solé G, Desnuelle C, Delmont E, Gauthier-Darnis M, Puget S, et al. Home versus hospital immunoglobulin treatment for autoimmune neuropathies: A cost minimization analysis. Brain Behav. 2018 Feb 26;8(2). DOI: 10.1002/brb3.923

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.32785
Artículos originales

Development and Evaluation of Moisturizing Cream containing *Salvia hispanica* and *Aloe vera* and its Antioxidant Potential

Desarrollo y evaluación de una crema humectante con *Salvia hispánica* y *Aloe vera* y su potencial antioxidante

Rutuja K. Deore[1] 0009-0007-2254-370X
Khemchand R. Surana[2] 0000-0001-8918-1159
Sunil K. Mahajan[2] 0009-0004-2148-5948
Rushikesh L. Bachhav [1] 0009-0002-1873-6764
Deepak D. Sonawane[3] 0009-0004-1908-5750

[1]Department of Pharmaceutical Quality Assurance, SSS's Divine College of Pharmacy Satana, Nashik, Maharashtra, India- 423301 Affiliated to Savitribai Phule Pune University, Pune.

[2]Department of Pharmaceutical Chemistry, SSS's Divine College of Pharmacy, Satana, Nashik, Maharashtra, India – 423301 Affiliated to Savitribai Phule Pune University, Pune.

[3]Department of Pharmaceutics, SSS's Divine College of Pharmacy, Nashik, Maharashtra, India – 423301 Affiliated to Savitribai Phule Pune University, Pune.

Correspondence

Dr. Khemchand R. Surana
khemchandsurana772@gmail.com

Received: 08.02.2025
Accepted: 21.05.2025
Published: 20.06.2025

Funding

Not applicable

Conflict of interests

The authors declare no conflict of interest.

Resumen

Introducción: Los bioactivos presentes en las semillas de *Salvia hispánica* (chía) y el aloe vera hidratan, protegen y restauran la piel. El objetivo de este trabajo es formular y evaluar una crema herbal multifuncional con mucílago de semilla de *Salvia hispánica*, gel de aloe vera, manteca de karité y aceite de coco para hidratar y restaurar la piel.

Métodos: El extracto de semilla de *Salvia hispánica* se obtuvo mediante la extracción del mucílago. Se preparó gel de aloe vera y se incorporó a la fórmula junto con manteca de karité y aceite de coco. La fórmula final de la crema se evaluó en cuanto a parámetros fisicoquímicos como pH (6), extensibilidad (7,5 g/s), consistencia, lavabilidad y ausencia de irritación. Se realizó un ensayo de depuración de peróxido de hidrógeno para determinar la actividad antioxidante.

Resultados: La crema formulada demostró actividad antioxidante y reveló una significativa capacidad de eliminación de peróxido de hidrógeno del 95,16 %, lo que indica un alto potencial de eliminación de radicales. La formulación presentó características fisicoquímicas deseables.

Conclusión: Este estudio descubrió que la crema tiene actividad antioxidante con su composición natural y componentes vegetales beneficiosos, podría ser una solución eficaz y sostenible para el cuidado de la piel.

Palabras clave: *Aloe vera, Salvia hispanica*, Chía, Crema hidratante, antioxidante.

Abstract

Introduction: Bioactive chemicals in *Salvia hispanica* (Chia) seeds and *Aloe vera* hydrate, protect, and restore skin. The goal of this work will formulate and evaluate a multifunctional herbal cream with *Salvia hispanica* seed mucilage, *Aloe vera* gel, shea butter, and coconut oil to hydrate and restore skin.

Methods: *Salvia hispanica* seed extract was obtained through mucilage extraction. *Aloe vera* gel was prepared and incorporated into the formulation along with shea butter and coconut oil. The final cream formulation was evaluated for physicochemical parameters such as pH (6), spreadability (7.5 g/sec), consistency, washability, and non-irritancy. The hydrogen peroxide scavenging assay for antioxidant activity performed.

Results: The formulated cream demonstrated antioxidant activity revealed a significant hydrogen peroxide scavenging activity of 95.16 %, indicating high radical scavenging potential. The formulation exhibited desirable physicochemical characteristics.

Conclusion: This study found that the cream is antioxidant activity with its natural composition and helpful plant-based components; it could be an effective and sustainable skincare solution.

Keywords: *Aloe vera*, *Salvia hispanic*a, Chia, Moisturizing cream, antioxidant.

Highlights

Developed a novel skin hydrating cream with *Salvia hispanica* seeds and *Aloe vera* for skin hydration and healing properties.

The formulated cream is also having antioxidant activity.

The formulated moisturizing cream containing *Salvia hispanica* seed extract and *Aloe vera* exhibited promising physicochemical and pharmacological properties, confirming its potential as an effective herbal skincare product.

The transepidermal water loss (TEWL) assessment demonstrated the creams efficacy in reducing moisture loss over time.

Introduction

The growing consumer desire for plant-based skincare products has sparked an increased interest in researching the medicinal properties of diverse botanical components. Among these, *Salvia hispanica* seeds (*Salvia hispanica L.*) and *Aloe vera* (*Aloe barbadensis Miller*) have received a lot of attention for their diverse bioactive components and demonstrated skin health advantages [1]. *Salvia hispanica* seeds, a nutrient-dense superfood, contain high levels of important fatty acids, particularly omega-3,

along with antioxidants, vitamins, and minerals, all of which have proven to aid in skin barrier function, hydration, and healing. External stimuli constantly subject the skin, the largest organ in the human body, to dryness, irritation, and damage. As a result, formulations that provide hydration, healing, and anti-inflammatory benefits are in high demand [2]. Combining *Salvia hispanica* seed extract and *Aloe vera* cream provides a unique opportunity to create a multifunctional formulation that tackles these common skin issues while taking advantage of the synergistic effects of both components [3].

Salvia hispanica seeds contain polysaccharides that, when moistened, produce a cream-like structure, increasing the skin's water retention capacity, while antioxidants protect the skin from oxidative stress. *Aloe vera*, recognized for its high vitamin, enzyme, and amino acid content, not only hydrates but also speeds up wound healing by encouraging cell regeneration and lowering inflammation. When combined, these components may provide an herbal and effective treatment for enhancing skin hydration, reducing inflammation, and boosting skin restoration [4].

The purpose of this study is to develope and evaluates cream based on *Salvia hispanica* seeds and *Aloe vera*, with a focus on skin hydration and healing characteristics. Authors conducted *in vitro* tests to analyse the physicochemical properties, stability, and efficacy of the formulation. This study adds to the expanding amount of information supporting plant-based skincare formulations and their potential role in improving skin health through herbal and sustainable methods [5, 6].

Methods

Materials

Material

Salvia hispanica seeds procured from the sudarshan scientific, Nashik, India and Authenticated by the HH Sri Sri Murlidhara Swamiji College of Horticulture, Malegaon, Nashik, India (Ref. No MGV/HHSSM-SCOH/410/2024-25). All required solvents and procured from the Locha Chem, India. *Aloe vera* is collected from the medicinal garden and authenticated by the Department of Pharmacognosy, SSS's Divine College of Pharmacy in Satana, Nashik (India) (Ref. No DCOP/PG/004/2023-24, Dated 20/02/2023 and GPS Location https://maps.app.goo.gl/LicmfoiGtqqAUNTn6)

Procedure

Extraction for *Salvia hispánica* Mucilage

Clean the seeds with a fine sieve or distilled water. Dry them in air before using. Weigh 10 g of chia seeds for soaking. Stir gently and let the seeds soak at room temperature for 2–4 h to hydrate and expand the mucilage. After soaking, gently stir the mixture with a manual stirrer to separate the mucilage from the seed. To remove seed husks from mucilage, filter the mixture through a fine mesh sieve, cheese cloth, or muslin cloth. Refilter as needed to increase clarity. Heat the mucilage extract in a water bath or pan on low heat (40–50°C) to reduce water content if it is excessively dilute. Prevent degradation by not overheating. Pre-heat the oven to 40–50°C for drying. To preserve mucilage function, keep the temperature below 50°C. Pour mucilage onto a parchment- or silicone-lined flat drying tray. Spread it thinly to dry evenly. Dry the mucilage on the tray in the oven. Depending on layer thickness and wetness, the process may take 4–8 h. Check occasionally to avoid overheating or drying. When dried and brittle, mucilage is ready. Remove dry mucilage from tray. Use a mortar and pestle or grinder to pulverize it. Store powdered mucilage in an airtight container. Keep cool, dry, and out of direct sunlight and moisture [7, 8].

Extraction of *Aloe Vera*

Run water over the leaves of fresh, thick, mature aloe vera to remove dirt and debris. Trim leaf bases and tips. Cut the side spines with a knife. Trimmed leaves should stand upright in a jar for 15–30 min to drain the yellow sap (aloin), which can irritate skin and digestion. Use a knife or peeler to delicately remove the green skin to reveal the clear aloe vera gel. Scrape out the inner gel using a spoon or knife, avoiding skin and sap. Put the gel in a clean container. Add a little distilled water to make it usable. A

pinch of activated charcoal can eliminate pollutants and improve clarity. Make a charcoal stove or fire. Put the aloe vera gel mixture in a stainless steel or heat-resistant container over heat. Heat the mixture gently, stirring occasionally. Never boil aloe vera because the beneficial components deteriorate. Heat for 15–20 min below 60°C to preserve characteristics of gel. To remove charcoal and undissolved particulates, filter it through a fine mesh strainer or muslin cloth. Put filtered aloe vera gel in clean, sealed containers. Refrigerate for a week or freeze for longer [9, 10].

Phytochemical Evaluation

1. **For Identification of Alkaloids**

Mayer's Test: Mix 1 ml of the extract with a few drops of Mayer's reagent (potassium mercuric iodide). When alkaloids are present, look for a white or creamy precipitate.

Dragendorff's Test: Mix 1 ml of the extract with a few drops of the reagent (potassium bismuth iodide). A reddish-brown precipítate confirms the presence of alkaloids.

2. **For Identification of Carbohydrates**

Molisch's Test: Mix 1 ml of the extract with two to three drops of Molisch's reagent (α-naphthol in ethanol). Without mixing, carefully place a few drops of strong sulfuric acid around the test tube's side. A violet ring at the interface indicates the presence of carbohydrates.

3. **For Identification of Steroids**

Salkowski's Test: Dissolve a tiny quantity of extract in 2 ml of chloroform. By the tube's side, add 2 ml of sulfuric acid concentrate. Look for signs of steroids, such as a yellow-green fluorescence and a red hue in the chloroform layer.

4. **For Identification of Proteins**

Biuret Test: Mix the extract with 1 ml of 1 % copper sulphate ($CuSO_4$) solution and 1 ml of 10 % sodium hydroxide. Mix well, then look for a violet or pinkish tint, which is a sign that proteins are present.

5. **For Identification of Phenols**

Ferric Chloride Test: Add a few drops of 1 % ferric chloride solution to a tiny amount of the extract. A blue, green, or purple colouring indicates the presence of phenolic chemicals.

6. **For Identification of Tannins**

Ferric Chloride Test: Mix the extract with a few drops of a 5 % ferric chloride solution. Tannins are indicated by a dark blue or green-black colouring.

7. **For Identification of Saponins**

Foam Test: For 15 min, vigorously shake 2 ml of the extract with 5 ml of distilled water. Saponins are present if steady foam forms and lasts for 15 to 20 min.

8. **For Identification of Flavonoids**

The Shinoda Test: Mix 1 ml of the extract with a few magnesium turnings. A few drops of strong hydrochloric acid should be added carefully. Flavonoids are indicated by a pink, orange, or red hue.

9. **For Identification of Glycosides**

Borntrager's Test: Bring the extract to a boil with diluted sulfuric acid and then let it cool and strain it. Separate the organic layer by shaking the filtrate after adding an equivalent volume of benzene or chloroform. Apply a solution of ammonia to the organic layer. Glycosides are present when the ammoniacal layer turns pink or crimson.

10. **For Identification of Mucilage**

Ruthenium Red Test: Mix 1 ml of the extract with a few drops of Ruthenium Red solution. Keep an eye out for any red or pink colouring, which denotes the presence of mucilage [11, 12].

Formulation Table

The foumulation of cream containing *Salvia hispanica* and *Aloe vera* described in table 1.

Table 1. Formulation (F2) of *Salvia hispanica* and *Aloe vera* Cream

Sr. No	Ingredient	Quantity	Uses
1	*Aloe vera* Cream	30 ml	Moisturizing and Soothing Agent
2	*Salvia hispanica* Mucilage	10 g	Hydrating, Exfoliating
3	Shea Butter	15 g	Emollient
4	Coconut Oil	8 ml	Moisturizing, CarierOil
5	Emulsifying Wax	5 g	Emulsifier
6	Glycerin	10 ml	Humectant, Hydrating Agent
7	Jojoba Oil	5 ml	Nourishing
8	Tea TreeOil	1 ml	Preservative
9	Rose Oil	5 ml	Hydrating
10	Preservative	1 g	Preservative
11	Distilled Water	Q. S	Solvent, Hydration Base

Manufacturing Process

Phase A (Oil Phase): In a heat-resistant container, combine shea butter (15 g), coconut oil (8 g), jojoba oil (10 g), and emulsifying wax (5 g). Heat gently in a double boiler until completely melted.

Phase B (Water Phase): In a separate container, mix *Aloe vera* (30 g), glycerine (10 g), and distilled water. Heat this phase to a similar temperature as the oil phase to allow emulsification.

Combine Phases: Slowly add the water phase (Phase B) to the oil phase (Phase A), stirring continuously to form an emulsion. Continue stirring until the mixture cools and thickens.

Add Active Ingredients and Preservatives: Once the emulsion has cooled to 40°C or below, add Chia seed Extract (10 g), tree tea Oil (1 g), rose oils (5 g), and Preservative (1 g). Stir well to ensure all ingredients are evenly dispersed.

Packaging: Transfer the cream into airtight containers and store in a Cool, Dry place. Multiple batches are prepared and stored at room temperature until further use [13, 14].

Evaluation Of Moisturizing Cream

Physical Evaluation

a. Color: A visual inspection revealed the cream's color.
b. Odor: It was discovered that the smell of cream was distinctive.
c. State: A visual examination revealed that the state was cream.
d. Consistency: Cream was manually rubbed on the hand to test the formula's consistency. The consistency of the cream is smooth.
e. pH: The pH of the cream was measured using a digital pH meter. The cream solution was made with 100 ml of distilled water and allowed to sit for 2 h. Three measurements of the solution pH were made, and the average was calculated.
f. Spredability: Separability is the ability to be separated or divided from something. It can also refer to the state of being disconnected. Better Spredability has been shown by reduced separation times between the two slides.
g. Washability: after applying the formulation to the skin, the ease of washing with water was determined.

h. Non-irritancy test: The formulation of herbal cream was assessed for non-irritancy. There was no redness or irritation in the preparation.

i. Viscosity: Using a Brooke field viscometer set to 25°C and spindle number 63 at rpm, the viscosity of the cream was measured.

j. Phase separation: A suitable wide-mouth container was used to transfer the manufactured cream. After 24 h, the separation between the oil and aqueous phases was visible and was set away for storage.

k. After feel: The amount of residue and emolliency slipperiness following the application of the prescribed quantity of cream was considered to be satisfactory. Table 2 displays the observation of all parameters [15, 16].

Trans Epidermal Water Loss

Clean the testy area with distilled water and let it dry for 30 min. Before testing, mark the test site. Baseline measurement Use the TEWL device to measure baseline water loss before applying the cream. Record at least three consistent readings. Apply a measurement amount of cream allows cream to absorb. Measure TEWL at regular intervals (30 min, 1 h, 2 h) [17].

$$TEWL\ \% = \frac{Baseline\ Treatment - Post\ Treatment}{Baseline\ Treatment} \times 100$$

Pharmacological Evaluation:

Antioxidant Activity

Preparation of Phosphate Buffer Saline (pH 7.4)

To prepare phosphate-buffered saline (PBS) with a pH of 7.4: Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate, and 8.0 g of sodium chloride in sufficient water to make a total volume of 1000 ml. Adjust the pH as necessary to achieve the desired level of 7.4.

Preparation of 40 mM hydrogen peroxide: To prepare a solution, 0.228 ml of hydrogen peroxide (H_2O_2) is dissolved in 50 ml of phosphate buffer with a pH of 7.4.

Procedure: The study assessment the hydrogen peroxide scavenging ability of newly prepares *Salvia hispanica* cream using a standard assay. Initially a 40 mM hydrogen peroxide solution was prepared in a 10 % phosphate buffer saline at pH 7.4 different concentration of cream (ranging from 10 to 1000 ppm were dissolved in water and then mixed with the hydrogen peroxide solution. After 10 minutes, the absorbance at 230 nm was measured against blank solution to determine the scavenging activity. Ascorbic acid served as the control standard in this experiment, which were performed in triplicate under dark condition to prevent light induced degradation [18, 19]. The percentage of hydrogen peroxide scavenged was then calculated using a specific formula.

Results And Discussion

Evaluation of Formulated Cream containing *Salvia hispanica* and *Aloe vera:*

Evaluation Parameter of Formulated cream described in table 2

Table 2. Evaluation Parameter of Formulated cream

Sr. No.	Parameter	Observation
1	Appearance	White withgreen tones
2	Aroma	Sweet and floral
3	Physical Form	Semi-solid
4	Texture	Smooth and uniform
5	Spredability	7.5 g.cm/s
6	Ease of Washing	Easilywashable
7	Post-Application Feel	Leaves an emollient effect
8	pH Value	5.5
9	Irritancy Assessment	Non-irritating
10	Phase Stability	No separation observed
11	Viscosity	10,500 Pa

The cream composed of *Aloe vera* cream and *Salvia hispanica* seed mucilage, showed a semi-solid state with a smooth, emollient after-feel. The pH of the cream was measured at 5.5, ensuring its compatibility with skin pH. The Spredability was found to be 7.5 g/cm, indicating good ease of application. No phase separation was observed, indicating a stable emulsion. The non-irritancy test confirmed that the cream is safe for topical use, showing no signs of redness or irritation. The cream was easy to wash off, and its viscosity 10500 Pa, tested using a Brookfield viscometer, supported the semi-solid consistency and smooth application.

Physicochemical and Phytochemical evaluation of *Salvia hispanica* Mucilage and *Aloe vera* cream

Table 3. Physicochemical and Phytochemical evaluation of *Salvia hispanica* Mucilage and *Aloe vera* cream

Sr. No	Parameter	Observation	
		***Salvia hispanica* Mucilage**	***Aloe vera* cream**
1	Colour	Greyish White	White Transparent
2	Test	Mucilaginous	Better
3	Odour	Odourless	Odourless
4	Appearance	Flaky Appearance	Transparent
5	Solubility	Turbid Solution in Water	Water Soluble
6	pH	6	5.5

Sr. No	Parameter	Observation	
		***Salvia hispanica* Mucilage**	***Aloe vera* cream**
7	Test For Alkaloids	+ Ve	-
8	Test For Carbohydrate	+ Ve	-
9	Test For Steroid	+Ve	- Ve
10	Test For Proteins	+ Ve	-
11	Test For Phenol	- Ve	-
12	Test For Tannins	+ Ve	-Ve
13	Test For Saponins	- Ve	+ Ve
14	Test For Flavonoid	+ Ve	-/+ Ve
15	Test For Glycoside	+ Ve	- Ve
16	Test For Mucilage	+ Ve	-
17	Test For Terpenoid	-	+ Ve

The *Salvia hispanica* seed mucilage exhibited a greyish-white colour and flaky appearance. It formed a turbid solution in water and had a pH of 6. The tests for alkaloids, carbohydrates, steroids, proteins, tannins, flavonoids, and glycosides were positive. The negative results for phenols and saponins suggest that the mucilage lacks these secondary metabolites, while the positive tests for mucilage and other phytochemicals confirm its suitability for use in cosmetic formulations.

Aloe vera cream appeared white and transparent, with a pH of 5.5. It was water-soluble and tested positively for terpenoids, tannins, and saponins, while it tested negatively for cardiac glycosides, steroids, and Phlobotannins. Its transparency and chemical properties make it a good candidate for use in moisturizing and hydrating formulations (Table 3).

Trans Epidermal Water Loss

The TEWL Test was performed and the result in table 4 indicated changes in TEWL over a 4 h period after cream application. TEWL the baseline is 14g/m^2/h. after 30 min is showing 14.28 %, after 1 h shows 21.42 %, at the 4 h shows 13g/m^2/h shown in Figure 1.

Table 4. Result of Trans Epidermal water loss

Time	Baseline TEWL	Post application TEWL	% TEWL	SD	SE	*P* value
0 min	14	-	-	-	-	-
30 min	-	12	14.28	1	0.5774	0.0742
1 h	-	11	21.42	1	0.5774	0.0351
2 h	-	10	28.57	1	0.5774	0.0202
4 h	-	13	7.14	1	0.5774	0.2254

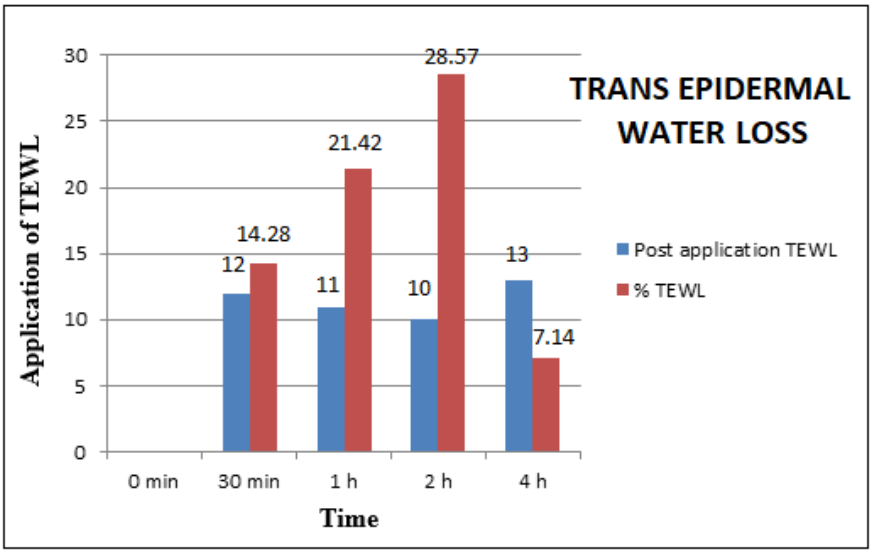

Figure 1. Graphical representation of results of trans epidermal water loss

Pharmacological Evaluation

Antioxidant Activity

The absorbance values and percentage inhibition data highlight the scavenging activity of the test sample, standard, and control across concentrations. At 10 ppm, the test sample showed 95.18 % scavenging activity, closely matching the standard's 97.16 %. Both exhibited a decline in inhibition at higher concentrations, with the test sample showing 90.22 % at 50 ppm and the standard 87.74 %. These results indicate the test sample having strong and consistent scavenging potential, nearly comparable to the standard across all concentrations (Table 5 and figure 2).

Table 5. Effect of component on Radial scavenging Activity

Concentration	Absorbance			% Scavenging Activity		
	Control	Standard	Test	Control	Standard	Test
10 ppm	5.043	0.143	**0.243**	0	97.16	**95.18**
20 ppm	5.043	0.347	**0.280**	0	93.11	**94.44**
30 ppm	5.043	0.446	**0.355**	0	91.15	**92.96**
40 ppm	5.043	0.609	**0.423**	0	87.92	**91.61**
50 ppm	5.043	0.618	**0.493**	0	87.74	**90.22**

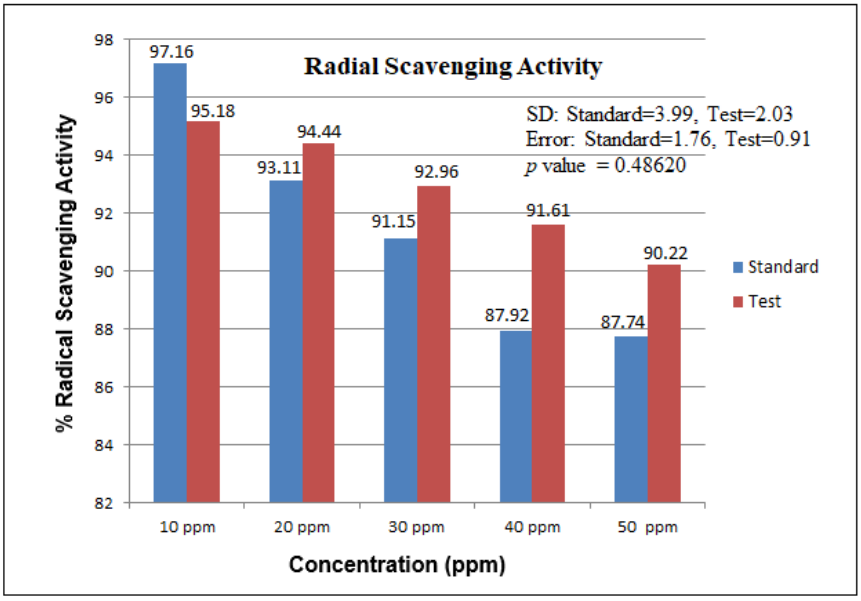

Figure 2. Graphical Representation of % Inhibition of scavenging activity

Discussion

Zia-ud *et al.*, (2021) reported that chia seeds are nutritious and healthy. They include antioxidant phytochemicals that reduce cholinesterase and may prevent neurodegenerative diseases. Minerals, omega-3s, fibers, and proteins in seeds boost skeletal muscle insulin sensitivity and lipid metabolism. Chia seeds boost omega-3s and lower saturated fat, which are essential for a balanced diet. The high fiber content of chia seeds can prevent many diseases. Products with chia seeds may be healthier and more functional [20, 21].

In this study, the formulated moisturizing cream containing *Salvia hispanica* seed extract and *Aloe vera* exhibited promising physicochemical and pharmacological properties, confirming its potential as an effective herbal skincare product. The physical evaluation demonstrated that the cream possessed a semi-solid consistency with a smooth texture, a sweet and floral aroma, and an emollient after-feel. The pH value of 5.5 aligns well with the natural pH of the skin, ensuring compatibility and minimizing the risk of irritation. The ease of washing and absence of phase separation further indicate good formulation stability, an essential attribute for commercial skincare products. The viscosity of 10,500 Pa supports the cream semi-solid state, which facilitates uniform application and absorption.

The phytochemical analysis of *Aloe vera* cream confirmed the presence of beneficial compounds such as terpenoids, tannins, and saponins, which contribute to its soothing and moisturizing effects. The physicochemical characterization of *Salvia hispanica* mucilage revealed a greyish-white, flaky appearance with mucilaginous properties. It formed a turbid solution in water and exhibited a pH of 6, making it compatible with the skin.

The trans-epidermal water loss (TEWL) assessment demonstrated the cream's efficacy in reducing moisture loss over time. At baseline, TEWL was recorded at 14 g/m^2/h, which decreased significantly after application, reaching 10 g/m^2/h at the 2 h mark. This reduction indicates enhanced skin hydration, which is a crucial parameter for an effective moisturizing cream. The statistical analysis further supported the significance of the observed changes, with *p* values confirming the reliability of the results.

The antioxidant activity of the cream, assessed through its radical scavenging activity, indicated that it had strong free radical neutralization potential. The test sample showed 95.18 % scavenging activity at 10 ppm, which was comparable to the standard (97.16 %). Even at higher concentrations, the test formulation maintained substantial antioxidant potential, with 90.22 % scavenging activity at 50 ppm. This demonstrates the cream's potential in protecting the skin against oxidative stress, which is a key factor in premature aging and skin damage.

Conclusion

The research shows that both *Salvia hispanica* and *aloe vera* are powerful natural ingredients. The physicochemical tests indicated that chia seed extract forms a turbid solution in water and has a relatively low pH, indicating its potential for skin-care applications. The cream formulation, which combined chia seed mucilage and aloe vera, demonstrated excellent physical properties with a smooth consistency, emollient after-feel, and stability under different conditions. The pH of cream was measured at 6, which is compatible with the skin's natural pH. In pharmacological evaluations, the cream exhibited promising antioxidant activity. The cream closely matched the standard antioxidant in the antioxidant test, exhibiting a scavenging activity of 95.18 % at 10 ppm.

References

1. Enes BN, Moreira LD, Toledo RC, Moraes ÉA, de Castro Moreira ME, Hermsdorff HH, Noratto G, Mertens-Talcott SU, Talcott S, Martino HS. Effect of different fractions of chia (*Salvia hispanica* L.) on glucose metabolism, in vivo and in vitro. J Funct Food. 2020;71:104026. Doi: 10.1016/j.jff.2020.104026

2. Din ZU, Alam M, Ullah H, Shi D, Xu B, Li H, Xiao C. Nutritional, phytochemical and therapeutic potential of chia seed (*Salvia hispanica* L.). A mini-review. Food Hydrocoll Health. 2021;1:100010. Doi: 10.1016/j.fhfh.2021.100010

3. Güzel S, Ülger M, Özay Y. Antimicrobial and antiproliferative activities of chia (*Salvia hispanica* L.) seeds. Int J Sec. Meta. 2020;7(3):174-80. Doi: 10.21448/ijsm.722574

4. Lucas-González R, Roldán-Verdu A, Sayas-Barberá E, Fernández-López J, Pérez-Álvarez JA, Viuda-Martos M. Assessment of emulsion creams formulated with chestnut (*Castanea sativa M*.) flour and chia (*Salvia hispanica* L) oil as partial fat replacers in pork burger formulation. J Sci Food Agric. 2020; 100(3):1265-73. Doi: 10.1002/jsfa.10138

5. Ebrahim R, Abdelrazek A, El-Shora H, El-Bediwi AB. Effect of ultraviolet on molecular structure and photochemistry compounds for *Salvia hispanica* medical seeds. Egypt Acad J Biolog Sci. (D-Histology and histochemistry) 2022;14(1):127-35. Doi: 10.21608/eajbsd.2022.232507

6. Guiotto EN, Tomás MC, Haros CM. Development of highly nutritional breads with by-products of chia (*Salvia hispanica* L.) seeds. Food. 2020;9(6):819. Doi:10.3390/foods9060819

7. Oteri M, Bartolomeo G, Rigano F, Aspromonte J, Trovato E, Purcaro G, Dugo P, Mondello L, Beccaria M. Comprehensive chemical characterization of chia (*Salvia hispanica* L.) seed oil with a focus on minor lipid components. Food. 2022;12(1):23.Doi: 10.3390/foods12010023

8. Motyka S, Koc K, Ekiert H, Blicharska E, Czarnek K, Szopa A. The current state of knowledge on *Salvia hispanica* and Salviae hispanicae semen (chia seeds). Mole. 2022;27(4):1207.doi:10.3390/molecules27041207

9. Banan ZM, Yaghobfar A, Mojab F. The chemical composition of Salvia macrosiphon seed. Iran J Pharm Sci. 2023;19(2):166-75. Doi: 10.22037/ijps.v19i2.43809

10. Dziadek K, Kopeć A, Dziadek M, Sadowska U, Cholewa-Kowalska K. The changes in Phytochemical compounds and antioxidant activity of chia (*Salvia hispanica* L.) herb under storage and different drying conditions: A comparison with other species of sage. Mole. 2022;27(5):1569. Doi: 10.3390/molecules27051569

11. Madaan R, Bala R, Zandu SK, Singh I. Formulation and characterization of fast dissolving tablets using *Salvia hispanica* (chia seed) mucilage as superdisintegrant. Acta Pharm Sci. 2020;58(1):69-82. Doi: 10.23893/1307-2080.APS.05805

12. Saleh HE, Chiad JS, Shawkat SM. Extraction, Characterization, and Evaluation the Activity of Chia Seed (*Salvia hispanica* L.) as an Antibacterial for the Treatment of Gingivitis. Iraqi J Industr Res. 2022;9(3):110-8. Doi: 10.53523/ijoirVol9I3ID216

13. Ramakrishna S, Gopikrishna UV. Formulation and Evaluation of Herbal Hair Cream. SchInt J Tradit Complement Med. 2022; 5(2):28-32. Doi: 10.36348/ssijtcm.2022.v5i02.002

14. Kulkarni AT, Agarkar BS, Sawate AR, Kshirsagar RB. Determination of physicochemical properties of chia seeds (*Salvia hispanica* L.). J Pharmacogn Phytochem. 2020;9(2):1858-61. Doi: NA

15. Sonawane PR, Sonawane SN, Aher SN, Surana KR, Patil VR, Mahajan SK, Patil DM. In silico Evaluation of Anti-Inflammatory Potential of Pyrimidine based Molecules. Adv Biores. 2024;15(4):196-207. Doi: 10.15515/abr.0976-4585.15.4.197206

16. Surana KR, Jadhav PS, Shewale HS, Wagh DB, Mahajan SK, Musale JV. Insilico and Biological Evaluation of Anti-Inflammatory Activity of synthesized Benzimidazoles Derivatives. Biosci Biotechnol Res Asia. 2024;20(3):1241-53. Doi: 10.13005/bbra/330

17. Fakir JS, Ahire CM, Surana KR, Kalam A, Ahamad AA, Davanage MD. Formulation and Evaluation of Antibacterial and Anti-Inflammatory Emulgel Containing Eugenia caryophyllus Buds Extract. Biosci Biotechnol Res Asia. 2024;21(3):1183-96.Doi: 10.13005/bbra/3296.

18. Li J, Fan G, Chen Y, Luo J, Zhang R. Optimization and characterization of antioxidant-rich cream incorporating *Aloe vera* gel. J Cosmet Dermatol. 2023;22(2):221-228. Doi: 10.1111/jocd.15267

19. Din ZU, Alam M, Ullah H, Shi D, Xu B, Li H, Xiao C. Nutritional, phytochemical and therapeutic potential of chia seed (*Salvia hispanica* L.). A mini-review. Food Hydrocoll Hlth. 2021;1:100010 Doi: 10.1016/j.fhfh.2021.100010

20. Guzel KS, Ulger M, Kahraman A. Phytochemical analysis, antioxidant and antimicrobial activities of Salvia virgata mericarps. Bot Serb. 2021;45(2):223-31. Doi: 10.2298/BOTSERB2301019G

21. Madaan R, Bala R, Zandu SK, Singh I. Formulation and characterization of fast dissolving tablets using *Salvia hispanica* (chia seed) mucilage as superdisintegrant. Acta Pharm. Sci. 2020;58(1):69-82. Doi: 10.23893/1307-2080.APS.05805

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.31529
Artículos originales

Formulation and evaluation of stiripentol oral suspension

Formulación y evaluación de la suspensión oral de estiripentol

Ujjwala Kandekar[1] 0000-0003-3520-9675
Dinesh Bitale[1]
Limbaji Chounde[1]
Ajit Gaikwad[2]

[1]Department of Pharmaceutics, JSPM's Rajarshi Shahu College of Pharmacy & Research Tathawade, Pune Maharashtra, India

[2]Formulation Research Scientist, Callidus Research Laboratories Pvt. Ltd. Pune, Maharashtra, India

Correspondence

Ujjwala Kandekar
Ujja2303@gmail.com

Received: 09.09.2024
Accepted: 26.03.2025
Published: 20.06.2025

Funding

This work has not been funded by any government and non-government organization.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

We would like to thank Rajarshi Shahu College of Pharmacy and Research, Tathawade, Pune and Callidus Research Laboratories, Pune for availing research facilities to carry out current research work.

Resumen

Introducción: El trabajo de investigación se llevó a cabo para desarrollar una suspensión estable y eficaz de estiripentol para el tratamiento de la epilepsia.

Método: Se utilizó el estudio FT-IR para evaluar la compatibilidad entre el fármaco y el excipiente y también se examinó la apariencia física de la mezcla del fármaco y el excipiente después de un mes de estudio de estabilidad. La suspensión se preparó mediante un agitador mecánico y se evaluó la viscosidad, el pH, el volumen de sedimentación, el potencial zeta y los estudios de liberación de fármaco in vitro. La formulación optimizada se evaluó adicionalmente para determinar el potencial zeta y el índice de polidispersidad.

Resultados: Los resultados de FT-IR confirmaron la compatibilidad del fármaco y los excipientes. La apariencia física de la mezcla no se alteró durante las condiciones de almacenamiento aceleradas. La viscosidad, el pH y el volumen de sedimentación de la formulación oscilaron entre 22,92 ±1,2 cPs y 54,8 ± 2,1 cPs; 5,32 ± 0,04 y 6,01 ± 0,1 y 83,19 ± 0,9 % y 98,87 ± 1,2 % respectivamente. El potencial zeta y el índice de polidispersidad de la formulación optimizada fueron -52,1 mV y 0,198 respectivamente. Estos resultados fueron indicativos de una suspensión monodispersa estable. La formulación optimizada fue estable a temperaturas y humedad más altas y en presencia de luz, lo que indica una buena vida útil.

Conclusiones: El estudio demostró que la suspensión oral de estiripentol puede formularse como una forma farmacéutica estable y eficaz para el tratamiento de la epilepsia.

Palabras clave: Estiripentol, suspensión, viscosidad, volumen de sedimentación, potencial zeta

Abstract

Introduction: The current research work was carried out to develop a stable and effective suspension of stiripentol for treating epilepsy.

Method: FT-IR study was used to evaluate the compatibility between drug and excipient and the physical appearance of drug and excipient mixture was also examined after one month of stability study. The suspension was prepared by a mechanical stirrer and evaluated for viscosity, pH, sedimentation volume, zeta potential and in-vitro drug release studies. The optimized formulation was further evaluated for zeta potential and polydispersity index.

Results: FT-IR results confirmed the compatibility of drug and excipients. The physical appearance of the mixture was not altered during the accelerated storage conditions. Viscosity, pH and sedimentation volume of formulation ranged between 22.92 ±1.2 to 54.8 ± 2.1 cPs, 5.32 ± 0.04 to 6.01 ± 0.1 and 83.19 ± 0.9 % to 98.87 ± 1.2 % respectively. The zeta potential and polydispersity index of the optimized formulation was -52.1 mV and 0.198 respectively. These results were indicative of stable monodispersed suspension. The optimized formulation was stable at higher temperatures, humidity and in the presence of light, indicative of good shelf-life.

Conclusions: The study demonstrated that stiripentol oral suspension can be formulated as a stable and effective dosage form for the treatment of epilepsy.

Keywords: Stiripentol, suspension, viscosity, sedimentation volume, zeta potential

Highlights

Stiripentol is recommended to treat Dravets syndrome in infants. In market it is available as capsule and powder for reconstitution which is not convenient for administration in infants.

It belongs to BCS class II; hence solution formulation is difficult to develop. The suspension formulation of the drug was developed for ease of administration.

Different formulations were prepared and optimized formulation was selected on the basis of drug release. Optimized formulation was found stable and suitable for oral administration.

Introduction

Epilepsy is the most common, heterogeneous neurological disorder; globally around five million individuals are diagnosed each year. Unpredictable and re-current interruptions of normal brain functions characterize it. It results from the imbalance between excitatory and inhibitory neurotransmitters with-

in certain central nervous system regions (CNS). It is associated with psychological comorbidity, mild degree of convulsions and short-term loss of consciousness. The risk of premature death in epileptic patients is up to three times higher than common population [1]. It might be a result of genetics, brain tumor, brain injury, bacterial or viral meningitis/viral encephalitis, stroke etc. Epilepsy requires prolonged treatment that might be extended to the patient's entire life. Despite inventive approaches to seizure control, administration of antiepileptic drugs remains integral [2]. Various antiepileptic drugs such as have been used to treat epilepsy. Depending on the type of seizure various antiepileptic drugs are available to treat epilepsy such as valproic acid, lamotrigine, phenytoin, carbamazepine. Among them, stiripentol is a novel orally active antiepileptic drug structurally dissimilar to other antiepileptics and exhibits both in-direct and direct anticonvulsant activity. It stimulates ɤ- amino butyric acid (GABA) transmission by augmenting its release, restricts synaptosomal uptake and hinders GABA transaminase-mediated degradation of GABA. It has been approved by the European Medicines Agency (EMEA) in 2008 as an adjuvant therapy with clobazam and valproate to treat Dravet's syndrome. It is used as adjuvant therapy in combination with other antiepileptics as it also exhibits the potential to restrict cytochrome P450 [3]. The anticonvulsant activity of stiripentol is age-dependent and it was found more effective in younger kids [4]. Chiron et al. (2023) have reported real-world retrospective study regarding effectiveness of stiripentol before 2 year of age in patients with Dravet syndrome. Stiripentol was introduced alongside valproate and clobazam in 93 % of cases at the age of 13 months, with a median dosage of 50 mg/kg per day. They have concluded that stiripentol treatment is both safe and advantageous, leading to a substantial decrease in prolonged seizures, such as status epilepticus, as well as a reduction in hospital admissions and mortality rates during the crucial early years of life [5]. Wheless and Weatherspoon (2025) overviewed stiripentol in detail with respect to mechanism, efficacy, side effects and tolerability, prescribing and dosing consideration, combined therapy with stiripentol [6].

Currently, 205 mg and 500 mg capsules and powder for reconstitution of stiripentol are available in the market. For ease of consumption in pediatrics or infants, it is utmost necessary to develop an oral liquid formulation of stiripentol. The current research work was focused on the development of stable oral suspension of stiripentol.

Material and methods

Material

Stiripentol was purchased from Nuray Chemicals Pvt Ltd., India, Sodium Lauryl Sulphate was obtained from BASF India Ltd, Methyl Paraben, Sucralose, Citric acid monohydrate and Aspartame were procured from Merck Life Science Pvt Ltd., Sodium Carmellose was purchased from Ashland India Pvt, Veegum K was obtained from Vanderbilt Minerals, LLC. Sodium Citrate was obtained from Finar Limited, India. Lemon Flavour was procured from FONA International Inc. All other materials, reagents and chemicals used were of analytical grade.

Methods

Drug-excipient compatibility study by FTIR

The spectra of stiripentol, physical mixture of stiripentol and excipients were recorded by FTIR equipment (Apha Bruker, Germany) using an attenuated total reflectance (ATR) accessory [7]. For this purpose, drug-excipient mixtures dry (excipients mixed in a 1:1 ratio) were packed in glass vials, stoppered with a butyl rubber stopper and sealed with aluminium caps. These packed samples were then exposed to 60 °C and 40 °C/ 75 % RH for 30 days. Then samples were withdrawn and scanned by FTIR and analysed for visual appearance. Single spectra of each sample were collected in wavelength range from 4000 to 400 cm^{-1} by averaging 10 scans at a resolution of 4 cm^{-1}.

Formulation of suspension

Different formulations were prepared by varying the amount of sodium lauryl sulphate, crosscarmallose sodium and Veegum as listed in Table 1. All materials were weighed accurately using a calibrated weighing balance (Sartorious MCA225P-20IN-U-QP1, Germany) and shifted through a 30 # sieve using a vibratory shifter (S.S. Engineering Works, India).

Preparation of suspending and thickening agent dispersion

Veegum K and carmellose sodium were added in a sufficient quantity of purified water and stirred at 3500 rpm by homogenizer (Remi, India) for 180 min to generate smooth slurry (Solution A).

Preparation of preservative solution

Purified water was heated up to a temperature of 70 °C to 80 °C and methyl paraben was added in heated water under continuous stirring till a clear solution was obtained. Solution was cooled up to 50 °C to 60 °C and glycerol was added with continuous stirring. The resulting solution was added in solution A and stirring was continued for 30 min.

Preparation of buffering solution

Citric acid monohydrate and trisodium citrate dihydrate were dissolved in purified water to form a clear solution. This buffering solution was added to solution A and stirred for 30 min.

Preparation of stiripentol dispersion

An accurately weighed quantity of sodium lauryl sulfate was dissolved in water slowly with stirring at a slow speed to generate clear solution. Stiripentol was slowly added to this solution with constant stirring at 2000 rpm for 60 min to ensure complete wetting. Prepared dispersion was incorporated in solution A with stirring at 2500 rpm for 120 min. Sucrose, aspartame, sucralose, and lemon flavour were added to bulk dispersion and continuously stirred for 10 min. The pH of the formulation was checked. Purified water was added to attain final bulk volume and dispersion was homogenized at 2000 rpm.

Table 1: Formulation of suspension/ 5 ml

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Stiripentol	30	30	30	30	30	30	30	30	30
Sodium lauryl sulphate	0.110	0.110	0.075	0.038	0.038	0.038	0.110	0.110	0.038
Methyl paraben	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Glycerol	30	30	30	30	30	30	30	30	30
Carmellose sodium	0.075	0.225	0.150	0.075	0.225	0.075	0.075	0.225	0.225
Veegum K	4.8	10.8	7.8	4.8	10.8	10.8	10.8	4.8	4.8
Sucrose	60	60	60	60	60	60	60	60	60
Aspartame	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sucralose	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Lemon flavour	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Citric acid	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Sodium citrate	3	3	3	3	3	3	3	3	3
Purified water	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

Evaluation of suspension

Percentage yield of all formulations

Viscosity, pH, sedimentation volume

Viscosity of formulations was determined at 25 °C using Brookfield viscometer and circulating detector (DV2TLVTJO, Brookfield Engineering Laboratories, Germany) at 25 rpm using Spindle SC4-18. pH of stiripentol suspension was determined by pH meter (Orion Star A211, Thermo Scientific Orion, India) at 25 °C temperature [8]. Sedimentation volume (F) is the ratio of the final volume of sediment (Vu) to the original volume of sediment (Vo) before settling. The suspension (40 ml) was transferred to 50 ml measuring cylinders and the volume of sediment was noted at 24 h. Sedimentation volume (F), was calculated using the equation (1) [9].

$$F=(Vu/Vo) \quad (1)$$

Dissolution study

Drug release of suspension was carried out by using the paddle method specified in USP type II (Paddle Type) dissolution apparatus (Electrolab, India) by using 900 ml of phosphate buffer pH 6.8 and 1 % SLS as dissolution media. A suspension sample (5 ml) was transferred to the bottom of a vessel using a syringe. Paddle speed and bath temperature were set at 75 rpm and 37 ± 0.5 °C respectively. Aliquots (5 ml) were withdrawn at fixed intervals of 5, 10, 15, 30, 45, and 60 min and 5 ml buffer were added after each sample removal to maintain sink conditions. All the samples were analysed by UV-visible spectrophotometer (Shimadzu 1800, Shimadzu Corporation, Japan) at λ_{max} of 265 nm. The dissolution study was conducted in triplicate [10].

Drug release kinetic

The release kinetic of optimized batch was analyzed by different kinetic models to get into insight of probable release mechanism. Zero order kinetic model is given by equation (2), where drug release expected to release independent on concentration.

$$M_t/M_\infty = kt \quad (2)$$

Where, M_t is the cumulative released amount at time t, M_∞ is the amount of drug released at infnite time, M_t/M_∞ is the percentage of drug released at time t, k is a kinetic constant. First-order kinetic is denoted by equation (3) which represents concentration dependent release.

$$Ln(M_t/M_\infty) = kt \quad (3)$$

Higuchi and Korsemeyer-Peppas model is given by equation (4 and 5), respectively.

$$M_t/M_\infty = kt^{1/2} \quad (4)$$

$$Mt/M\infty = kt \quad (5)$$

Where, n is the release exponent which indicates the drug release mechanism.

Appearance of optimized batch

Optimized suspension was observed at weekly intervals for 4 weeks for physical changes such as crystal growth, aggregation and caking [9].

Particle size distribution and zeta potential

The size distribution (PDI) of the optimized suspension was measured by Malvern Zetasizer (Malvern Instrument, Malvern, UK). The suspension was diluted 100 folds in deionized water and transferred in a cuvette for analysis at 25°C [11].

Stability study

Stability studies were performed as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. These were conducted at storage conditions at 25 °C/60 % RH (Relative Humidity) and 40 °C/75 % RH for three months [12]. At the end of every month optimized formulation was evaluated for release of drug, viscosity and pH.

Photostability study

Pure stiripentol and optimized formulation were exposed to light providing an overall illumination of not less than 1.2 million lux h and an integrated near ultraviolet energy of not less than 200-watt h/square meter.

Statistical analysis

Data analysis and graphs were made in Microsoft® Excel® (Microsoft Office Professional Plus 2013, Microsoft Corporation, USA)

Results and Discussion

Stiripentol is a crystalline insoluble drug; hence it was formulated as a suspension. Various ingredients used in formulation were selected in the view to maintain stability, palatability and ease of handling. Sodium lauryl sulphate was selected as a surfactant as it is the most frequently used anionic surfactant and functions to reduce surface tension. Carmellose sodium was used as a viscosity-modifying agent, which alters the viscosity and enhances the stability of suspension by hindering sedimentation rate. It is water soluble, anionic, biodegradable, non-toxic and linear polysaccharide of un-hydro glucose. Besides, it is thermostable up to 140˚C, follows Newtonian flow at lower concentration (below 1 %) and non-Newtonian at high concentration (above 1 %). It do not form gelatinous, irreversible gel as that of gums [13]. Veegum K (magnesium aluminum silicate) was used as a suspending agent as it improves stability of suspension owing to its colloidal nature. It is having ability to suspend even high density particulate; it enhances re-dispersability and maintains pourability. As it is oral suspension and intended to be used in infants, organoleptic additives are integral. Aspartame, sucrose and sucralose were used as sweetening agent to mask the unpleasant taste or to increase palatability of oral suspension. Orange flavour was used for to enhance their taste and aroma. To maintain the pH of formulation during storage, citric acid monohydrate and sodium citrate were used as buffering agent in suspension.

Fourier transformation infrared spectroscopy (FTIR)

Major functional groups present in stiripentol showed characteristic peaks in FT-IR spectrum (Figure 1). FT-IR spectrum of stiripentol was characterized by a sharp peak at 3549.46 cm^{-1} corresponding to O-H group. Peaks at 2953.97 and 2890.02 cm^{-1} was indicative of H-C = C-H and aliphatic C-H respectively. Peak at 1380.75 and 861.99 cm^{-1} represented C-H ethyl and CH_3 vibrations. Peaks at 1246.35 and 796.76 cm^{-1} were corresponding to bending vibration of C-H phenyl group. Vibration of CH_2-O and C-C phenyl was detected at 1036.16 cm^{-1}. Presence of CH_3 vibration was detected at 997.06 $cm^{-1}$and 923.96 cm^{-1}. FT-IR spectra of physical mixture showed 3661.99 cm^{-1} corresponding to O-H group, Peaks of H-C = C-H and aliphatic C-H were found at 2925.22 and 2854.96 cm^{-1} respectively. C-H ethyl and CH_3 vibrations were present at 1367.36 and 856.44 cm^{-1} respectively. Peaks at 1220.73 and 795.29 cm^{-1} were confirmed bending vibration of C-H phenyl group. Peaks of CH_2-O and C-C phenyl vibrations were detected at 1035.95 cm^{-1}. CH_3 vibrations were detected at 998.65 cm^{-1} and 920.64 cm^{-1}. The presence of FT-IR peaks corresponding to stiripentol was indicative of compatibility of drug with added excipients [14].

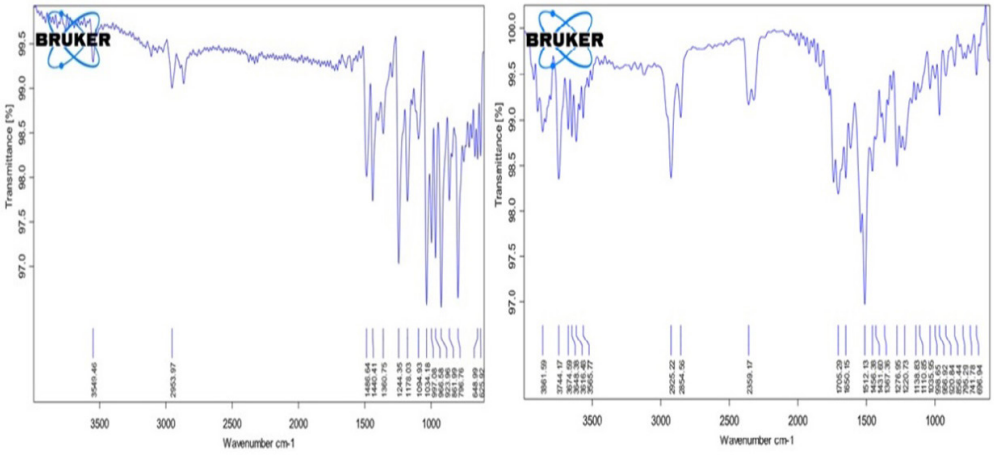

Figure 1. A) FTIR of pure drug and B) physical mixture of excipients

Physicochemical appearance for drug-excipient compatibility study

Mixtures of drug and excipients were stored at higher temperature and humidity to test the compatibility and examined visually for appearance at the end of one month. List of excipient and ratios along with compatibility study results are summarized in Table 2.

Table 2: Physicochemical appearance for drug-excipient compatibility study.

Sr. No	Description	Ratio	Initial	40°C/75 % RH (4 week)	60°C/75 %RH (4 week)
1	Stiripentol	Stiripentol 500 mg: 0 mg	Off white powder	No significant change	No significant change
2	Stiripentol: sodium lauryl sulphate	500 mg:10mg	Off white powder	No significant change	No significant change
3	Stiripentol: methyl paraben	500 mg:10 mg	Off white powder	No significant change	No significant change
4	Stiripentol: glycerol	500 mg: 1000 mg	Off white powder	No significant change	No significant change
5	Stiripentol: carmellose sodium	500 mg:10 mg	Off white powder	No significant change	No significant change
6	Stiripentol: veegum K	500 mg: 200 mg	Off white powder	No significant change	No significant change
7	Stiripentol: sucrose	500 mg: 2000 mg	Off white powder	No significant change	No significant change
8	Stiripentol: aspartame	500 mg:10 mg	Off white powder	No significant change	No significant change
9	Stiripentol: sucralose	500 mg:30 mg	Off white powder	No significant change	No significant change
10	Stiripentol: citric acid monohydrate + sodium citrate	500 mg:10 mg:50 mg	Off white powder	No significant change	No significant change
RH= Relative Humidity					

No significant changes were observed in appearance of all samples up to period of 30 days when stored at 60 ^{0}C and at 40 °C/75 % RH. In addition to FT-IR this test also confirmed the compatibility between drug and excipients.

Viscosity

Key criteria for oral liquids are ease of pourability and inhibition of spillage from the container. Ideally, liquid should not be spilled off during removal from container owing to high fluidity and it should be too thick to pose the difficulty in removal of accurate dose from the container. To accomplish this a shear thinning or pseudoplastic behavior is essential; specifically, the dispersions must exhibit high viscosity when at rest to prevent the sedimentation of drug particles, while simultaneously demonstrating reduced viscosity during agitation to facilitate easy pouring from a container. This criterion can be maintained by modifying viscosity. Viscosity of suspension is also one of the determinants of stability. The viscosity of all formulations was in the range of 22.92 ± 1.2 to 54.8 ± 2.1 cPs (Table 3). The difference in viscosity was solely attributed to the presence of Veegum K. It is composed of three lattice layers of octahedral alumina and two tetrahedral silica sheets which can be separated upon hydration in water. Once, it undergoes hydration weakly positive edges are attracted to negatively charged faces and creates three dimensional colloidal structure that exhibits thixotropic behaviour [15]. As concentration of suspending agent Veegum K is highest (10.8 mg) in formulation F2, F5, F6 and F7, more clumpy mass was generated and measurement of viscosity was difficult. These results revealed major impact of suspending agent on viscosity, Formulation F3 showed higher viscosity as it contained a moderate amount of Veegum K (7.8 mg). The ideal pH range of oral formulation is ranged between 3 to 9 [16]. pH of all formulations ranged from 5.32 ± 1.84 to 6.1 ± 1.16. Results were indicative of an almost neutral pH of formulation that can be well tolerated by body. Comparative profile of pH all batches is given in Table 3. Sedimentation refers to settling of solid particles under gravitation force in liquid at bottom of the container. As it is a suspension formulation, it is very important to check rate of sedimentation owing to basic consideration for suspension stability. Preferably, suspensions should not settle quickly and should maintain uniform and accurate dose. For ideal suspension sedimentation value range is 0.5-1 and value near to 1 denotes better stability [17]. Comparative profile of sedimentation volume all batches is given in Table 3. An increase in the concentration of the suspending agent resulted in a corresponding rise in sedimentation volume, which was attributed to enhanced viscosity of medium with rise in concentration that retards the rate of sedimentation. Sedimentation volume of formulation batches was ranged from 0.47 ± 0.06 to 0.96 ± 0.02 at the end of 24 h. Results demonstrate that formulations F1, F4, F8 and F9 exhibited more settling owing to lower concentration of suspending agent. Formulation F2, F3, F5, F6 and F7 revealed good sedimentation volume which was within acceptable limit of 0.5 to 1.

Table 3: Sedimentation volume, pH, and viscosity of all formulations.

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Viscosity (cPs)	22.92 ± 1.2	**	54.8 ± 2.1	33.72 ± 1.8	**	**	**	26.58 ± 3.8	25.85 ± 4.6
Sedimentation volume (%)	0.49 ± 0.09	0.87 ± 0.02	0.85 ± 0.05	0.47 ± 0.06	0.96 ± 0.02	0.87 ± 0.09	0.94 ± 0.04	0.58 ± 0.02	0.65 ± 0.03
pH	6 ± 1.19	5.8 ± 1.26	5.5 ± 1.33	5.8 ± 2.07	6.1 ± 1.16	6.01 ± 1.28	5.68 ± 1.22	5.32 ± 1.84	5.67 ± 0.09

** Formulations composed of clumpy mass, so determination of viscosity was difficult

In-vitro drug release studies

In-vitro drug release studies were performed by using USP type II dissolution apparatus of all formulation (Figure 2). Inverse correlation was found between concentration of suspending agent and drug release. Drug release from various formulations ranged from 64.86 % to 98.56 %. The formulations F1, F4, F8 and F9 having lower concentration of suspending agent showed 98.56 %, 94.75 %, 86.46 % and

85.57 % drug release respectively. While, formulations F2, F5, F6 and F7 having high concentration of suspending agent showed 64.86 %, 69.34 %, 72.65 % and 76.59 % release of drug respectively. Formulation (F3) composed of intermediate concentration of suspending agent showed 97.57 % drug release. Formulation F3 was selected as desirable formulation based on drug release studies.

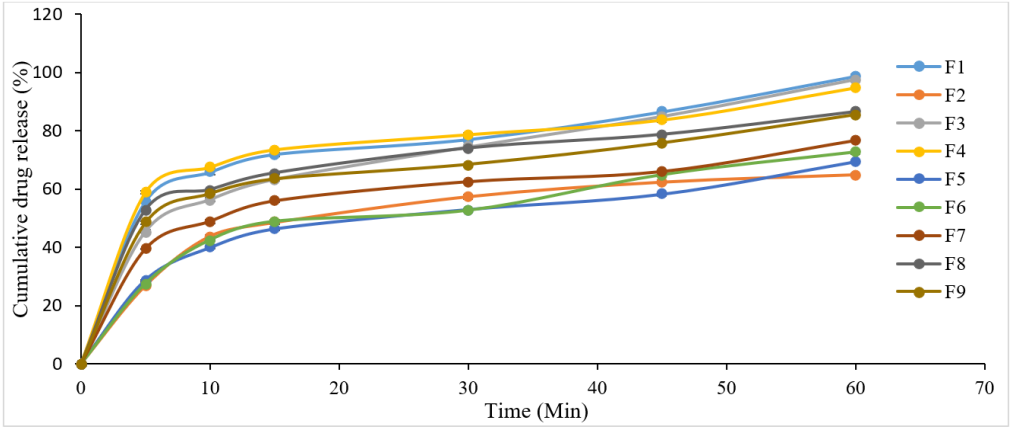

Figure 2. Drug release of all formulations

Drug release kinetic

The optimized formulation was fitted into different kinetic models. R^2 value obtained for zero order, first order, Higuchi and Korsemeyer-Peppas were 0.9634, 0.8438, 0.8439 and 0.8149 respectively. These results were indicative of zero order type of drug release.

Appearance of optimized batch

To check any undesirable physical changes, instability and loss of aesthetic appeal optimized suspension (Formulation F3) was observed at weekly intervals for 4 weeks for crystal growth, caking and aggregation. No undesirable physical changes were observed in the optimized formulation. These observations proved that suspension was physically stable and its aesthetic appeal was maintained. It also reflected the correct excipients selection.

Particle size distribution, polydispersity index (PDI) and zeta potential

As it is suspension formulation particle size is of crucial importance. It is one of determinant of quality, stability, palatability and bioavailability. Average particle size of suspension was 713.5 ± 2.24 nm. Homogeneity of particle can be computed form value of polydispersity index. It is an indicator of size variation among the disperse system. PDI scale ranging from 0.0–1.0, while values ≤0.1 demonstrate highly monodispersed particles, 0.3 to 0.5 value indicative of moderately monodispersed and value of 0.5 to 1 is regarded as polydispersed particles. For suspension formulation monodispersed particles are recommended. PDI of F3 formulation was 0.198± 3.24, confirmed the uniform particle size throughout the formulation. Charge of the particles was denoted by value of zeta potential. Zeta potential (positive or negative) values have a substantial contribution in stabilizing suspended particles. Similar charges attributed to electrostatic repulsion between particles and ultimately avoids clumping within the suspended solids [18]. The obtained value for F3 formulation was -52.1 ± 2.84 mV, depicting a stable system as the values of zeta potential within ± 30 and -30 are not considered to be stable [19]. The obtained results were in agreement with research carried out by Adeleke et al. (2020) [20], where researchers reported PDI value of 0.37 ± 0.04 and zeta potential −41.10 ± 5.57 mV for isoniazid reconstitute dry suspension.

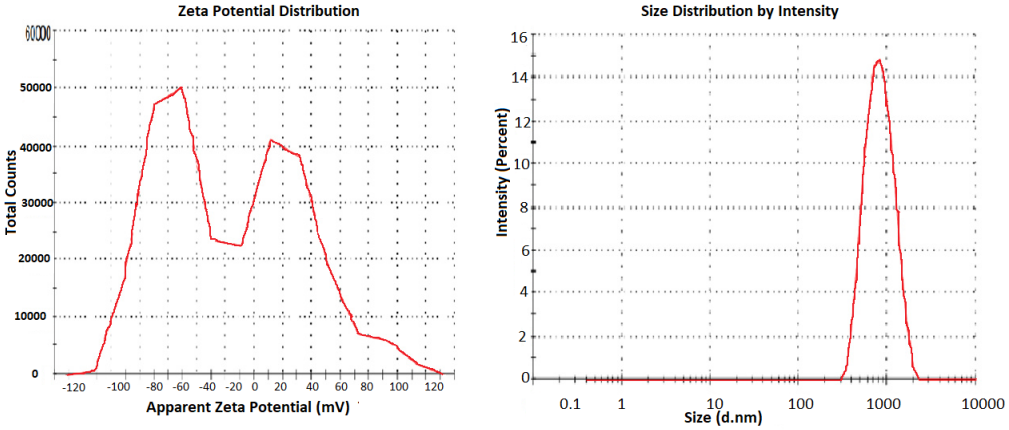

Figure 3. (a) Zeta potential, (b) Particle size of optimized formulation

Stability study of optimized formulation

The purpose of stability testing is to provide evidence on how quality of a drug substance or drug product varies with time under influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage condition, re-test periods and shelf life to be established. Formulation F3 was studied for stability. F3 was stored at temperature 40 °C/45 % RH and 25 °C/60 % RH for 1 month, 2 month and 3 month. Results obtained from that all were within the limits. There was no significant change in viscosity of suspension as compared to initial values (Table 4). *In-vitro* drug release obtained at storage conditions at 40 °C/45 % RH after 1 month, 2 month, and 3 month was 94.17 ± 2.28 %, 93.42 ± 3.06 % and 95.94 ± 2.53 % respectively. Whereas, 96.14 ± 2.16 % drug release was observed at storage conditions of 25 °C/60 % RH at the end of 3rd month (Figure 4). pH and viscosity for formulation ranged between 5.6 ± 0.2 to 6.1 ± 0.3 and 54.8 ± 2.13 cPs to 62.79 ± 4.2 cPs respectively. Stability data was indicative of insignificant deviations from initial values [21] .

Table 4: Stability data of optimized batch.

Parameters	Initial	40°C /75 %RH			25°C/60 %RH
	0M	1M	2M	3M	3M
pH	5.6 ± 0.2	5.8 ± 0.8	6.1 ± 0.3	5.8 ± 0.2	5.7 ± 0.1
Viscosity (cPs)	54.8 ± 2.13	56.48 ± 3.44	62.79 ± 4.2	58.31 ± 3.6	56.85 ± 3.2
*All the readings were carried out in triplicate. RH= Relative Humidity					

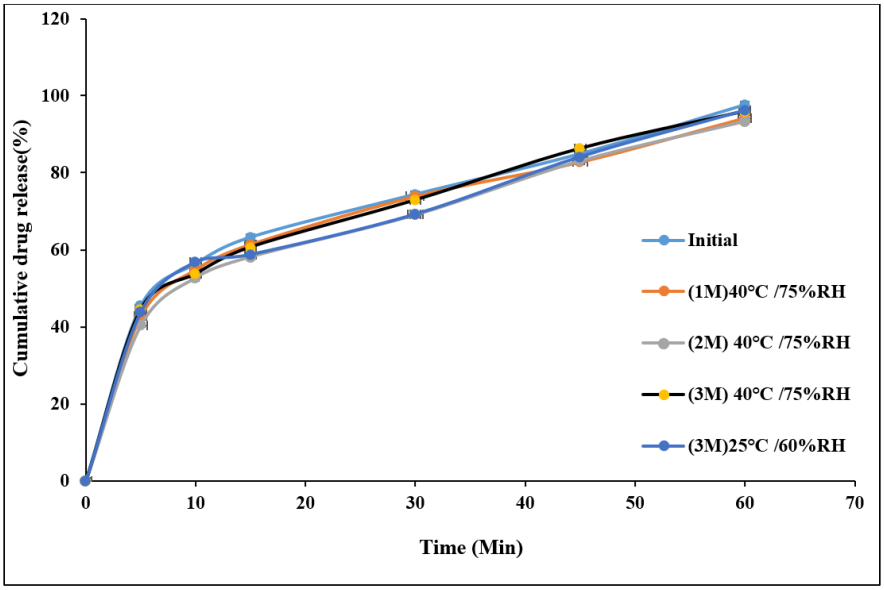

Figure 4. Stability data for drug release of optimized batch

Photostability study of optimized formulation

Pure stiripentol and optimized formulation was exposed to light and evaluated for appearance, assay and related substance in open condition, closed condition and control sample (Table 5). Appearance of pure drug and optimized formulation was white to beige colour which signifies stability under exposed light. Assay (%) obtained from pure drug and finished product at various conditions was in specified limit i.e., 95 % to 105 %. Related substances such as piperonal impurity, stiripentol stage-1 impurity was not more than 10 %. Any individual unspecified impurity was not detected when directly exposed to light. All overall results obtained from photostability data gives confirmation of stability of optimized formulation at in presence of light.

Table 5: Photostability data of optimized batch (F3).

Test parameters	Stability product specification	Stiripentol			Finished product		
		Open condition (Petri dish)	Closed Condition (Amber colour vials with stopper)	Control sample (Petridish wrapped with aluminium foil)	Open condition (Direct exposure) transparent Bottle	Closed condition (Amber colour bottle with CRC cap)	Control sample (Wrapped with aluminium foil)
Appearance		White to beige colour powder	White to beige colour powder	White to beige colour powder	Complies	Complies	Complies

Test parameters	Stability product specification	Stiripentol			Finished product		
		Open condition (Petri dish)	Closed Condition (Amber colour vials with stopper)	Control sample (Petridish wrapped with aluminium foil)	Open condition (Direct exposure) transparent Bottle	Closed condition (Amber colour bottle with CRC cap)	Control sample (Wrapped with aluminium foil)
Assay (%)	95.0 % -105.0 %.	98.8	98.7	98.3	100.8	99.6	99.6
Methyl paraben	80-120 %	-	-	-	98.8	98.0	98.2
Related substances (%)							
Piperonal impurity	NMT 0.15 %	ND	ND	ND	ND	ND	ND
Stiripentol stage-I impurity	NMT 0.15 %	ND	ND	ND	ND	ND	ND
Any individual unspecified impurity	NMT 0.10 %	0.04	0.04	0.04	0.04	0.04	0.04
Total impurities	NMT 1.0 **%**	0.04	0.04	0.04	0.04	0.04	0.04
**ND- Not detected, *NMT- Not more than							

Conclusion

Stiripentol liquid oral suspension is not available in market. Hence, current study was aimed to develop stable liquid suspension. During the development of formulation Veegum K and cross carmellose sodium had major impact on viscosity, sedimentation volume and drug release. Optimized formulation was selected based on optimum viscosity, sedimentation volume and higher drug release. The optimized formulation was stable with uniform particle size. The pH and viscosity of the optimized formulation was suitable for oral administration. The study concluded that stiripentol oral suspension was formulated using a combination of suitable excipients to achieve good stability, uniformity and sustained release of the drug.

References

1. Hameed Z, Saleem S, Mirza J, Mustafa MS,Qamar Ul I. Characterisation of ictal and interictal states of epilepsy: A system dynamic approach of principal dynamic modes analysis. PLoS One. 2018; 13(1):e0191392. doi:10.1371/journal.pone.0191392.

2. Rho JM,Sankar R. The pharmacologic basis of antiepileptic drug action. Epilepsia. 1999; 40(11):1471-83.

3. Fisher JL. The effects of stiripentol on GABA(A) receptors. Epilepsia. 2011; 52 Suppl 2(2):76-8. doi:10.1111/j.1528-1167.2011.03008.x.

4. Uchida Y, Terada K, Madokoro Y, et al. Stiripentol for the treatment of super-refractory status epilepticus with cross-sensitivity. Acta Neurol Scand. 2018; 137(4):432-37. doi:10.1111/ane.12888.

5. Chiron C, Chemaly N, Chancharme L,Nabbout R. Initiating stiripentol before 2 years of age in patients with Dravet syndrome is safe and beneficial against status epilepticus. Dev Med Child Neurol. 2023; 65(12):1607-16. doi:10.1111/dmcn.15638.

6. Wheless J,Weatherspoon S. Use of Stiripentol in Dravet syndrome: A guide for clinicians. Pediatr Neurol. 2024; doi:10.1016/j.pediatrneurol.2024.10.015.

7. Wang Y, Xu S, Xiao Z, et al. Stiripentol enteric solid dispersion-loaded effervescent tablets: Enhanced dissolution, stability, and absorption. AAPS PharmSciTech. 2022; 23(5):141. doi:10.1208/s12249-022-02261-5.

8. Vázquez-Blanco S, González-Freire L, Dávila-Pousa MC,Crespo-Diz C. pH determination as a quality standard for the elaboration of oral liquid compounding formula. Farm Hosp. 2018; 42(6):221-27. doi:10.7399/fh.10932.

9. Oppong EE, Osei-Asare C,Klu MW. Evaluation of the suspending properties of shea tree gum. Int J Pharm Pharm Sci. 2016; 8(7):409-13.

10. Shah PP,Mashru RC. Formulation and evaluation of taste masked oral reconstitutable suspension of primaquine phosphate. AAPS PharmSciTech. 2008; 9(3):1025-30. doi:10.1208/s12249-008-9137-6.

11. Giupponi G,Pagonabarraga I. Determination of the zeta potential for highly charged colloidal suspensions. Philos Trans A Math Phys Eng Sci 2011; 369(19):2546-54. doi:10.1098/rsta.2011.0024.

12. Patel MS, Patel AD,Damor S. Design and development of dual release reconstitutable oral suspension of cefpodoxime proxetil for pediatric patient using risk-based quality by design approach. J Pharm Innov. 2022; 17(3):955-78. doi:10.1007/s12247-021-09577-y.

13. Rahman MS, Hasan MS, Nitai AS, et al. Recent developments of carboxymethyl cellulose. Polymers. 2021; 13(8):1345. doi: 10.3390/polym13081345

14. Almutairi M, D R L, Ghabbour H, Joe IH,Attia M. Spectroscopic identification, structural features, Hirshfeld surface analysis and molecular docking studies on stiripentol: An orphan antiepileptic drug. Journal of Molecular Structure. 2018; 1180(1):doi:10.1016/j.molstruc.2018.11.088.

15. Pongjanyakul T,Puttipipatkhachorn S. Polymer-magnesium aluminum silicate composite dispersions for improved physical stability of acetaminophen suspensions. AAPS PharmSciTech. 2009; 10(2):346-54. doi:10.1208/s12249-009-9215-4.

16. Attebäck M, Hedin B,Mattsson S. Formulation optimization of extemporaneous oral liquids containing naloxone and propranolol for pediatric use. Sci Pharm. 2022; 90(1):15. doi:10.3390/scipharm90010015.

17. Owusu FW, Asare CO, Enstie P, et al. Formulation and in vitro evaluation of oral capsules and suspension from the ethanolic extract of cola nitida seeds for the treatment of diarrhea. Biomed Res Int. 2021; 2021(1):1-7. doi:10.1155/2021/6630449.

18. Cheng Z, Kandekar U, Ma X, et al. Optimizing fluconazole-embedded transfersomal gel for enhanced antifungal activity and compatibility studies. Front Pharmacol. 2024; 15(1):1353791.

19. Júnior JAA,Baldo JB. The behavior of zeta potential of silica suspensions. New J GC. 2014; 4(02):1-9. doi:10.4236/njgc.2014.42004.

20. Adeleke OA, Hayeshi RK,Davids H. Development and evaluation of a reconstitutable dry suspension containing isoniazid for flexible pediatric dosing. Pharmaceutics. 2020; 12(3):286.

21. Santoveña A, Suárez-González J, Martín-Rodríguez C,Fariña JB. Formulation design of oral pediatric Acetazolamide suspension: dose uniformity and physico-chemical stability study. Pharm Dev Technol. 2017; 22(2):191-97. doi:10.1080/10837450.2016.1175475.

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.32353
Artículos originales

Therapeutic Potential of Fabaceae Species: A Phytochemical and Bioactivity Investigation

Potencial terapéutico de las especies de Fabaceae: Una investigación fitoquímica y de bioactividad

Alveena Sabir[1]
Naila Safdar[1]
Wasim Akhtar[2]
Iram Fatima[3]
Gul-e-Saba Chaudhry [4] 0000-0002-4555-8572

[1]Department of Biotechnology, Fatima Jinnah Women University, Rawalpindi.

[2]Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan.

[3]PCSIR Labs Complex, Plot no. 16, Sector H-9, Islamabad-44000, Pakistan.

[4]Institute of Climate Adaptation and marine Biotechnology, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Malaysia.

Correspondence

Gul-e-Saba Chaudhry , Naila Safdar
gul.saba@umt.edu.my, nailahussain@fjwu.edu.pk

Received: 19.12.2024
Accepted: 13.04.2025
Published: 20.06.2025

Funding

This research work was supported by Higher Education Commission (HEC) under start-up research grant program (SRGP).

Conflict of interests

The authors declare no conflict of interest

Resumen

Introducción: La creciente prevalencia de enfermedades crónicas y dolencias infecciosas requiere la exploración de nuevos agentes terapéuticos. Este estudio tiene como objetivo elucidar la composición fitoquímica y las actividades biológicas de especies seleccionadas de Fabaceae.

Métodos: El potencial antioxidante se evaluó con el ensayo DPPH, mientras que la protección del ADN y la actividad antidiabética se probaron mediante ensayos de inhibición. Los potenciales anti-leishmania y anti-dengue se evaluaron contra *Leishmania* major y *Aedes aegypti*.

Resultados: *Sophora mollis*, *Mucuna pruriens* y *Sesbania sesbans* exhibieron el mayor contenido de fitoquímicos. El poder de captura de DPPH de *M. pruriens* fue el más alto (14,09 ± 3,60 µg/ml), y la capacidad de protección del ADN de los extractos de metanol de *Pueraria tuberosa* fue la más alta. El extracto metanólico de *S. sesban* efectivamente inhibió la leishmaniasis (82,99 % de inhibición) y el dengue. (70,10 ppm LD50). Mediante GC/MS se identificó 37 compuestos, con predominancia de 9-octadecenamida (Z) y tetradecanamida.

Conclusión: Los extractos de hojas en metanol de especies seleccionadas de Fabaceae revelaron propiedades biológicas significativas en comparación con los extractos de metanol-DMSO y metanol-glicerol. Además, llevar a cabo los efectos sinérgicos de los compuestos antileishmaniales, anti-dengue, antidiabéticos y antioxidantes, puede conducir al desarrollo de estrategias terapéuticas más efectivas y completas.

Palabras clave: Fabaceae; Extractos de Plantas; Antioxidantes; Agentes Antiparasitarios

Abstract

Introduction: The increasing prevalence of chronic diseases and infectious ailments necessitates the exploration of novel therapeutic agents. This study aims to elucidate selected Fabaceae species' phytochemical composition and biological activities.

Methods: Antioxidant potential was assessed with the DPPH assay, while DNA protection and antidiabetic activity were tested via inhibition assays. Anti-leishmanial and anti-dengue potentials were evaluated against *Leishmania major* and *Aedes aegypti*.

Results: *Sophora mollis*, *Mucuna pruriens*, and *Sesbania sesbans* exhibited the highest phytochemical content. The DPPH-scavenging power of *M. pruriens* was highest (14.09 ± 3.60 µg/ml), and the DNA protection ability of *P. tuberosa* methanol extracts was highest. The methanol extract of *S. sesbans* effectively inhibited leishmaniasis (82.99 % inhibition) and dengue (70.10 ppm LD50). GC/MS identified 37 compounds, with 9-octadecenamide (Z) and tetradecanamide predominating.

Conclusion: The methanol leaves extracts of selected Fabaceae species revealed significant biological properties compared to the methanol-DMSO and methanol-glycerol extracts. Furthermore, carrying out the synergistic effects of antileishmanial, anti-dengue, antidiabetic, and antioxidant compounds, may lead to the development of more effective and comprehensive therapeutic strategies.

Keywords: Fabaceae; Plant Extracts; Antioxidants; Antiparasitic Agents

Highlight

This study evaluates the phytochemical composition and biological activities of selected Fabaceae species, including antioxidant, DNA protection, antidiabetic, anti-leishmanial, and anti-dengue properties.

Sophora mollis, *Mucuna pruriens*, and *Sesbania sesbans* exhibited significant bioactivity, with *M. pruriens* showing the highest antioxidant activity and *S. sesbans* demonstrating strong anti-leishmanial (82.99 %) and anti-dengue (70.10 ppm LD50) effects.

GC/MS analysis identified 37 bioactive compounds, with 9-octadecenamide (Z) and tetradecanamide predominating. These findings underscore the therapeutic potential of these species, offering a foundation for integrated antiviral, antidiabetic, and antioxidant strategies.

Introduction

Oxygen is an essential component of life. However, excessive production of reactive oxygen species (ROS), such as hydroxyl radical (OH-) and nitric oxide (NO), along with alterations in DNA and protein, may cause oxidative stress [1]. If affected cells fail to repair completely, then it may cause chronic diseases like diabetes, Alzheimer's disease and ageing. The plants can scavenge ROS due to the compounds and antioxidant molecules, including polyphenols that can scavenge free radicals, chelating free metals, thereby protecting the integrity of cell membranes and free-radical mediated oxidative stress[2].

Besides these, investigations on the antidiabetic activities of wild plants have also gained considerable attention due to their strong potential as natural antidiabetic agents. [1] As the mechanisms of these diseases are complex, the rate of response of synthetic drugs is low and more likely to cause adverse effects. Hence, it is necessary to explore novel plants for treating such diseases in which immune responses are vital for disease development[3].

The Fabaceae family is second to Graminae, with approximately 750 genera and includes various economically and medicinally important flowering plants. In this context, some studies have highlighted the biological significance of Fabaceae species. For instance[4] , proposed antimicrobial, hypoglycemic, anti-tumour, antioxidant and anti-Parkinson properties of *Mucuna pruriens* seed extract. Similarly, *Lablab purpureus* leaves and flowers treat cholera, diarrhea, nausea, inflammations and uterus inflammation[5,6], *Pueraria tuberosa* and *Sesbania sesbans* are used in traditional medicines to cure blood and urinary diseases and as cardiotonic, demulcent, anthelmintic, diuretic and galactagogue[7,8]. Anti-mutagenic effects of *P. tuberosa* have been described, and [9] flavonoids' presence has been attributed to them. Furthermore, the authors [10] revealed the antibacterial, cytotoxic, antipyretic, analgesic and anti-tumour potential of different compounds extracted from *Sophora mollis*.

Hence, the objectives of the current study were to prepare the methanol, methanol-dimethyl sulfoxide (DMSO) and methanol-glycerol extracts of fresh leaves of five Fabaceae species commonly grown in Pakistan. The five species were: *M. pruriens*, *S. sesbans, S. mollis*, *P. tuberosa* and *L. purpureus*. Subsequently, the phytochemical content, antioxidant, DNA damage protection, anti-diabetic, anti-leishmanial and anti-dengue potential of the selected leaf extracts was determined using standard bioassays. Lastly, the concentration of different hydrocarbons, fatty acids and esters was evaluated using GC/MS method.

Methods

Extracts preparation

Fresh leaves of five selected species were collected and their accession numbers were assigned from the Herbarium of the National Agriculture Research Centre (NARC), Islamabad. The extracts were weighed to determine the extract yield (Table 1).

Table 1. Selected Fabaceae species, their accession numbers, extract yield and acronyms.

Plant species	Accession numbers	Methanol extracts		Methanol-DMSO extracts		Methanol-Glycerol extracts	
		Yield	Acronym	Yield	Acronym	Yield	Acronym
***Mucuna pruriens* (Linn.) DC.**	RAW101497	24.25	MPM	27.10	MPMD	50.50	MPMG
***Sesbania sesbans* (L.) Merrill**	RAW101498	72.35	SSM	43.65	SSMD	97.40	SSMG
***Sophora mollis* (Royle) Baker**	RAW101499	29.75	SMM	33.00	SMMD	72.50	SMMG

Plant species	Accession numbers	Methanol extracts		Methanol-DMSO extracts		Methanol-Glycerol extracts	
		Yield	Acronym	Yield	Acronym	Yield	Acronym
***Pueraria tuberosa* (Roxb. ex Willd.) DC.**	RAW101500	27.60	PTM	39.00	PTMD	92.00	PTMG
***Lablab purpureus* (L.) Sweet**	RAW101501	31.50	LPM	39.75	LPMD	72.25	LPMG

*DMSO: Dimethyl sulfoxide; The extract yield was measured in percentage (%).

Determination of phytochemicals

For total phenolic contents (TPC), total flavonoid contents (TFC) and total alkaloid contents (TAC), the procedure was the same as the previously reported method(11,12, 13).

Antioxidant assay

In this assay, 0.1 mM DPPH solution was made by adding 3.94 mg of DPPH in 100 mL of methanol, and the absorbance (0.98 ± 0.02) was set at 517 nm. The procedure was followed as per the previously reported method[14].

DNA damage protection assay

Briefly, 14 mg of iron sulphate heptahydrate (FeSO4.7H2O) was added to 25 ml of distilled water (dH2O), and 0.2 µg/µl pBR322 plasmid was diluted using 50 mM of phosphate buffer saline (PBS). Then, 3 µl of pBR322 plasmid DNA was added in Fenton reagent and plant extracts, and the volume was made up to 15 µl using dH2O. The reaction mixture was incubated (30 min) at 37 °C, and then 1 % agarose gel was prepared. Subsequently, 5 µl of ethidium bromide (EtBr) was added to the gel and transferred to the gel tank, using 1x Tris-borate EDTA (TBE) as a running buffer. The reaction mixtures (10 µl) were loaded on the gel, and 2 µL of loading dye and electrophoresis were conducted. After 20-30 min, DNA bands were observed under UV light[15].

Antidiabetic assays

α-amylase inhibition activity and α-glucosidase inhibition activity

The α-amylase inhibition activity and α-glucosidase inhibition activity were performed per the reported method[16,17].

Anti-leishmanial assay

For parasites culture, *L. major* isolates were obtained from the Department of Zoology, University of Peshawar (Pakistan) and grown in RPMI-1640 culture medium with 10 % heat-inactivated fetal calf serum (HIFCS) in the presence of penicillin and streptomycin solution (100 µg/mL each) at 23 °C. The MTT (3-(4,5-dimethylthiazole-2yl)-2,5-diphenyltetrazolium bromide) assay was done per the previously reported protocol[18].

Anti-dengue assay

A. aegypti larvae were collected with an aquatic net and then transferred to the laboratory. The larvae were fed dog biscuits and yeast powder (3:1) and maintained at 28 ± 2 °C. Afterwards, the 4th instar larvae were visually detected by a relatively larger size, and larvicidal activity was performed following the WHO protocol[19]. Finally, % mortality and LD50 values were determined.

Gas chromatography-mass spectrometry (GCMS) analysis

For GC/MS, 70 µL of plant oil was accumulated by adding 10 grams of powdered plant material in 100 mL of ultrapure dH2O and then performing microwave-assisted hydro-distillation for 40 min. GCMS instrument (Agilent technologies – GC7890B and MS5977A) was equipped with DB-5MS fused capillary column. Various compounds were recognized with the help of NIST and WILEY database[20,21].

Statistical analysis

All assays were performed twice and mean ±SD were calculated. The least significant difference (LSD) was observed using Statistix 8.1, and a *P*-value (< 0.05) was used to establish statistical significance.

Results and Discussion

Plant extraction

The polarity of extraction solvents plays a critical role in plants' biological activities[22]. In this study, DMSO and glycerol were chosen as green solvents in combination with the strongly polar solvent (methanol) to determine the phytochemicals and biological activities of five species.

Total phenolics, flavonoids and alkaloids content

Our study showed a large variation in TPC, TFC and TAC among selected extracts. TPC and TFC were observed highest in *S. mollis* (32.35 ± 0.19 mg gallic acid equivalent/g and 15.00 ± 0.78 mg rutin equivalent/g) and *M. pruriens* (31.62 ± 0.22 mg GAE/g and 16.66 ± 0.78 mg RE/g) methanol extracts while lowest in the methanol-glycerol extracts of *P. tuberosa* (7.75 ± 0.26 mg GAE/g and 3.33 ± 0.78 mg RE/g) and *M. pruriens* (8.05 ± 0.07 mg GAE/g and 3.05 ± 0.39 mg RE/g) (Figure 1).

Similarly, the highest TAC was also recorded in SMM (9.61 ± 0.54 mg AE/g) and MPM (9.61 ± 0.54 mg AE/g) and lowest in MPMG (2.69 ± 0.54 mg AE/g) and PTMG (3.07 ± 0.00 mg AE/g). However, all phytochemical contents were found in descending order of methanol extracts > methanol-DMSO extracts > methanol-glycerol extracts.

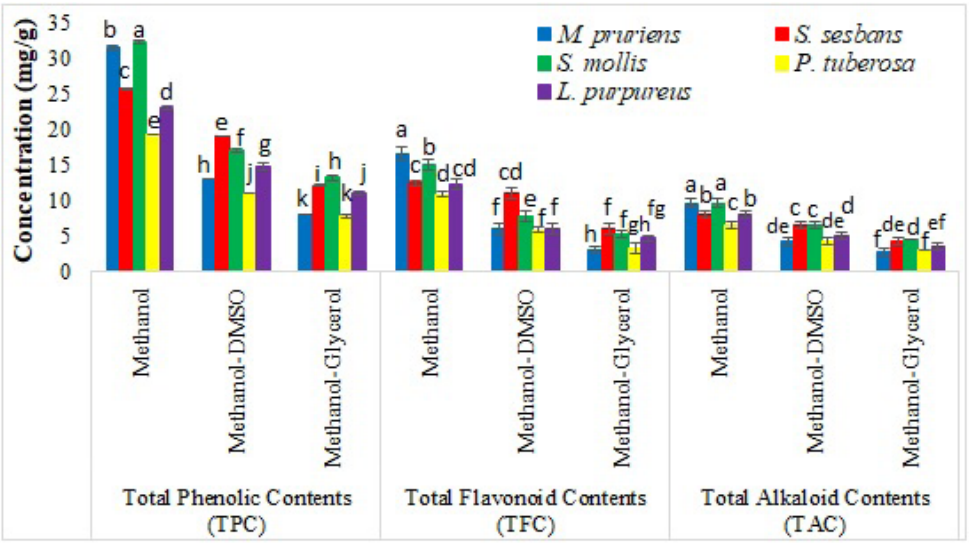

Figure 1. Phytochemicals observed in five Fabaceae extracts. Data represents mean ± SD and alphabetical values (a-k) are significantly different by $P < 0.05$.

Antioxidant activity

The antioxidant activity revealed DPPH activity in descending order of methanol extracts > methanol-DMSO extracts > methanol-glycerol extracts. The lowest IC50 value was recorded in MPM, *i.e.* 14.09 ± 3.60 µg/mL, indicating the highest scavenging activity, while the highest IC50 value was found in PTMG, *i.e.* 1772.66 ± 10.01 µg/mL showing the lowest antioxidant capacity (Figure 2a). The correlation test showed that DPPH activity was strongly correlated with TPC (r = 0.845), TFC (r = 0.842) and TAC (r = 0.842) (Figure 2b). It can be suggested that the DPPH scavenging activity of leaf extracts is directly associated with the concentration of phenolic, flavonoid and alkaloid content in leaves. Similarly, our results are comparable to the other studies that TPC and DPPH activity were 10.96 ± 0.21 mg GAE/g and 61.51 ± 0.33 % in *S. sesbans*, 2.89 ± 0.01 mg GAE/g and 28.21 ± 1.29 % in *L. purpureus* and 39.96 ± 0.00 mg GAE/g and 40-60 % in *M. pruriens* grown in Thailand and India(23, 6).

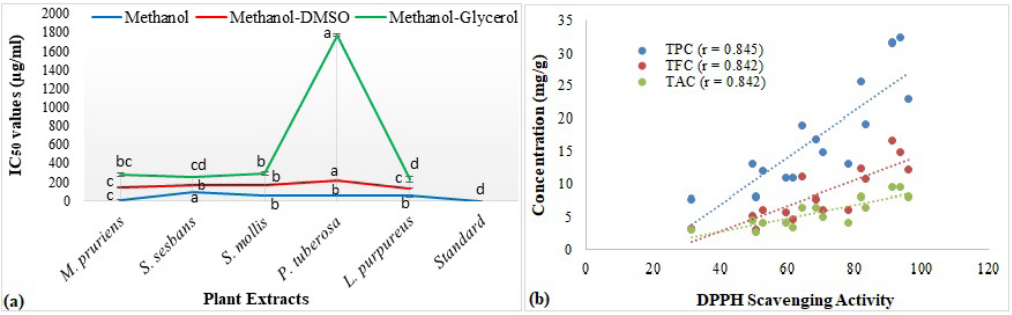

Figure 2. Antioxidant activity of plant extracts assessed at different concentrations and its correlation with phytochemicals (a) IC50 values determined in DPPH assay (b) Correlation between DPPH activity and phytochemicals.

DNA damage protection assay

In this assay, untreated plasmid expressed two bands while strand scission was observed on treating DNA with Fenton reagent. The gel photographs showed that PTM (3631.88) and SSMD (2050.85) exhibited highest protection, whereas SSM (1283.59), MPM (1088.60), SMM (1044.59), PTMD (886.41) and SSMG (211) possesses lowest protection against oxidative DNA damage, as corroborated by densitometric analysis (Figure 3b). However, all other extracts were found to be ineffective in protecting DNA from damage (Figure 3a). In the current study, methanol extracts protected DNA by scavenging the oxidation products that damage the DNA. It can be inferred that the abundance of TPC, TFC and TAC present in leaves acted directly on the oxidative agents and prevented the chain reaction of oxidative stress which alleviated oxidative damage in the methanol extracts.

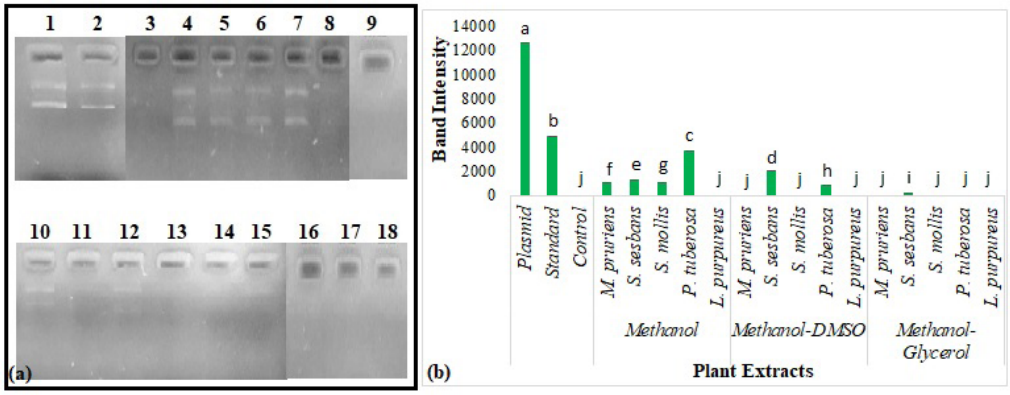

Figure 3. Effects of Fabaceae extracts showing DNA damage protection ability (a) Gel photograph displaying DNA bands (Lane 1: plasmid; Lane 2: plasmid + reagent + standard; Lane 3: plasmid + reagent; Lane 4: plasmid + reagent + MPM; Lane 5: plasmid + reagent + SSM; Lane 6: plasmid + reagent + SMM; Lane 7: plasmid + reagent + PTM; Lane 8: plasmid + reagent + LPM; Lane 9: plasmid + reagent + MPMD; Lane 10: plasmid + reagent + SSMD; Lane 11: plasmid + reagent + SMMD; Lane 12: plasmid + reagent + PTMD; Lane 13: plasmid + reagent + LPMD; Lane 14: plasmid + reagent + MPMG; Lane 15: plasmid + reagent + SSMG; Lane 16: plasmid + reagent + SMMG; Lane 17: plasmid + reagent + PTMG; Lane 18: plasmid + reagent + LPMG) (b) Densitometry calculation of DNA bands.

α-Amylase and α-glucosidase inhibition assays

In current study, α-amylase assay revealed lowest IC50 value in SMM (10.62 ± 7.71 µg/mL) and SSM (17.01 ± 11.94 µg/mL) indicating highest α-amylase inhibition potential (Figure 4a). In α-glucosidase assay, highest activity was recorded in the methanol-glycerol and methanol-DMSO extracts of *S. sesbans* (*i.e.* 0.02 ± 0.01 µg/mL and 0.03 ± 0.03 µg/mL IC50 values). In contrast, lowest α-glucosidase inhibitory potential was found in all extracts of *P. tuberosa* (IC50 values 13.82 to 26.36 µg/mL) (Figure 4c). Comparatively, α-amylase activity was significantly correlated with TPC (r = 0.490) and TFC (r = 0.470) as compared to the TAC (r = 0.394) (Figure 4b). Likewise, α-glucosidase inhibitory activity also depicted moderately positive correlation with TPC (r = 0.497), TFC (r = 0.446) and TAC (r = 0.483) (Figure 4d). The r value of 0.4 indicates a moderately positive correlation but not a stronger one. The results further corroborate previous studies[22] that elucidated > 80 % α-glucosidase inhibitory activity in *S. sesbans* and *L. purpureus* extracts. Similarly, Gulati et al. [4] documented significantly lower IC50 values for both α-amylase (< 25 µg/mL) and α-glucosidase (< 5 µg/mL) inhibitory activities in *M. pruriens* grown in Ethiopia.

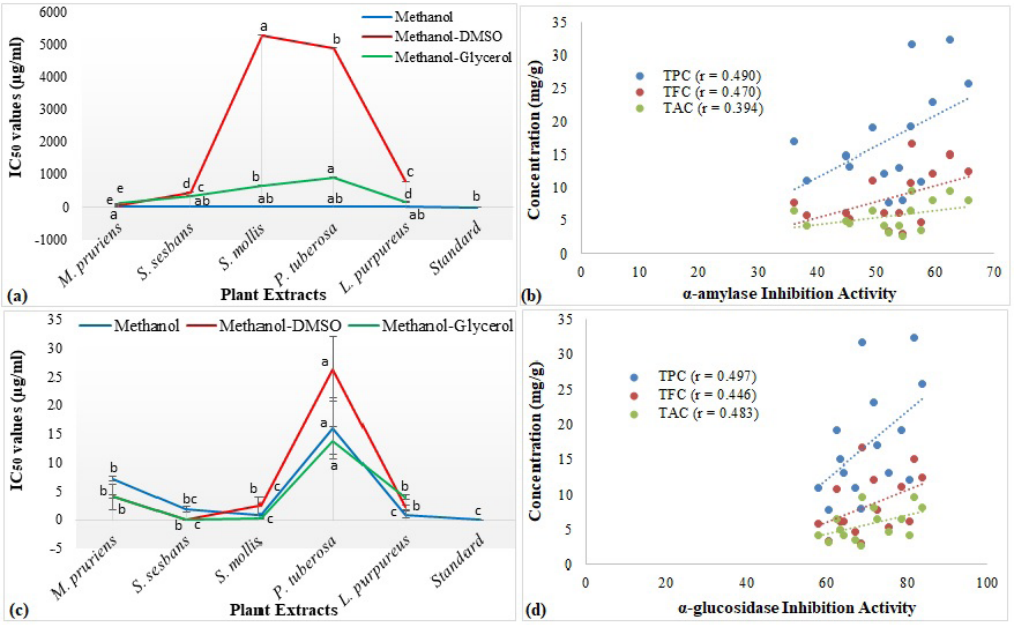

Figure 4. Antidiabetic activity of plant extracts and its correlation with phytochemicals (a) α- amylase inhibition assay (b) Correlation of α- amylase inhibition activity with phytochemicals (c) α- glucosidase inhibition assay (d) Correlation of α- glucosidase inhibition activity with the phytochemicals. * r is correlation co-efficient and IC50 stands for half-maximal inhibitory concentration. * Acarbose was used as a standard.

Anti-leishmanial and anti-dengue assays

All examined species showed significant biological activities in their methanol extracts. Hence, the methanol extracts were further tested to examine anti-leishmanial and anti-dengue potential of these species. The anti-leishmanial activity was found in descending order of *S. sesbans* (82.99 % inhibition) > *L. purpureus* (69.31 % inhibition) > *M. pruriens* (68.85 % inhibition) > *P. tuberosa* (66.92 % inhibition) > *S. mollis* (36.27 % inhibition) (Table 2). In the anti-dengue assay, only *S. sesbans* and *L. purpureus* were found to be effective in controlling the growth of *A. aegypti* as they displayed LD50 values of 70.10 and 200.00 ppm, respectively (Table 3).

Table 2. Percentage inhibition and IC50 values of methanol leaves extracts as determined in anti-leishmanial activity.

Plant extracts	Percentage inhibition observed at different concentrations			IC_{50} values (µg/ml)
	250 µg/ml	500 µg/ml	1000 µg/ml	
M. pruriens	33.11	39.78	68.85	566.10
S. sesbans	21.59	59.10	82.99	397.70
S. mollis	6.99	17.74	36.27	1368.00

Plant extracts	Percentage inhibition observed at different concentrations			IC_{50} values (µg/ml)
	250 µg/ml	500 µg/ml	1000 µg/ml	
P. tuberosa	25.45	40.17	66.92	617.20
L. purpureus	21.56	49.61	69.31	514.70

*IC50: Half-maximal inhibitory concentration.

Table 3. Larvicidal activity examined against dengue vector *Aedes aegypti* L. after exposure to the selected methanol extracts.

Plant Extracts	Percentage mortality at different concentrations (ppm)			R-square	LD_{50}	95 % CI
	50	100	200			
M. pruriens	0	0	0	-	-	-
S. sesbans	40	60	80	0.99	70.10	38.62 - 127.24
S. mollis	0	0	0	-	-	-
P. tuberosa	0	0	0	-	-	-
L. purpureus	0	30	50	1.00	200.00	105.37 - 379.60
Standard (Permethrin)	60	80	100	1.00	37.09	20.02 - 68.71

LD50: Lethal dose; CI: Confidence interval.

Previously[24], determined anti-plasmodial flavones from the roots of *S. mollis*. Hence, the antileishmanial activity of leaves examined in the current study was ineffective in inhibiting leishmanial parasites' growth. The current study confirmed the parasite inhibitory potential of *M. pruriens* using *L. major* promastigotes.

The decreased parasite growth can be ascribed to the increased production of ROS and reduced level of arginase that can be metabolized to nitric oxide (NO), a microbicidal agent responsible for the intracellular parasite removal[25].

GC/MS analysis

A total of 37 compounds, *viz.* hydrocarbons, fatty acids, alcohols, esters and carbohydrates, were identified, and differences in their peak area were observed in the GC/MS chromatograms. Among these, 9-octadecenamide, (Z) and tetradecanamide were found to be dominant compounds ranging from 11.94 to 63.89 % in all species. Similarly, 13-Docosenamide was also present in higher concentration (20.87 %) in *L. purpureus* only. However, all other compounds were found < 12 % as shown in Table 4.

Table 4. Concentration (%) of compounds observed in five Fabaceae species using GCMS method.

Names of Compounds	CC	Concentration (%)				
		M. pruriens	*S. sesbans*	*S. mollis*	*P. tuberosa*	*L. purpureus*
α-D-glucopyranoside, methyl	C9	-	-	1.68	-	10.19
o- Xylene	C4	-	-	1.48	0.96	-
p-Xylene	C4	3.46	1.46	0.78	-	3.76
1-Cyclohexene, 1-ethynyl	C3	-	0.47	-	-	-
1-Docosene	C3	-	-	-	-	0.40
1-Heptanol, 6-methyl	C7	-	-	0.51	-	0.69
2-Amino-5-methylamino-1,3,4-thiadiazole	C4	-	-	-	-	1.11
2-Cyclohexen-1-one, dimethyl-	C3	-	0.38	-	-	-
2-O-Methyl-D-mannopyranosa	C10	-	-	1.65	-	-
2-Pentanol, acetate	C6	8.92	-	8.03	2.46	-
3-Ethyl-2,6,10-trimethylundecane	C1	-	-	1.17	-	-
5-methyl-5-propyl, Nonane	C1	-	-	-	-	0.63
8-Methylnonanoic acid	C5	4.76	-	-	-	-
9-Octadecenamide, (Z)	C12	57.76	63.36	62.46	63.89	43.54
13-Docosenamide	C12	-	-	-	-	20.87
16-Hexadecanoyl hydrazide	C5	-	-	-	-	0.03
Acetic acid, hydrazide	C5	-	4.14	-	-	-
Bicyclo[2.1.1]hexan-2-ol, 2-ethenyl-	C2	10.15	-	-	-	-
Butyric acid hydrazide	C5	-	-	-	-	3.75
Carbamodithioic acid, phenyl-, methyl ester	C13	-	0.06	-	-	-
cis-11-Eicosenamide	C12	-	-	-	12.03	-
Cyclohexane	C2	-	-	-	-	1.63
Cyclohexane, 1,1-dimethoxy	C2	-	2.09	1.51	-	-
Cyclohexanone	C2	-	2.39	2.30	1.66	-
Dodecane, 2,6,10-trimethyl	C1	-	0.78	-	-	-
Glycoaldehyde dimer	C10	-	-	-	-	0.37
Heptane, 3,4-dimethyl-	C1	-	-	0.74	-	-
Hexadecanal	C11	-	-	0.86	-	-
Hexadecanoic acid, 15-methyl-, methyl ester	C5	-	0.55	-	-	-
Hydrazinecarboxamide	C13	-	-	0.71	-	-

Names of Compounds	CC	Concentration (%)				
		M. pruriens	*S. sesbans*	*S. mollis*	*P. tuberosa*	*L. purpureus*
Nonane, 3,7-dimethyl-	C1	-	-	-	0.39	-
Nonyl chloroformate	C6	-	0.38	-	-	-
Oxirane, hexadecyl	C8	-	-	-	-	0.84
Pentadecanal	C11	-	0.80	2.57	-	-
Phenol, 2,4-bis(1,1-dimethylethyl)-	C4	-	0.47	-	-	-
Sulfurous acid, hexyl octyl ester	C6	-	-	-	1.07	-
Tetradecanamide	C12	14.94	34.56	13.45	17.53	11.94

*Compounds are listed in alphabetical order; CC: Chemical class; C1: Linear alkanes; C2: Cycloalkanes; C3: Unsaturated hydrocarbons; C4: Aromatic/heterocyclic hydrocarbons; C5: Fatty acids; C6: Esters; C7: Alcohols; C8: Cyclic ether; C9: Carbohydrates; C10: Aldehyde; C11: Fatty aldehyde; C12: Fatty amides; C13: Other compounds.

The 9-octadecenamide (Z) is used as a hypolipidemic agent and for treating atherosclerosis, while tetradecanamide is considered for anti-mycobacterial and anti-tubercular activities[26]. Previously[27], proposed anti-leishmanial properties of 13-docosenamide, which have been detected significantly (20.87 %) in *L. purpureus* leaves. Cis-11-eicosenamide (12.03 %) was detected only in *P. tuberosa* and hexadecanoic acid-methyl ester was recorded in minor concentration in *S. sesbans* (0.55 %) only.

Conclusion

The obtained results confirmed that the extracts prepared with green solvents differed significantly in their chemical composition, which is directly related to their biological activities. It can be concluded that the strongly polar i.e. methanol extracts of *S. mollis, M. pruriens* and *S. sesbans* exhibited the strongest degree of biological activities due to the presence of the highest amount of phenolic, flavonoid and alkaloid contents. The methanol extract of *M. pruriens* displayed the highest antioxidant potential, *P. tuberosa* showed the highest DNA protection ability, and *S. sesbans* revealed the highest anti-leishmanial and anti-dengue potential. Thus, their methanol extracts could be promising candidates as natural bioactive agents in relevant fields, yet the antidiabetic activities in animal models need to be studied.

References

1. Zahra SA, Iqbal J, Abbasi BA, Shahbaz A, Kanwal S, Shah SL, Ahmad P, Mahmood T. Antimicrobial, cytotoxic, antioxidants, enzyme inhibition activities, and scanning electron microscopy of *Lactuca orientalis* (Boiss.) Boiss. seeds. Microsc Res Tech. 2021; 84(6):1284-1295. doi: 10.1002/jemt.23687.

2. Jimoh MA, Idris OA, Jimoh MO. Cytotoxicity, phytochemical, antiparasitic screening, and antioxidant activities of *Mucuna pruriens* (Fabaceae). Plants. 2020; 9(9):1249. doi: 10.3390/plants9091249.

3. Fatima I, Safdar N, Akhtar W, Ayaz A, Ali S, Elansary HO, Moussa IM, Zaman, W. Green solvent-based extraction of three Fabaceae species: A potential antioxidant, anti-diabetic, and anti-leishmanial agents. Heliyon. 2024; 10(13). doi: 10.1016/j.heliyon.2024.e33668.

4. Gulati V, Harding IH, Palombo EA. Enzyme inhibitory and antioxidant activities of traditional medicinal plants: potential application in the management of hyperglycemia. BMC Complement Altern Med. 2012; 12(1):1-9. doi: 10.1186/1472-6882-12-77.

5. Al-Snafi AE. The pharmacology and medical importance of *Dolichos lablab* (*Lablab purpureus*)-A review. IOSR J Pharm. 2017; 7(2):22-30.

6. Rai KK, Rai N, Pandey-Rai S. Unlocking pharmacological and therapeutic potential of Hyacinth Bean (*Lablab purpureus* L.): Role of omics-based biology, biotic and abiotic elicitors. Legume Res. 2021; 2. doi: 10.5772/intechopen.99345.

7. Oh SR, Kinjo J, Shii Y, Ikeda T, Nohara T, Ahn KS, Kim JH, Lee HK. Effects of triterpenoids from *Pueraria lobata* on immunohemolysis: D-glucuronic acid plays an active role in anticomplementary activity *in vitro*. Planta Med. 2000; 66:506-510. doi: 10.1055/s-2000-8614.

8. Mythili T, Ravindhran R. Phytochemical screening and antimicrobial activity of *Sesbania sesban* (L.) Merr. Asian J Pharm Clin Res. 2012; 5(4):18-23.

9. Miyazawa M, Sakano K, Nakamura S, Kosaka H. Antimutagenic activity of Isoflavone from *Pueraria lobata*. J Agric Food Chem. 2001; 49(1):336-341.doi: 10.1021/jf000255w.

10. Quradha MM, Khan R, Adhikari A, Rauf A, Rashid U, Bawazeer S, Al-Awthan YS, Bahattab O, Mubarak MS. Isolation, biological evaluation, and molecular docking studies of compounds from *Sophora mollis* (Royle) Graham Ex Baker. ACS Omega. 2021; 6(24):15911-15919. doi: 10.1021/acsomega.1c01532.

11. Um M, Shin GJ, Lee JW. Extraction of total phenolic compounds from yellow poplar hydrolysate and evaluation of their antioxidant activities. Ind Crops Prod. 2017; 97:574-581. doi: 10.1016/j.indcrop.2016.12.062.

12. Xie Y, Zheng Y, Dai X, Wang Q, Cao J, Xiao J. Seasonal dynamics of total flavonoid contents and antioxidant activity of *Dryopteris erythrosora*. Food Chem. 2015; 186:113-118. doi: 10.1016/j.foodchem.2014.05.024.

13. Ajayi AF, Akhigbe RE, Adewumi OM, Okeleji LO, Mujaidu KB, Olaleye SB. Effect of ethanolic extract of *Cryptolepis sanguinolenta* stem on *in vivo* and *in vitro* glucose absorption and transport: mechanism of its antidiabetic activity. Indian J Endocrinol Metab. 2012; 16:S91. doi: 10.4103/2230-8210.94265.

14. Wu N, Zu Y, Fu Y, Kong Y, Zhao J, Li X, Li J, Wink M, Efferth, T. Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L. J Agric Food Chem. 2010; 58(8):4737-4743. doi: 10.1021/jf904593n.

15. Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* Var. saboten. J Agric Food Chem. 2002; 50(22):6490-6496. doi: 10.1021/jf020388c

16. Kwon YI, Apostolidis E, Shetty K. Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. J Food Biochem. 2008; 32(1):15-31. doi: 10.1111/j.1745-4514.2007.00165.x.

17. Elya B, Basah K, Mun'im A, Yuliastuti W, Bangun A, Septiana EK. Screening of α-Glucosidase inhibitory activity from some plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. J Biomed Biotechnol.2012; 2012:1-6. doi: 10.1155/2012/281078.

18. Essid R, Rahali FZ, Msaada K, Sghair I, Hammami M, Bouratbine A, Aoun K, Limam F. Antileishmanial and cytotoxic potential of essential oils from medicinal plants in Northern Tunisia. Ind Crops Prod. 2015; 77:795-802. doi: 10.1016/j.indcrop.2015.09.049.

19. World Health Organization. Department of Control of Neglected Tropical Diseases, World Health Organization. Epidemic, & Pandemic Alert. Dengue: guidelines for diagnosis, treatment, prevention and control. World Health Organization, 2009.

20. NIST. NIST standard reference database number. 2011; 69. https://webbooknistgov/chemistry/.

21. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream (IL): Allured Publ Corp. 2007;4.

22. Lima PJM, Da-Silva RM, Neto CACG, Gomes-e-Silva NC, Souza JEDS, Nunes YL, Sousa-dos-Santos JC. An overview on the conversion of glycerol to value-added industrial products via chemical and biochemical routes. Biotechnol Appl Biochem. 2021. doi: 10.1002/bab.2098.

23. Wongsa P, Chaiwarit J, Zamaludien A. In vitro screening of phenolic compounds, potential inhibition against α-amylase and α-glucosidase of culinary herbs in Thailand. Food Chem. 2012; 131(3):964-971. doi: 10.1016/j.foodchem.2011.09.088.

24. Zhang GP, Xiao ZY, Rafique J, Arfan M, Smith PJ, Lategan CA, Hu LH. Antiplasmodial isoflavanones from the roots of *Sophora mollis*. J Nat Prod. 2009; 72(7):1265-1268. doi: 10.1021/np900144c.

25. Badirzadeh A, Taheri T, Taslimi Y, Abdossamadi Z, Heidari-Kharaji M, Gholami E, Sedaghat B, Niyyati M, Rafati S. Arginase activity in pathogenic and non-pathogenic species of *Leishmania* parasites. PLoS Negl Trop Dis. 2017; 11(7):e0005774. doi: 10.1371/journal.pntd.0005774.

26. Mali JK, Sutar YB, Pahelkar AR, Verma PM, Telvekar VN. Novel fatty acid-thiadiazole derivatives as potential antimycobacterial agents. Chem Biol Drug Des.2020; 95(1):174-181. doi: 10.1111/cbdd.13634.

27. Clementino LC, Torres FAE, Velasquez AMA, Villela L, Mutue TF, Colepicolo P, Graminha MA. Bioguided study of the Antarctic alga *Himantothallus grandifolius* (A. Geep & ES Geep) indicates 13E-Docosenamide as potential antileishmanial agent. J App Pharm Sci. 2020; 10(12):098-103. doi: 10.7324/JAPS.2020.101213

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.33046
Artículos originales

Optimizing Complex Pharmaceutical Formulations Using Lexicographic Goal Programming: A Case Study on Ultradeformable Liposomes

Optimización de Formulaciones Farmacéuticas Complejas Mediante Programación por Metas Lexicográficas: Un Caso de Estudio en Liposomas Ultradeformables

Sonia Valverde-Cabeza [1]
Pedro Luis González-R [2] 0000-0002-1812-0671
María Luisa González-Rodríguez [1] 0000-0002-2450-1622

[1]Universidad de Sevilla, Faculty of Pharmacy, Department of Pharmacy and Pharmaceutical Technology, Seville, Spain.

[2]Universidad de Sevilla, School of Engineering, Department of Industrial Engineering and Management Science, Seville, Spain

Received: 09.03.2025
Accepted: 27.05.2025
Published: 20.06.2025

Correspondence

María Luisa González-Rodríguez
malugoro@us.es

Funding

No fundings received.

Conflict of interests

None

Resumen

Introducción: La optimización de formulaciones farmacéuticas requiere enfoques de estudio avanzados para garantizar calidad, seguridad y eficacia. Entre ellos, aunque la función de deseabilidad y la Programación por Metas Ponderada equilibran múltiples respuestas, carecen de priorización jerárquica. La Programación por Metas Lexicográfica supera esta limitación al optimizar objetivos de forma secuencial. Este estudio presenta a este tipo de programación como un enfoque innovador para optimizar nanoliposomas ultradeformables.

Método: El estudio sigue seis etapas estructuradas, incluyendo la identificación de factores clave, el desarrollo de un Diseño de Experimentos, la definición de niveles de aspiración, la asignación de prioridades y la optimización secuencial. Se enfocó en parámetros críticos como tamaño de vesícula, potencial zeta y eficacia de encapsulación, asegurando una visión jerárquica integral.

Resultados: La Programación por Metas Lexicográfica priorizó primero el potencial zeta y la eficacia de encapsulación, seguidos por el tamaño de vesícula y las respuestas de menor prioridad. Preservó los atributos críticos sin compromisos, minimizando eficientemente las desviaciones tanto para objetivos de maximización como de minimización.

Conclusiones: La Programación por Metas Lexicográfica proporciona un marco jerárquico sólido para la optimización de formulaciones en el campo farmacéutico, gestionando eficazmente los compromisos entre objetivos. Al garantizar que los objetivos clave se cumplen primero, se alinea con los principios de Calidad por Diseño. Este método es especialmente ventajoso para formulaciones complejas, como los sistemas de administración de fármacos liposomales, que requieren un control preciso sobre la estabilidad de las vesículas, su tamaño y la encapsulación del fármaco.

Palabras clave: Toma de decisiones asistida por ordenador; Técnicas de Apoyo para la Decisión; Diseño de Medicamentos; Liposomas

Abstract

Introduction: Optimizing pharmaceutical formulations requires advanced approaches to ensure quality, safety, and efficacy. While the desirability function and Weighted Goal Programming balance multiple responses, they lack hierarchical prioritization. Lexicographic Goal Programming overcomes this limitation by optimizing objectives sequentially. This study introduces this tool as an innovative approach for optimizing ultradeformable nanoliposomes.

Method: The study follows six structured steps, including identifying key factors, developing a Design of Experiments, defining aspiration levels, assigning priorities, and sequential optimization. It focuses on critical parameters such as vesicle size, zeta potential, and encapsulation efficiency, ensuring a comprehensive hierarchical approach.

Results: Lexicographic Goal Programming prioritized zeta potential and encapsulation efficiency first, followed by vesicle size and lower-priority responses. It preserved critical attributes without compromise, efficiently minimizing deviations for both maximization and minimization objectives.

Conclusions: Lexicographic Goal Programming provides a robust hierarchical framework for optimization in the pharmaceutical field, effectively managing trade-offs. Ensuring key objectives are met first, it aligns with Quality-by-Design principles. This method is particularly advantageous for complex formulations, such as liposomal drug delivery systems, requiring precise control over vesicle stability, size, and drug encapsulation.

Keywords: Decision Making Computer-Assisted, Decision Support Techniques; Pharmaceutical Design; Liposomes.

Highlights

The optimization of formulations in Pharmaceutical Technology, which have multiple responses, is typically approached using multi-objective techniques, frequently using the desirability function or Weighted Goal Programming as a global criterion. However, these techniques do not capture hierarchical optimization priorities in responses, which could be a requirement in practice.

To our knowledge, this is the first time that the Lexicographic Goal Programming technique has been applied to the optimization of pharmaceutical formulations.

The optimization of pharmaceutical formulations using Lexicographic Goal Programming improves decision-making by prioritizing critical objectives, ensuring stability and efficacy without compromising

essential attributes in complex formulations such as ultra-deformable nanoliposomes. This methodology could be applied and standardized in the optimization of other formulations where this optimization process is complicated.

Introduction

No borrar esta línea (sub-sección del nivel 1 tiene 2 líneas en blanco después) Optimization of nano pharmaceutical formulations often involves multiple, potentially conflicting objectives such as maximizing drug entrapment, minimizing particle size, ensuring adequate stability, and meeting regulatory criteria[1,2]. Quality-by-design (QbD) principles promote systematic design and risk assessment to ensure robust and high-quality drug products[3–5]. Conventional optimization techniques, including Weighted Goal Programming (WGP), typically aggregate all objectives simultaneously, which may not adequately reflect the importance hierarchy among different responses[6].

Lexicographic Goal Programming (LGP) addresses this shortcoming by introducing a tiered approach. To each objective is assigned a distinct priority, and higher-priority goals are optimized first. The method proceeds to subsequent objectives only after fully or nearly satisfying higher-priority objectives. This study explores the application of LGP to timolol-loaded ultra-deformable nanoliposomes, which are eye drop formulations designed to enhance ocular bioavailability by improving permeability across corneal barriers[7]. By prioritizing critical parameters such as zeta potential (to maintain stability) and drug entrapment efficiency (to ensure therapeutic efficacy), the approach ensures that the final formulation meets stringent performance requirements.

Timolol, a beta-blocker commonly used for glaucoma management, can be formulated into ultra deformable liposomes to enhance ocular penetration and reduce systemic absorption[8]. These vesicles often require balancing size distribution, surface charge and drug encapsulation efficiency to achieve optimal therapeutic effects while minimizing side effects[9]. WGP-based optimization has been used in earlier studies to strike a compromise among these parameters[10]. However, the lexicographic method offers a more decision-driven approach[11]. By systematically addressing the highest-priority responses, such as preventing vesicle aggregation (via stable zeta potential) and maximizing drug entrapment, LGP can yield a more robust solution.

This research aimed to demonstrate the effectiveness of LGP in resolving multi-objective conflicts for QbD-based timolol-loaded ultra-deformable nanoliposomes. The approach highlights how sequential decision-making can achieve lower deviations in high-priority objectives and potentially improve overall performance. Additionally, adaptability of the method to incorporate both minimization (z_1, z_2) and maximization goals (z_3, z_4, z_5, z_6), is showcased.

Methods

The methodology follows a structured, step-by-step process, detailed below.

Identifying Factors and Responses

According to González-Rodríguez et al.[12], five key formulation factors influence the quality attributes of liposomes, including cholesterol amount (F_1), edge activator amount (F_2), the phase in which timolol is added (F_3), the presence of stearylamine (F_4), and the type of edge activator (F_5). This study focuses on optimizing six critical responses: minimizing vesicle size (z_1) to enhance ocular penetration and polydispersity index (z_2) to ensure uniform particle distribution, while maximizing zeta potential (z_3) for colloidal stability, deformability index (z_4) to facilitate transit through ocular membranes, phosphorus content (z_5) to maintain bilayer integrity, and drug entrapment efficiency (z_6) to enhance therapeutic efficacy.

Design of Experiments (DoE)

A fractional factorial Taguchi L16 orthogonal array (see Table 1) was employed to systematically analyze the impact of formulation factors on each response[13].

Table 1. Taguchi L16 orthogonal array. T20: Tween® 20, Deo: sodium deoxycholate.

Run	F_1 Cholesterol (µmol)	F_2 EA (mg)	F_3 TM Phase	F_4 SA	F_5 Edge-activator
1	20	10	Lipid	Yes	T20
2	20	10	Aqueous	No	Deo
3	20	12	Lipid	Yes	Deo
4	20	12	Aqueous	No	T20
5	27	10	Lipid	No	Deo
6	27	10	Aqueous	Yes	T20
7	27	12	Lipid	No	T20
8	27	12	Aqueous	Yes	Deo
9	20	10	Lipid	Yes	Deo
10	20	10	Aqueous	No	T20
11	20	12	Lipid	No	T20
12	20	12	Aqueous	Yes	Deo
13	27	10	Lipid	No	T20
14	27	10	Aqueous	Yes	Deo
15	27	12	Lipid	Yes	Deo
16	27	12	Aqueous	No	T20

Each experimental run measured (z_1–z_6), and regression models were developed to quantify their dependencies on formulation factors. Adjusted R-squared values confirmed model accuracy, and any anomalies were addressed to ensure data reliability.

Defining Aspiration Levels

The aspiration levels for each response were set based on the decision maker's preferences to serve as a guide for the optimization process and ensure the best possible performance of the liposomal formulation. Table 2 presents the target values for each response, defining whether they should be minimized or maximized based on their impact on formulation quality (e.g., using "≤" or "≥"). Zeta potential was set to be maximized above 12 mV to maintain colloidal stability, preventing particle aggregation. Drug entrapment efficiency was prioritized for maximization, requiring values above 4.5 % to ensure enough drug loading. Vesicle size was targeted for minimization, with an aspiration level below 160 nm to facilitate ocular penetration. Similarly, polydispersity index was set to be minimized below 0.155 to ensure uniform size distribution and improve formulation consistency. The deformability index was aimed to be maximized above 0.24 ml/min to enhance vesicle flexibility, improving penetration through biological membranes. Lastly, phosphorus content was maximized above 30 mg to maintain bilayer integrity, ensuring structural stability.

Table 2. Aspiration levels.

Response	Criterion	Target
Vesicle size (z_1)	Minimize (≤)	≤ 160 nm
Polydispersity index (z_2)	Minimize (≤)	≤ 0.155
Zeta potential (z_3)	Maximize (≥)	≥ 12 mV
Deformability index (z_4)	Maximize (≥)	≥ 0.24 ml/min
Phosphorus content (z_5)	Maximize (≥)	≥ 30 mg
Drug entrapment efficiency (z_6)	Maximize (≥)	≥ 4.5 %

These aspiration levels provide a structured reference for optimization, ensuring that key formulation attributes align with pharmaceutical quality standards, even if they are not fully met. In such cases, LGP finds the closest possible solution while respecting the priority hierarchy.

Assigning Priorities

In LGP, the highest-priority goals are satisfied first, ensuring that no solution compromises the top-tier objectives while optimizing lower-tier ones. The primary goal is zeta potential (z_3), which ensures stability, followed by drug entrapment efficiency (z_6) to maintain therapeutic efficacy. Next, vesicle size (z_1) is prioritized as it influences bioavailability, while the deformability index (z_4) assists in ocular penetration. Phosphorus content (z_5) reflects bilayer integrity, and finally, the polydispersity index (z_2) contributes to sample uniformity. This hierarchical approach guarantees that critical formulation attributes are preserved before addressing secondary considerations.

Optimization

LGP solves a series of optimization problems in a predetermined hierarchy. Therefore, if response i (for the set of m responses) has a higher priority than response i+1, i.e. $P_i >> P_{i+1}$, the model can be summarized as follows:

$$\text{Lexmin } G = [d_{P_1}, d_{P_2}, \ldots d_{P_n}] = [n_3, n_6, p_1, n_4, n_5, p_2]$$

Subjected to:

$$z_i(\mathbf{F}) + n_i - p_i = T_i, \forall i \in m$$

$$n_i, p_i \geq 0, \forall i \in m$$

$$F_1, F_2 \in R \text{ and } F_3, F_4, F_5 \in \{0, 1\}$$

where F = (F_1, ..., F_5) represents the sets of decision variables (factors) and z_i(F) represents the actual function value for the i-th goal. The variables n_i and p_i represent the negative and positive deviations (d_{pj}) from the aspiration level T_i and priority j.

Since z_1 and z_2 are minimization responses, their positive deviations p_i (excess over T_i) are minimized. Conversely, for maximization responses z_3 to z_6, their negative deviations n_i (shortfall from T_i) are minimized.

The objective function G first minimizes the deviation associated with the highest-priority goal, then proceeds to the second priority, and so forth[11]. The final solution ensures that higher priorities remain as close as possible to their targets, with lower priorities optimized within that feasible boundary. This stepwise approach is particularly valuable in multi-objective formulation scenarios, where exceeding or missing certain targets can drastically affect product quality[14].

LINGO software was used for solving sequential optimization problems, employing a global solver to ensure optimum solutions for each priority level[15]. Alternative tools could handle the problem similarly, but the robust capability of LINGO for non-linear and mixed-integer programming made it well-suited for these liposomal models.

Results

According to the methodology, the results obtained based on the established priorities are presented below.

Achieving Priority 1: Zeta Potential (z_3)

The first objective minimized n_3, the negative deviation from the zeta potential aspiration level. See z_3 in Figure 1 and Figure 2. The solution yielded a zeta potential above 12 mV (e.g., 12.91 mV in one itera-

tion), confirming that the highest-priority stability requirement was met. All subsequent optimizations locked $n_3 = 0$, meaning no further compromise on zeta potential was allowed.

Achieving Priority 2: Drug Entrapment Efficiency (z_6)

Next, n_6 was minimized to increase entrapment above 4.5 %. The final optimized solution reached or exceeded this threshold (e.g., 4.52 %), ensuring adequate drug delivery. With zeta potential already set, the second priority had to meet its target without reducing z_3 below 12 mV, exemplifying the strength of the hierarchical approach.

Achieving Priority 3: Vesicle Size (z_1)

Subsequently, p_1 was minimized to keep vesicle size below 160 nm. While the solution approached this target, slight deviations were allowed only if they did not compromise the first two priorities. This often resulted in sizes around 152–155 nm, close to the aspiration level, highlighting the trade-off management inherent to LGP.

Remaining Goals

After fixing the first three responses, the model minimized deviations in deformability (z_4), phosphorous content (z_5), and polydispersity index (z_2) in sequence. Minor fluctuations were noted, but overall, each metric remained within acceptable limits. For example, polydispersity might have a slight deviation if optimizing it would conflict with maintaining the higher-priority goals[6]. This structured step-by-step optimization demonstrates the power of lexicographic approaches for managing complex, multi-objective problems.

The evolution of each response over the steps is shown in Figure 1.

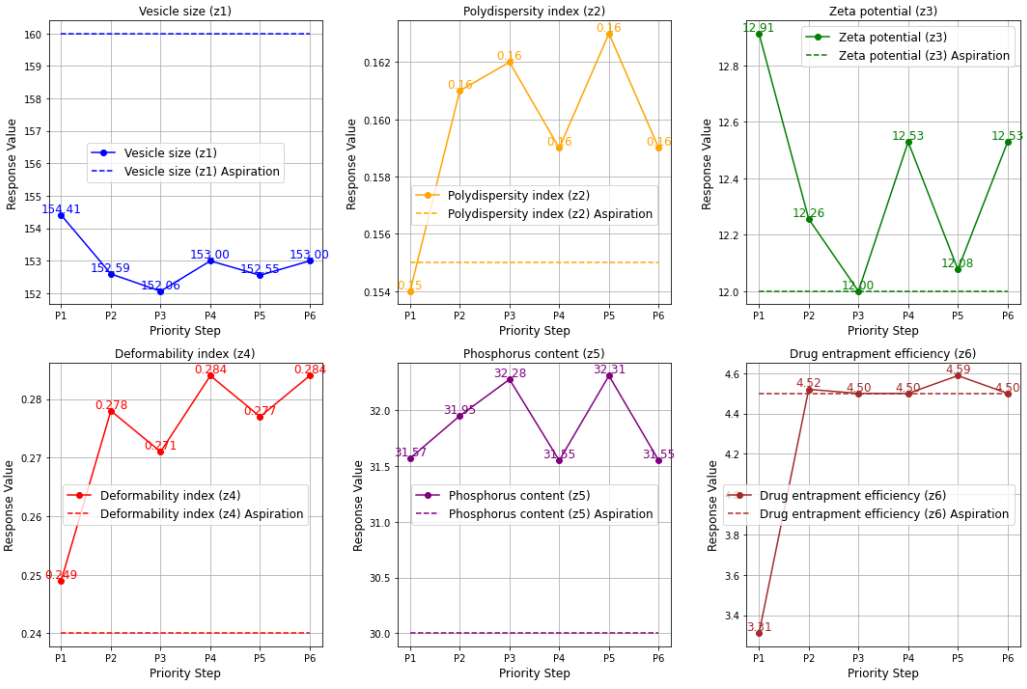

Figure 1. z_i evolution through priority steps in optimization.

In addition, Figure 2 shows the deviations on each response through the priority steps.

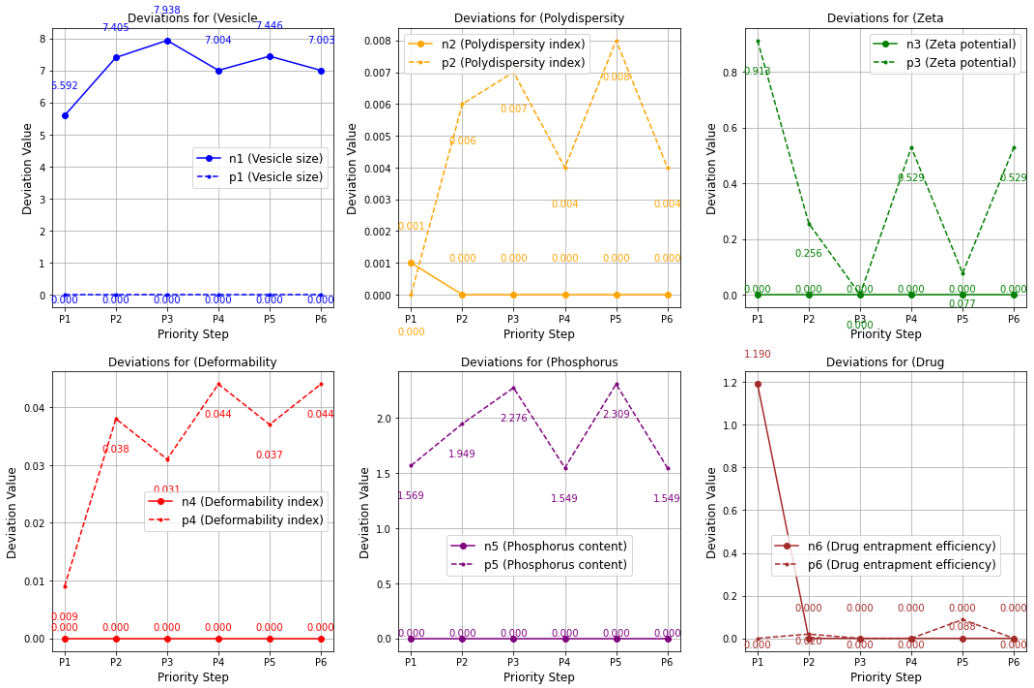

Figure 2. Deviations n_i and p_i evolution through priority steps in optimization.

Discussion

The sequential, hierarchical nature of LGP highlights its utility in tackling complex pharmaceutical optimization challenges, particularly when critical formulation objectives cannot be compromised. Consistent with prior research, emphasizing high-impact parameters—such as zeta potential (z_3) for stability and drug entrapment efficiency (z_6) for therapeutic efficacy—ensures that key formulation attributes are maintained throughout the optimization process[16]. This ensures that the most essential parameters, particularly those linked to colloidal stability and therapeutic potency, achieve near-ideal or acceptable levels before the process attends to lower-priority goals. As a result, the system avoids the simultaneous balancing of all objectives at once, which could dilute the emphasis on crucial variables. The lock-in of high-priority achievements (e.g., $n_3 = 0$ for z_3) provides a stable baseline upon which subsequent optimizations can be executed without eroding earlier successes.

While higher-priority goals often converge fully to their aspiration levels, lower-priority responses may display small deviations due to inherent constraints[11]. For instance, polydispersity index (z_2) may exceed its nominal threshold if pushing it further risks undermining drug entrapment efficiency or colloidal stability. These minor compromises underscore the real-world relevance of LGP. In practice, decision-makers may tolerate slight trade-offs in less critical attributes so long as primary objectives remain intact. Crucially, this stepwise acceptance of lower-priority deviations reflects a pragmatic understanding of limited resources, mechanical constraints, and time or cost considerations.

From a QbD perspective, LGP aligns well with risk-based frameworks that emphasize systematic identification and control of critical process parameters[1]. Because LGP assigns strict priorities and satisfies

them in an orderly, tiered fashion, it readily fits into the QbD approach of defining design spaces that reflect actual manufacturing priorities and constraints. This feature becomes especially relevant in liposomal formulations, where small shifts in composition or process conditions can produce outsized changes in vesicle stability or drug release profiles[14]. By directing resources toward high-impact attributes and locking them in place before refining lower-priority features, LGP creates a robust path for developers to meet their most pressing objectives while still optimizing ancillary characteristics. Ultimately, the integration of LGP into QbD methodologies offers a powerful synergy, enabling structured and evidence-based decision-making that reflects both scientific insights and regulatory expectations.

Conclusions

LGP presents a structured, sequential optimization methodology that aligns well with real-world pharmaceutical development, where certain product attributes cannot be compromised. This study confirms that a hierarchical approach—focusing first on essential responses like zeta potential and drug entrapment—can yield robust, near-optimal solutions for complex, multi-parameter formulations such as timolol-loaded ultra deformable liposomes.

By ensuring minimal deviations for top-tier objectives, LGP preserves stability and therapeutic efficacy while allowing secondary goals to be fine-tuned under fewer constraints. This hierarchical method stands in contrast to WGP, where simultaneous optimization may risk suboptimal performance in critical responses. Overall, LGP is a valuable tool in QbD-driven formulation design, effectively balancing the myriad objectives inherent to pharmaceutical product optimization.

Although the case study focused on timolol-loaded ultra-deformable liposomes, LGP can be generalized to other nanoparticles, micellar systems, or advanced platforms. Potential enhancements include integrating machine learning algorithms to refine the search space or coupling LGP with Bayesian approaches for improved uncertainty management[17]. Such expansions could further streamline pharmaceutical optimization processes.

References

1. ICH Expert Working Group. Pharmaceutical Development Q8(R2). ICH Harmonised Tripartite Guideline. 2009;8.

2. Destro F, Barolo M. A review on the modernization of pharmaceutical development and manufacturing – Trends, perspectives, and the role of mathematical modeling. Int J Pharm. 2022;620:121715. doi:10.1016/j.ijpharm.2022.121715

3. Beg S, Hasnain MS, Rahman M, Swain S. Introduction to Quality by Design (QbD): Fundamentals, Principles, and Applications. In: Beg S, Hasnain S, editors. Pharmaceutical Quality by Design: Principles and Applications. London: Academic Press. 2019;1-17. doi:10.1016/B978-0-12-815799-2.00001-0

4. Grangeia HB, Silva C, Simões SP, Reis MS. Quality by design in pharmaceutical manufacturing: A systematic review of current status, challenges and future perspectives. Eur J Pharm Biopharm. 2020;147:19-37. doi:10.1016/j.ejpb.2019.12.007

5. Alshaer W, Nsairat H, Lafi Z, Hourani OM, Al-Kadash A, Esawi E, Alkilany AM. Quality by Design Approach in Liposomal Formulations: Robust Product Development. Molecules. 2023;28(1):10. doi:10.3390/molecules28010010

6. Greco S, Ehrgott M, Figueira JR. Multiple Criteria Decision Analysis: state of the art surveys operations research & management science. Greco S, Ehrgott M, Figueira JR, editors. Methods. New York, NY: Springer. 2016. (International Series in Operations Research & Management Science; vol. 233). doi: 10.1007/978-1-4939-3094-4

7. Soni PK, Saini TR. Formulation design and optimization of cationic-charged liposomes of brimonidine tartrate for effective ocular drug delivery by design of experiment (DoE) approach. Drug Dev Ind Pharm. 2021;47(11):1847–66. doi: 10.1080/03639045.2022.2070198

8. Kim M, Jang H, Rho S. Risk factors for periorbital dermatitis in patients using dorzolamide/timolol eye drops. Sci Rep. 2021; 11(1):17896. doi:10.1038/s41598-021-97565-0

9. Yoon DJ, Kaur R, Gallegos A, West K, Yang H, Schaefer S, et al. Repurposing ophthalmologic timolol for dermatologic use: caveats and historical review of adverse events. Am J Clin Dermatol. 2021;22(1):89–99. doi:10.1007/s40257-020-00567-3

10. Valverde Cabeza S, González-R PL, González-Rodríguez ML. Enhancing quality-by-design through weighted goal programming: a case study on formulation of ultradeformable liposomes. Drug Dev Ind Pharm. 2025;Feb 27:1–12. doi:10.1080/03639045.2025.2470397

11. Jones D, Tamiz M. Practical Goal Programming. Springer New York, NY. 2010. doi:10.1007/978-1-4419-5771-9

12. González-Rodríguez ML, Arroyo CM, Cózar-Bernal MJ, González-R PL, León JM, Calle M, et al. Deformability properties of timolol-loaded transfersomes based on the extrusion mechanism. Statistical optimization of the process. Drug Dev Ind Pharm. 2016;42(10):1683–94. doi:10.3109/03639045.2016.1165691

13. Tavares Luiz M, Santos Rosa Viegas J, Palma Abriata J, Viegas F, Testa Moura de Carvalho Vicentini F, Lopes Badra Bentley MV, et al. Design of experiments (DoE) to develop and to optimize nanoparticles as drug delivery systems. Eur J Pharm Biopharm. 2021;165:127-148 doi:10.1016/j.ejpb.2021.05.011

14. Cunha S, Costa CP, Moreira JN, Sousa Lobo JM, Silva AC. Using the quality by design (QbD) approach to optimize formulations of lipid nanoparticles and nanoemulsions: A review. Nanomedicine. 2020;28:102206. doi: 10.1016/j.nano.2020.102206

15. Lindo. Lingo and optimization modeling [Internet]. Lindo Systems Inc. 2024 [cited 2024 Jan 19]. Available from: https://www.lindo.com/

16. Shah S, Dhawan V, Holm R, Nagarsenker MS, Perrie Y. Liposomes: advancements and innovation in the manufacturing process. Adv Drug Deliv Rev. 2020; 154–155:102–22. doi: 10.1016/j.addr.2020.07.002

17. Chang H, Domagalski N, Tabora JE, Tom JW. Bayesian data-driven models for pharmaceutical process development. Curr Opin Chem Eng. 2024; 45:101034. doi:10.1016/j.coche.2024.101034

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.31860
Artículos originales

Modulation of gene expression of antioxidant markers by Cynara scolymus extract in thioacetamide-induced liver injury in rats

Modulación de la expresión génica de marcadores antioxidantes por el extracto de Cynara scolymus en el daño hepático inducido por tioacetamida en ratas

Deena M. Eldeberky[1] 0009-0007-0424-0895
Abubakr M. El-Mahmoudy[1] 0000-0002-5855-7421
Faten I. Elsayed[1] 0000-0003-1745-3076
[1]Benha University, Faculty of Veterinary Medicine, Department of Pharmacology,13736 Moshtohor, Qalioubia, Egypt.

Correspondence
Deena M. Eldeberky
deenamagdy53@gmail.com

Received: 04.11.2024
Accepted: 23.03.2025
Published: 20.06.2025

Funding
This work hasn't been funded by anyone.

Conflict of interests
The authors declare no conflict of interest.

Resumen

Introducción: Este estudio evaluó el potencial antioxidante del extracto de *Cynara Scolymus* en la expresión génica de enzimas antioxidantes hepáticas en un modelo de daño hepática inducido por tioacetamida en ratas Wistar.

Método: Se administró tioacetamida en una dosis de 100 mg/kg, por vía intraperitoneal, dos veces por semana durante 8 semanas a 30 ratas Wistar macho. Se evaluó el potencial terapéutico de dos dosis de extracto de *Cynara Scolymus* (100 y 200 mg/kg), en comparación con la silimarina como tratamiento estándar mediante espectrofotometría y reacción en cadena de la polimerasa.

Resultados: El análisis espectrofotométrico de las muestras de homogeneizado hepático reveló una disminución significativa del marcador de peroxidación lipídica malondialdehído y un aumento significativo de las moléculas antioxidantes catalasa, glutatión peroxidasa y superóxido dismutasa (tasas de restauración del 87 %, 95 % y 81 % normalizadas frente a la silimarina). El análisis de la expresión génica reveló que el tratamiento con extracto de *Cynara Scolymus* (100 o 200 mg/kg, diario vía oral, durante 8 semanas) produjo un aumento dosis-dependiente de la expresión de enzimas antioxidantes, superando los efectos de la silimarina estándar. El extracto de *Cynara Scolymus* (100 mg/kg) aumentó la expresión de catalasa, glutatión peroxidasa y superóxido dismutasa hasta 1,85 veces, 1,76 veces y 1,92 veces, respectivamente. Estos efectos fueron mayores con 200 mg/kg, alcanzando 2,43 veces para la catalasa, 2,24 veces para la glutatión peroxidasa y 2,58 veces para la superóxido dismutasa.

Conclusiones: Estos hallazgos sugieren que *Cynara Scolymus* exhibe potentes efectos hepatoprotectores a través de la regulación de la expresión génica de enzimas antioxidantes, constituyendo un enfoque terapéutico prometedor para el tratamiento de lesiones hepáticas.

Palabras clave: *Cynara scolymus*; Expresión génica; Enzimas antioxidantes; Tioacetamida; Hepatoprotección; Catalasa; Glutatión peroxidasa; Superóxido dismutasa.

Abstract

Introduction: This study evaluated the antioxidant potential of *Cynara scolymus* extract on the gene expression of liver antioxidant enzymes in a model of thioacetamide-induced liver damage in Wistar rats.

Method: Thirty male Wistar rats were administered thioacetamide at a dose of 100 mg/kg, intraperitoneally, twice a week for 8 weeks. The therapeutic potential of two doses of *Cynara scolymus* extract (100 and 200 mg/kg) was evaluated in comparison with silymarin as a standard treatment using spectrophotometry and polymerase chain reaction.

Results: Spectrophotometric analysis of the homogenized liver samples revealed a significant decrease in the lipid peroxidation marker malondialdehyde and a significant increase in the antioxidant molecules catalase, glutathione peroxidase and superoxide dismutase (restoration rates of 87 %, 95 % and 81 % normalized against silymarin). Analysis of gene expression revealed that treatment with *Cynara scolymus* extract (100 or 200 mg/kg, daily by oral route, for 8 weeks) produced a dose-dependent increase in the expression of antioxidant enzymes, surpassing the effects of standard silymarin. The *Cynara Scolymus* extract (100 mg/kg) increased the expression of catalase, glutathione peroxidase and superoxide dismutase up to 1.85 times, 1.76 times and 1.92 times, respectively. These effects were greater with 200 mg/kg, reaching 2.43 times for catalase, 2.24 times for glutathione peroxidase and 2.58 times for superoxide dismutase.

Conclusions: These findings suggest that *Cynara scolymus* exhibits powerful hepatoprotective effects through the regulation of the gene expression of antioxidant enzymes, constituting a promising therapeutic approach for the treatment of liver damage.

Keywords: *Cynara scolymus*; Gene expression; Antioxidant enzymes; Thioacetamide; Hepatoprotection; Catalase; Agglutination peroxidase; Superoxide dismutase.

Highlights

This study provides evidence of the hepatoprotective potential of *Cynara scolymus* extract against thioacetamide-induced liver injury through the modulation of antioxidant defence mechanisms.

The study provides a strong foundation for the development of *Cynara Scolymus Extract*-based hepatoprotective nutraceuticals in clinical applications.

Introduction

Liver disease is a serious, worldwide problem with high morbidity and mortality. The main causes of liver diseases are excessive alcohol consumption, viruses, toxins, parasitic diseases, hepatitis, hepatotoxins, antibiotics, and chemotherapeutic agents including paracetamol, carbon tetrachloride (CCl4), and thioacetamide (TAA). TAA is a hepatotoxin frequently used to generate experimental liver damage.

The liver has a great capacity to detoxify toxic substances and produce useful end products. Acute liver injury can be caused by excess alcohol consumption or exposure to hepatotoxins and may progress to severe hepatic diseases such as hepatitis, cirrhosis, and cancer. Acute liver injury is linked to oxidative stress that produces harmful intermediates such as free radicals and redox-active reactants. It can then progress to acute liver failure, which results into hepatic encephalopathy and multiple organ failure, with a high mortality rate [1].

Drugs can cause liver disease in several ways. Some drugs are directly injurious to the liver; others are transformed by the liver into chemicals which subsequently may cause injury to this organ. There are three types of liver toxicity: dose-dependent toxicity, idiosyncratic toxicity, and/or drug allergy [2].

The incidence of hepatic adverse drug reactions (ADRs) remains unknown in the general population. Sgro et al (2002) reported that the main drugs implicated in ADRs were anti-infectious, psychotropic, hypolipidemic agents, and nonsteroidal anti-inflammatory drugs (NSAIDs) [3].

Jabbar et al (2023) investigated the protective effects of silymarin (20 and 40 mg/kg daily for 2 months) against TAA fibrosis in rats (200 mg/kg TAA three times weekly for two months) by histopathological and immunohistochemical assays. The results showed that silymarin is a hepatoprotective compound due to its inhibitory effects on fibrosis, hepatotoxicity, liver cell proliferation, up-regulation of HSP 70, and downregulation of α-smooth muscle actin (α-SMA) expression, inhibiting lipid peroxidation by decreasing malondialdehyde (MDA) production, while retaining the liver index (serum bilirubin, total protein, albumin, and liver enzymes) and antioxidant enzymes to normal [4].

The role of silymarin on the inflammatory response induced by carbon tetrachloride (CCl4) as an example of xenobiotics on liver tissues in male rats has been assessed [1]. Silymarin reduced CCl4 induced-liver inflammation by overcoming the oxidative stress process and inflammatory cytokines production.

TAA-induced hepatotoxicity has been reviewed [5]. TAA induces acute and chronic liver injury due to its effects on the synthesis of proteins, RNA, DNA and γ-glutamyl transpeptidase activity.

TAA administration is an established technique for generating rat models of liver fibrosis and cirrhosis. Oxidative stress is believed to be involved as TAA-induced liver fibrosis is initiated by thioacetamide S-oxide, which is derived from the biotransformation of TAA by the microsomal flavine-adenine dinucleotide (FAD)-containing monooxygense (FMO) and cytochrome P450 systems [6].

Treatment of mice and rats with TAA induced liver cell damage, fibrosis and/or cirrhosis, associated with increased oxidative stress and activation of hepatic stellate cells [7].

The protective effects of silymarin in TAA-induced liver damage have been investigated [8]. This study determined gene expression changes in the liver at the level of microRNA (miRNA), and found that treatment with silymarin before exposure to the toxin successfully altered its effects on the animals in the study.

Jimenez-Escrig et al. (2003) reported that artichoke (*Cynara scolymus L.*), an edible vegetable from the Mediterranean area, is a good source of natural antioxidants such as vitamin C, hydroxycinnamic acids, and flavones [9]. Extracts from *Cynara scolymus* leaves have long been used in folk medicine for their choleretic and hepatoprotective activities, which are often related to the cynarin content [10].

The hepato-curative effects of *Cynara Scolymus* extract (CSE) on CCl4-induced oxidative stress and liver injury has been investigated in rats [11]. CCl4 was administered at a dose of 0.2 ml/kg twice daily. CSE was given orally for 2 weeks at a dose of 1.5 g/kg after CCl4 application on the curative group. Significant decreases in serum alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) and

MDA levels were detected. Significant increase in superoxide dismutase (SOD) and catalase (CAT) activities were obtained. *C. scolymus* leaf extract application normalized the DNA % fragmentation, p53 and caspase 3 levels in the liver.

CSE has antioxidant and anti-inflammatory effects, and countless medicinal properties [12]. Artichokes inhibit the production of reactive oxygen species (ROS) and free radicals due to the phenolic acids and flavonoid compounds they contain, and also suppress the activation pathway of NF-κB. Therefore, CSE reduces oxidative stress and inflammatory factors. On the other hand, CSE has been shown to be effective on the in vitro inhibition of LDL oxidation.

In a previous study, we showed the hepatoprotective role of CSE on a TAA-induced liver injury model [13]. The objective of the present study was to delve deeper into the mechanism of action of CSE, focusing on the gene expression of antioxidant markers (CAT, GPX, SOD).

Methods

Cynara scolymus (Artichoke)

The Cynara scolymus plant is a traditional vegetable crop of the Mediterranean basin. *Cynara cardunculus L.* variety *C. scolymus L.* (artichoke) is cultivated for its fleshy heads. It belongs to the *Asteraceae* family [14]. The *Cynara scolymus* leaves were purchased from a medicinal plant farm at the Faculty of Agriculture of the University of Banha, Egypt. The leaves were collected in March 2023. The plant was identified by Dr. Mostafa Hamza Mohamed, Assistant Professor of Vegetable Crops, Horticulture Department, Faculty of agriculture, Benha University, (Voucher # 96647).

Drugs and chemicals

Thioacetamide (TAA)

TAA is used for experimental induction of liver injury in animal studies [15]. It was obtained from Alamia company, Benha, Qalioubia Governorate, Egypt. TAA powder was dissolved in saline at a concentration of 40 mg/ml and administered at the standard dose of 100 mg/kg body weight [17].

Silymarin

Silymarin was produced by Medical Union Pharmaceuticals (MUP), Abu Sultan, Ismailia, Egypt, under the commercial name Hepaticum®. It is presented as 50 mg / 5 ml suspension.

Reagents used for the assessment of antioxidant markers

Phosphate buffered saline (PBS) solution, pH 7.4 containing 0.16 mg/ml heparin. Cold buffer (100 ml potassium phosphate, pH 7.0, containing 2 ml EDTA per gram tissue). Other standard laboratory chemicals and solutions were also used: 70 % hydroethanolic alcohol, distilled water, normal saline solution (sodium chloride 0.9 %), among others.

Laboratory animals

Thirty male Wister albino rats weighing 150-170 g were obtained from the Faculty of Veterinary Medicine, Benha University, Egypt. The animals were kept in a standard environment with controlled temperature (25 °C), humidity (45–75 %), and photoperiod (12-hr/12-hr light/dark cycle). All animals had free access to food and water. The animals were housed in stainless steel wire mesh cages with wood chip bedding. They were fed a standard diet (vegetable feed with 19 % protein). The animals were given a 15-day acclimatization period. Research was conducted in accordance with the Committee of Experimental Animal Care and Procedure (Faculty of Veterinary Medicine, Benha University, Benha, Egypt).

Preparation of C. scolymus leaves extract

Leaves around the stems of *C. scolymus* were obtained and cut into smaller pieces and then dried at room temperature. The extraction was done by maceration of chopped leaves with 70 % v/v (ethanol/distilled H2O). The mixture was left for 72 hr. in a refrigerator with intermittent shaking. The extract was filtered through muslin mesh, concentrated at 70 °C for 3 days to determine the weight of crude extract, and then stored at 4 °C until needed. The high dose extract was prepared by dissolving 4 g in 200 ml saline solution (0.9 %). The low dose extract was prepared by dissolving 2 g in 200 ml saline solution (0.9 %). Concentrations were 40 mg/ml and 20 mg/ml, respectively. Both extracts were used to evaluate the hepatoprotective effect of artichoke extract in high (200 mg/kg) and low (100 mg/kg) doses in rats [16]. This procedure was repeated weekly to obtain fresh extracts. Yield was determined using the formula:

$$\text{Yield \%} = \frac{\text{wt. of extract}}{\text{wt. of plant}} \times 100$$

Silymarin (Hepaticum®)

Silymarin was administered at a dose of 100 mg/kg body weight [17].

Study design

The experiment was conducted according to the Guide for Care of Laboratory Animals and approved by the Ethical Animal Committee, Faculty of Veterinary Medicine, Benha University (Approval # 96647).

The rats were divided into 5 groups of 6 rats each, as follows:

- Group I: Negative control group. The rats received no drugs, only vehicle (saline solution orally and intraperitoneally).
- Group II: Positive control group (or diseased group). The rats received TAA at a dose of 100 mg/kg, interperitoneally, twice weekly for 8 weeks to develop liver injury.
- Group III: Standard group. This group was made up of rats with liver injury as in group II and they were treated with silymarin (100 mg/kg, orally, daily for 8 weeks).
- Group IV: Small artichoke dose. This group was made up of rats with liver injury as in group II and they were treated with **CSE** (100 mg/kg BW, orally, daily for 8 weeks).
- Group V: Large artichoke dose. This group was made up of rats with liver injury as in group II and they were treated with **CSE** (200 mg/kg BW, orally, daily for 8 weeks).

Sampling

The animals were sacrificed by cutting the carotid arteries with a scalpel and forceps under light ether anesthesia. The livers were examined macroscopically and then removed from the animals. Pieces (100 mg) were placed in Eppendorf tubes and submerged in Trizol for RNA isolation and PCR of hepcidin and interleukin-1 and -2.

Other liver pieces were placed in sterile tubes for homogenate preparation. For this purpose, liver pieces were washed in ice-cooled saline, dried, weighed and kept in buffer saline, and then homogenized with an electric homogenizer. Then the homogenate was centrifuged at 4 °C for excluding debris. The transparent supernatant was used for the evaluation of antioxidant markers [18].

The remaining parts of the liver lobes were preserved in 10 % formalin for histopathological examination.

Measurements

Spectrophotometric analysis

SOD was determined spectrophotometrically using a kit from Biodiagnostic (29 Tahreer St., Dokki, Giza, Egypt), based on the methodology described by Nishikimi et al (1972) [19]. GPx was determined spectrophotometrically with a kit from Biodiagnostic, based on the methodology described by Paglia and Valentine (1967)[20]. CAT was determined spectrophotometrically using a kit from Biodiagnostic, based on the work by Aebi (1984) [21].

RT-PCR of catalase, GPX, SOD in hepatocytes

Total cellular RNA was extracted from the hepatic specimens with Trizol reagent following the methodology described by Chomczynski & Sacchi (2006) [22]. RT was performed with RNA random primers using a RT-PCR kit (Takara Bio INC., Shiga, Japan) following the manufacturer's instructions.

PCR was performed using the following primers (Rikaken, Nagoya, Japan). Catalase, forward: 5′-GCAGATACCTGTGAACTGTCCCT-3′; catalase, reverse: 5′-GTAGAATGTCCGCACCTGAGTGA-3′; GPX, forward: 5′- CGGTTTCCCGTGCAATCAGT-3′; GPX, reverse: 5'-ACACCGGGGACCAAATGATG -3′ for GPX; SOD, forward: 5′-GGTCCACGTTTCTTGTTCTGC-3′; SOD, reverse: 5′- CAATCACACCACAAGCCAAGC-3′.

The PCR products were separated in 1.5 % agarose gel electrophoresis starting with 70 V for 20 min and continuing with 120 V until the fragments reached the desired separation levels. The DNA bands were visualized with an ultraviolet trans-illuminator (UVP, CA, USA). Quantification of amplified DNAs was carried out by densitometric analysis using Scion Image 4.02 software (Scion Corporation, Maryland, USA).

Histopathological examination

After euthanizing the animals, a small portion of each liver was fixed in 10 % formalin. Each piece was dehydrated in an increasing gradient of ethanol and finally cleared into toluene. The pieces of liver were then embedded in molten paraffin wax. Sections were cut with a microtome at 5 mm thickness and stained with hematoxylin and eosin (H&E) and examined under a light microscope at magnification powers of 200x and 400x.

Data Presentation and Analysis

Data of spectrophotometric analysis are expressed as mean ± standard error of the mean of 6 separate observations. Observations were compared with an ANOVA followed by *a posteriori* Tukey *post-hoc* test ($P < 0.05$ was considered statistically significant). Gene expression was done in triplicates and calculated as fold-change compared to the control. All statistics and graphs were generated using GraphPad Prism® version 8 software (GraphPad Inc., CA, USA).

Results

Thioacetamide-induced liver injury and effects of CSE

Figure 1 shows macroscopic and microscopic images of livers from all study groups. Those from TAA-intoxicated rats show diffuse degenerative lesions, including vacuolation, fibrotic and hyperplastic changes at both macroscopic (a) and microscopic (a/) levels. Adjunct administration of artichoke extracts significantly protected the liver tissue from the toxic effect of thioacetamide in a dose-dependent manner (d & d/; e & e/, respectively) reaching results similar to those of the Silymarin group (c & c/).

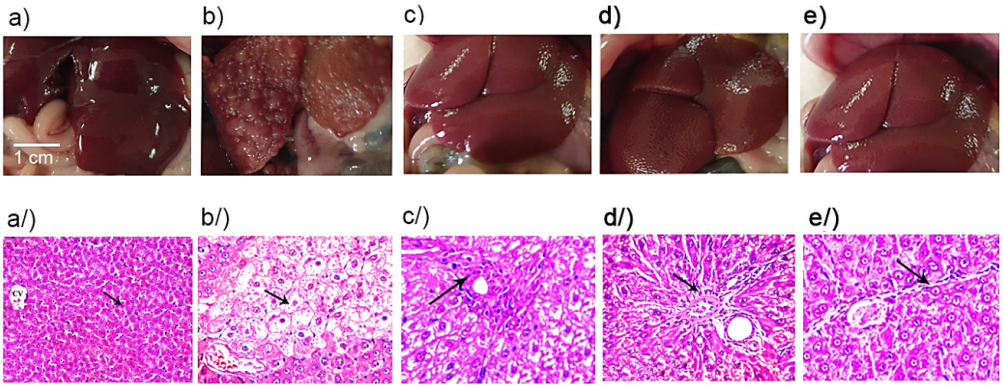

Figure 1. Normal liver tissue (a & a/) and the effects of thioacetamide without (b & b/) or with Silymarin (c & c/), or artichoke extract at small or high dose (d & d/; e & e/). (a) shows normal histology (x 200). (b) shows extensive lesions, namely, hydropic degeneration (arrow) of hepatocytes characterized by swollen, pale, vacuolated cytoplasm (arrow, x400) with mild hyperplasia. (c) shows mild peri-portal fibrous connective tissue proliferation (arrow, x400), with mild leukocyte infiltration. (d & e) show fine strands of fibrous connective tissue (arrow) in the hepatic parenchyma (arrow, x400). CV, central vein. Staining, H&E.

Effect of CSE on liver CAT, GPx and SOD

As shown in table 1, control liver samples showed normal levels of antioxidant markers CAT, SOD and GPx, while those of group 2 revealed a significant decrease in these enzymes compared with control samples. Administration of CSE along with thioacetamide induced significant increases (improvement) in these parameters in comparison with the TAA group. This improvement was dose-dependent and parallel to that in the silymarin group. The potential of protection produced by CSE were 49.26 % after small dose and 87.024 % after large dose (CAT); 49.18 % and 95.44 % (GPx); and 57.93 % and 81.84 % (SOD), as effective as silymarin, respectively.

Table 1: Effects of CSE on liver CAT, GPX and SOD

Group	CAT (IU/g wet wt)	GPx (IU/g wet wt)	SOD (IU/g wet wt)
Control (saline)	5.24 ± 0.32^{a}	72.46 ± 1.4^{a}	126 ± 2.36^{a}
TAA 100 mg/kg	1.97 ± 0.11^{d}	41.34 ± 1.28^{c}	56.39 ± 4.67^{d}
TAA 100 mg/kg + Sil 100 mg/kg	4.19 ± 0.19^{b}	70.35 ± 1.56^{a}	107.9 ± 1.95^{b}
ISD (TAA 100 mg/kg + CSE 100 mg/kg)	3.07 ± 0.07^{c}	55.61 ± 1.17^{b}	86.23 ± 1.08^{c}
ILD (TAA 100 mg/kg + CSE 200 mg/kg)	3.91 ± 0.09^{b}	69.03 ± 1.07^{a}	98.55 ± 2.39^{b}

Data are expressed as mean ± SEM of 6 observations/group. The values in the same column with different letters are significantly different from each other ($P < 0.05$). SOD, superoxidase dismutase; GPx, glutathione peroxidase; TAA, thioacetamide; CSE, artichoke extract; ISD, Intoxicated rats treated with small dose of CSE; ILD, Intoxicated rats treated with large dose of CSE.

The group treated with TAA revealed significant decreases in CAT, GPx and SOD gene expression by 0.42, 0.38 and 0.45 times, compared with those of control samples (table 2 and figure 3). Administration of CSE along with TAA normalized the downregulated gene expressions by 1.85 and 2.43 (CAT), 1.76 and 2.24 (GPx), and 1.92 and 2.58 (SOD) timesd, after small and large doses, respectively.

Table 2. RT-PCR of antioxidant markers

Group	CAT (fold change)	GPx (fold change)	SOD (fold change)
Control (saline)	1 ± 0.07	1 ± 0.08	1 ± 0.09
TAA 100 mg/kg	0.42 ± 0.01	0.38 ± 0.01	0.45 ± 0.01
TAA + Sil 100 mg/kg	0.85 ± 0.07	0.76 ± 0.06	0.9 ± 0.05
ISD (TAA + CSE 100 mg/kg)	1.85± 0.16	1.76 ± 0.15	1.92 ± 0.11
ILD (TAA + CSE 200 mg/kg)	2.43 ± 0.2	2.24 ± 0.1	2.58 ± 0.22

Data are expressed as fold change of 3 observations/group. SOD, superoxidase dismutase; GPx, glutathione peroxidase; TAA, thioacetamide; CSE, artichoke extract; ISD, Intoxicated rats treated with small dose of CSE; ILD, Intoxicated rats treated with large dose of CSE.

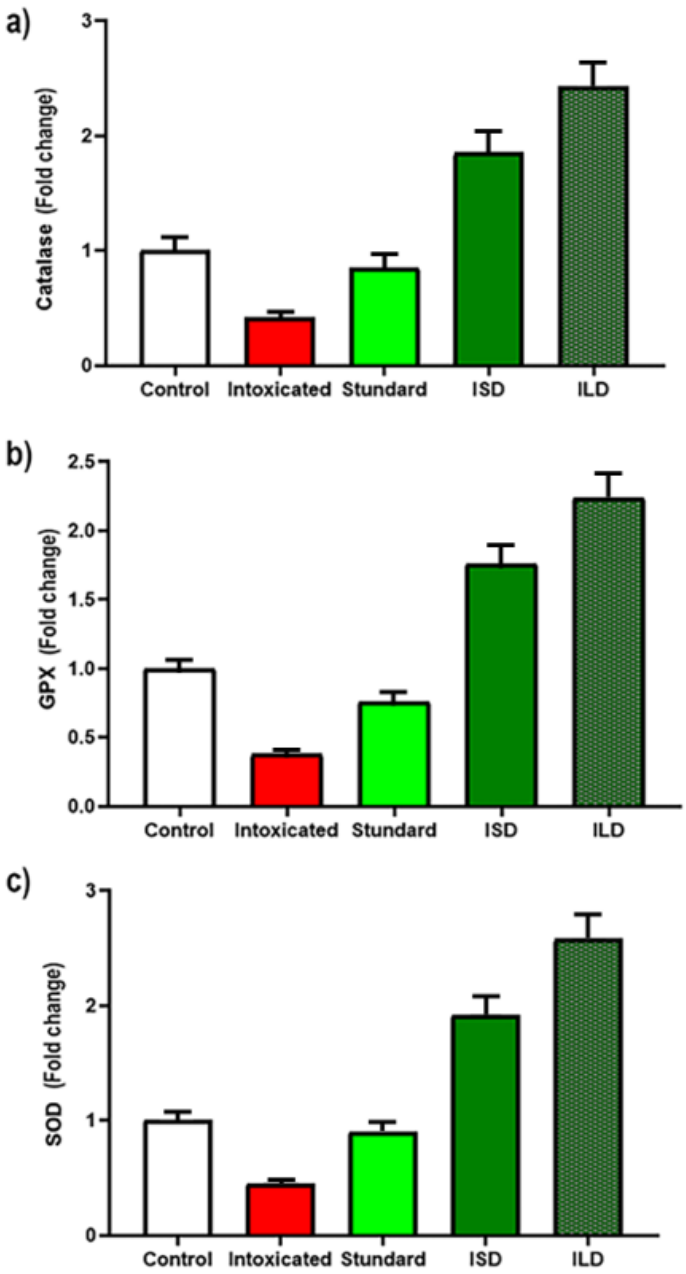

Figure 2. RT-PCR results of antioxidant markers of *CSE* in rats. Data are expressed as fold change of 3 observations/ group. SOD, superoxidase dismutase; GPx, glutathione peroxidase; Intoxicated (TAA, thioacetamide); ISD, Intoxicated rats treated with small dose of CSE; ILD, Intoxicated rats treated with large dose of CSE.

Discussion

Liver disease and associated health problems are a global issue that requires the combined efforts of all professionals and specialists to control it. TAA has been widely used in the development of suitable animal models of acute and chronic liver injury using various doses, times and routes of administration, particularly in drinking water, due to its similarity to human liver fibrosis and cirrhosis.

The present study demonstrates the significant modulatory effects of **CSE** on key antioxidant enzymes in TAA-induced hepatic injury, as evidenced by oxidative stress markers and RT-PCR gene expression analysis. These results were corroborated by the histological study of the liver samples.

In the present study, TAA significantly decreased CAT, SOD and GPx activities and CSE administration restored these values in a dose-dependent manner. The effects of artichoke extract could be attributed to its antioxidant effect; however, it is necessary to investigate the active ingredients of the extract that are responsible for the effect. The antioxidant effect found in the present study is consistent with that reported by El-boshy et al., (2017), who described the protective effect of artichoke leaf extract (300 mg/kg body weight) against cadmium-induced oxidative stress (100 mg/L), hepatorenal damage and immunosuppressive and hematological disorders in rats. Artichoke leaf extract significantly improved the immune response, the antioxidant system and hepatorenal function with a significant decrease in MDA and an increase in GSH, GPx, SOD and CAT [23]. Our data are also consistent with those of Colak et al., (2016), who reported that **CSE**(orally for 2 weeks at a dose of 1.5 g/kg) protected against CCl4 (0.2 mL/kg twice daily)-induced oxidative stress and hepatic injury in rats. Significant increases of SOD and CAT activities in the were found in the group treated with CSE. These results indicate that *C. scolymus* leaf extract has hepatocurative effects on CCl4-induced oxidative stress and hepatic injury by reducing lipid peroxidation [11].

However, our data disagree with those of Speroni et al. (2003), who reported the ineffectiveness of different preparations of *C. scolymus* (1 and 2 g/kg) in liver damage induced by CCl4 in rats. Pretreatment with either of those extracts or chlorogenic acid did not reduce MDA production [10].

Our findings also point to the modulation of the gene expression of the hepatic antioxidant enzymes following treatment with CSE in TAA-induced liver injury. In our work, TAA administration markedly suppressed the expression of key antioxidant enzymes (CAT reduced to 0.42 times, GPx to 0.38 times, and SOD to 0.45 times, compared to control values). This substantial downregulation of the antioxidant defense indicates severe oxidative stress and compromised cellular protective mechanisms in the liver tissue. The therapeutic potential of *CSE* was evidenced by its superior ability to restore and enhance antioxidant enzyme expression compared to the standard treatment, silymarin. Our results agree with those of Seoudi & Saleh (2018) who demonstrated that **CSE** significantly upregulated antioxidant enzymes in CCl4-induced liver damage, showing approximately 2-fold increases in SOD and CAT activities [24]. Moreover, our study is consistent with that of El-Boushy et al (2017), who reported that artichoke extract treatment resulted in a marked elevation of hepatic antioxidant enzymes, with SOD increasing by 2.1 times and GPx by 1.9 times, in an experimental model of liver injury [23].

However, our data may be inconsistent with those of Jabbar et al. (2023), who found a lower improvement in antioxidant enzymes with CSE treatment in TAA-induced liver injury. At 200 mg/kg, CSE only increased catalase 1.3 times, GPx 1.2 times and SOD 1.4 times, substantially less than our findings[4]. Finally, our results are different from those of Banaee et al. (2023), who reported that silymarin has superior antioxidant enzyme modulation compared to **CSE** [25].

Conclusion

This study provides evidence of the hepatoprotective potential of CSE against TAA-induced liver injury through the modulation of antioxidant defense mechanisms. Our findings demonstrate that CSE effectively counteracts TAA-induced oxidative stress, as evidenced by the significant restoration of key antioxidant enzymes. These findings establish CSE as a promising therapeutic agent for liver injury, mainly through its ability to improve cellular antioxidant defense mechanisms. More in-depth research into CSE active ingredients and molecular mechanisms is recommended for its potential clinical applications.

References

1. El-Kot SM, Wanas W, Hafez AM, Mahmoud NA, Tolba AM, Younis AH, et al. Effect of silymarin on the relative gene expressions of some inflammatory cytokines in the liver of CCl4-intoxicated male rats. Sci Rep. 2023;13(1):15245. Doi:10.1038/s41598-023-42250-7

2. Zimmerman BJ. Self-efficacy: An essential motive to learn. Contemporary educational psychology. 2000;25(1):82-91. Doi:10.1006/ceps.1999.1016

3. Sgro C, Clinard F, Ouazir K, Chanay H, Allard C, Guilleminet C, et al. Incidence of drug-induced hepatic injuries: a French population-based study. Hepatology. 2002;36(2):451-5. Doi:10.1053/jhep.2002.34857

4. Jabbar AA, Alamri ZZ, Abdulla MA, AlRashdi AS, Najmaldin SK, Zainel MA. Sinapic acid attenuate liver injury by modulating antioxidant activity and inflammatory cytokines in thioacetamide-induced liver cirrhosis in rats. Biomedicines. 2023;11(5):1447.Doi:10.3390/biomedicines11051447

5. Akhtar T, Sheikh N. An overview of thioacetamide-induced hepatotoxicity. Toxin Rev. 2013;32(3):43-6. Doi:10.3109/15569543.2013.805144

6. Low TY, Leow CK, Salto-Tellez M, Chung MC. A proteomic analysis of thioacetamide-induced hepatotoxicity and cirrhosis in rat livers. Proteomics. 2004;4(12):3960-74. Doi:10.1002/pmic.200400852

7. Kang JS, Wanibuchi H, Morimura K, Wongpoomchai R, Chusiri Y, Gonzalez FJ, et al. Role of CYP2E1 in thioacetamide-induced mouse hepatotoxicity. Toxicol appl pharmacol. 2008;228(3):295-300. Doi:10.1016/j.taap.2007.11.010

8. Teksoy O, Sahinturk V, Cengiz M, İnal B, Ayhancı A. The protective effects of silymarin on Thioacetamide-Induced liver damage: measurement of miR-122, miR-192, and miR-194 levels. Appl Biochem Biotechnol. 2020;191(2):528-39. Doi:10.1007/s12010-019-03177-w

9. Jimenez-Escrig A, Dragsted LO, Daneshvar B, Pulido R, Saura-Calixto F. In vitro antioxidant activities of edible artichoke (Cynara scolymus L.) and effect on biomarkers of antioxidants in rats. J Agric Food Chem. 2003;51(18):5540-5. Doi: 10.1021/jf030047e

10. Speroni E, Cervellati R, Govoni P, Guizzardi S, Renzulli C, Guerra M. Efficacy of different Cynara scolymus preparations on liver complaints. J Ethnopharmacol. 2003;86(2-3):203-11. Doi:10.1016/S0378-8741(03)00076-X

11. Colak E, Ustuner MC, Tekin N, Colak E, Burukoglu D, Degirmenci I, et al. The hepatocurative effects of Cynara scolymus L. leaf extract on carbon tetrachloride-induced oxidative stress and hepatic injury in rats. SpringerPlus. 2016;5:1-9. Doi:10.1186/s40064-016-1894-1

12. Keramati M, Musazadeh V, Ghadimi K. Antioxidant and Anti-inflammatory Effects of Artichoke or Cynara Scolymus L. as Promising Potential Therapeutic in Anemia. J Nutr Food Sec. 2022;7(1):129-35. Doi:10.18502/jnfs.v7i1.8544

13. El-Deberky D, Rizk M, Elsayd F, Amin A, El-Mahmoudy A. Protective potential of Cynara scolymus extract in thioacetamide model of hepatic injury in rats. Bionatura. 2021;6(2):1792-802. Doi:10.21931/RB/2021.06.02.20

14. Giorgi D, Pandozy G, Farina A, Grosso V, Lucretti S, Crinò P, et al., editors. Karyotype of globe artichoke (Cynara cardunculus var. scolymus): preliminary studies to define its chromosome morphology.

VIII International Symposium on Artichoke, Cardoon and their Wild Relatives 983; 2012. 10.17660/ActaHortic.2013.983.17

15. Bernacchi A, De Castro C, de Toranzo E, Marzi A, De Ferreyra E, De Fenos O, et al. Pyrazole prevention of CC14-induced ultrastructural changes in rat liver. Br J Exp Pathol. 1980;61(5):505. PMCID: PMC2041540

16. Zhu X, Zhang H, Lo R. Phenolic compounds from the leaf extract of artichoke (Cynara scolymus L.) and their antimicrobial activities. J Agric Food Chem. 2004;52(24):7272-8. https://doi.org/10.1111/j.1365-2621.2005.tb07106.x

17. Yan-Yu X, Yun-mei S, Zhi-peng C, Qi-neng P. Preparation of silymarin proliposome: a new way to increase oral bioavailability of silymarin in beagle dogs. Intl J Pharm. 2006;319(1-2):162-8. https://doi.org/10.1016/j.ijpharm.2006.03.037

18. Del Maestro R, McDonald W. Oxidative enzymes in tissue homogenates. Handbook of methods for oxygen radical research. 1985:291-6.

19. Nishikimi M, Roa N, Yogi K. Determination of superoxide dismutase in tissue homogenate. Biochem Bioph Res Commun. 1972;46:849-54. DOI:10.1007/s00216-011-5070-8

20. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Clin Lab Med. 1967;70(1):158-69. PMID: 6066618

21. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6. Doi:10.1016/S0076-6879(84)05016-3

22. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. Nature Protocols. 2006;1(2):581-5. Doi:10.1038/nprot.2006.83

23. El-Boshy M, Ashshi A, Gaith M, Qusty N, Bokhary T, AlTaweel N, et al. Studies on the protective effect of the artichoke (Cynara scolymus) leaf extract against cadmium toxicity-induced oxidative stress, hepatorenal damage, and immunosuppressive and hematological disorders in rats. Environ Sci Pollut Res. 2017;24:12372-83. Doi:10.1007/s11356-017-8876-x

24. Seoudi DM, Saleh EM. Assessment of hepatoprotective and apoptotic efficacy of cynara scolymus leaf extract. Intl J Bioscie. 2018;12(1):300-14. Doi:10.12692/ijb/12.1.300-314

25. Banaee M, Impellitteri F, Multisanti CR, Sureda A, Arfuso F, Piccione G, et al. Evaluating silymarin extract as a potent antioxidant supplement in diazinon-exposed rainbow trout: oxidative stress and biochemical parameter analysis. Toxics. 2023;11(9):737.Doi:10.3390/toxics11090737

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.32674
Artículos originales

Evaluation of [^{18}F]FDG distribution and uptake via micro PET/CT in a murine model of myocardial infarction

Evaluación de la distribución y captación de [^{18}F]FDG mediante microPET/CT en un modelo murino de infarto de miocardio

Mustafa Ezzeddin Ayoub[1] 0009-0007-6286-1788
Cesar Rios-Navarro[2, 3, 4] 0000-0002-6405-6279
Matilde Merino Sanjuan[5] 0000-0003-4956-0247
Ana Diaz[1,2] 0000-0002-5396-4466
Vicente Bodi[2,3,6,7] 0000-0001-6425-5828
Facundo Pérez Giménez[8] 0000-0002-2212-9812
Mónica Romero Otero[9] 0009-0003-8372-2307

[1]Unidad Central de Investigación Médica, Universitat de València. València, España.
[2]Centro de Investigación Biomédica en Red (CIBER)-CV. Madrid, España
[3]INCLIVA Instituto de Investigación Sanitaria. València, España.
[4]Departamento de Patología. Universitat de València. València, España.
[5]Departamento de Farmacia, Tecnología Farmacéutica y Parasitología. Universitat de València. València, España.
[6]Departamento de Cardiología. Hospital Clínico Universitario de València. València, España.
[7]Departamento de Medicina. Universitat de València. València, España.
[8]Departamento de Química Física. Universitat de València. València, España.
[9]Departmento de Radiofarmacia. Hospital Clínico Universitario de València. València, España.

Correspondence

Dra. Mónica Romero Otero
Correo: romero_monote@gva.es

Received: 27.01.2025
Accepted: 13.04.2025
Published: 20.06.2025

Funding

This work was supported by grants from “Instituto de Salud Carlos III” and “Fondos Europeos de Desarrollo Regional FEDER” (grant numbers PI20/00637, PI23/01150, CB16/11/00420 and CIBERCV16/11/00350) and Conselleria de Educación–Generalitat Valenciana (grant numbers PROMETEO/2021/008, CIAPOS/2023/247, and CIAPOS2023/248).

Conflict of interests

No conflict of interest is reported in the submission of this manuscript.

Resumen

Introducción: Evaluar la distribución de [^{18}F]Fluodesoxiglucosa ([^{18}F]FDG) en un modelo murino de infarto de miocardio (IM), utilizando Tomografía por Emisión de Positrones y Tomografía Computarizada (PET/CT) para validar este enfoque para su uso en la evaluación de nuevas terapias para el tratamiento del IM.

Método: El IM fue inducido por ligadura permanente de la arteria coronaria descendente anterior izquierda. El estudio incluyó un grupo control, un grupo con 7 días de infarto (infarto agudo) y otro con 21 días de infarto (infarto crónico). Antes de la eutanasia, aproximadamente 60 minutos después de la inyección de [^{18}F]FDG se obtuvieron las imágenes mediante el PET/CT. Los valores de la región de interés y del valor de captación estándar se obtuvieron mediante la CT para evaluar las diferencias entre los grupos en la captación de [^{18}F]FDG en el corazón, el cerebro y la vejiga.

Resultados: Se observó una distribución cardíaca homogénea del radiofármaco en el grupo sano, mientras que en los grupos infartados hubo ausencia de captación en la zona del infarto. Mediante el análisis de ANOVA y la prueba de Kruskal-Wallis, se detectaron diferencias estadísticamente significativas entre los grupos de infarto sano y agudo y los grupos de infarto agudo y crónico. No hubo diferencias significativas entre los tres grupos a nivel cerebral ni en la eliminación de radiofármacos.

Conclusiones: La técnica de microPET/CT tiene la sensibilidad para detectar IM en el modelo murino. Además, nos permite cuantificar la captación de [^{18}F]FDG por los órganos de los diferentes grupos estudiados.

Palabras clave: PET/TC; modelos animales; infarto agudo de miocardio; 18FDG

Abstract

Introduction: To evaluate [^{18}F]Fluodeoxyglucose ([^{18}F]FDG) distribution in a murine model of myocardial infarction (MI), using Positron Emission Tomography and Computed Tomography (PET/CT) to validate this approach for use in evaluating new therapies for MI treatment.

Method: MI was induced by permanent ligation of the left anterior descending coronary artery. The study included a sham group, a group with 7 days infarction (acute infarction) and another with 21 days infarction (chronic infarction). Prior to euthanasia, each group was imaged by PET/CT approximately 60 minutes after injection of [^{18}F]FDG. Total and maximal Region of Interest and Standard Uptake Value values were obtained using CT, which enables more precise delineation of the anatomy to assess between-group differences in [^{18}F]FDG uptake in the heart, brain and bladder.

Results: Following distribution of [^{18}F]FDG throughout the body, at 60 minutes after injection homogeneous cardiac distribution of the radiopharmaceutical was observed in the healthy group, while in the infarcted groups there was an absence of uptake in the infarct area. Using ANOVA analysis and the Kruskal-Wallis test, statistically significant differences were detected between the healthy and acute infarction groups and the acute and chronic infarction groups. There were no significant differences between the three groups in brain uptake or radiopharmaceutical clearance.

Conclusions: The microPET/CT technique has the sensitivity to detect infarcted areas in the murine model. Moreover, it allows us to quantify [^{18}F]FDG uptake and thus assess uptake by the organs in the different groups studied.

Keywords: PET/CT; Animal Models; Myocardial Infarction; 18FDG.

Highlights

Current scientific knowledge supports the use of microPET/CT to evaluate [^{18}F]FDG distribution in murine models of MI. This method allows identifying infarcted areas with high sensitivity, quantifying glucose uptake and evaluating differences depending on the state of the infarct. The study validates the use of microPET/CT to detect and quantify [^{18}F]FDG uptake in infarcted areas in a murine model, providing a precise tool to evaluate new therapies in MI. The results support the use of microPET/CT as an accurate tool to evaluate therapies in MI, facilitating preclinical research and improving the development of innovative cardiovascular treatments.

Introduction

Cardiovascular pathologies represent one of the leading causes of morbidity and mortality at the global level[1]. They are considered complex multifactorial pathologies involving both genetic and environmental factors, which makes it difficult to establish prevention criteria covering all risk factors[2]. Lifestyle habits (smoking, alcohol consumption, diets and lack of physical activity) and individual health factors (blood pressure, cholesterol, blood glucose) also exert a decisive influence on cardiovascular health[1].

Myocardial infarction (MI) is a cardiovascular pathology caused by thrombotic occlusion of a coronary artery. The first line of treatment for patients with ST-segment elevation MI is rapid coronary revascularization to restore nutrient and oxygen supply to the myocardium[3]. As a consequence of the ischemia-reperfusion process, an inflammatory response is triggered that facilitates the removal of necrotic cardiomyocytes and remnants of the infarcted tissue matrix[4] and the subsequent formation of a collagen-based fibrotic scar[5].

Animal models are an interesting method to study the pathophysiological aspects of disease and to evaluate new therapeutic and preventive options[2]. Over the last two decades, the mouse model of MI has become the preclinical model of choice to study the molecular mechanisms of human heart physiology and disease[6].

Despite substantial differences from humans in heart size, heart rate, and myocardial structure, other characteristics of this species position it as an interesting animal model, including the lower cost of procedures and the capacity to manipulate the genome[2]. In addition, the results obtained in the preclinical phase using this animal model can be transferred to clinical phase studies always taking in consideration the differences between animal model and , which has increased interest in this model, especially for developing new therapies[8].

Imaging techniques are non-invasive methods which enable us to assess not only myocardial metabolism and viability[9] but also the distribution, pharmacodynamics and toxicity of new drugs[10]. Among these, positron emission tomography (PET) with [^{18}F]Fluodeoxyglucose ([^{18}F]FDG), chosen for its sensitivity, is considered an effective technique to study myocardial viability[11]. [^{18}F]FDG is a radiopharmaceutical that behaves metabolically like glucose, but unable to continue with the glycolytic pathway because it lacks a hydroxide in position 2, it is retained inside the cell, which allows us to analyse the real consumption of glucose in the tissue under study (Figure 1).

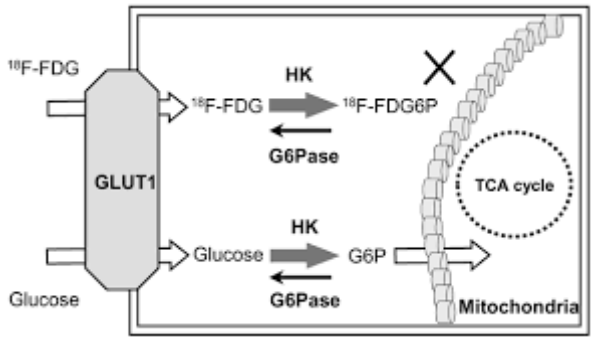

Figure 1. Uptake of [^{18}F]fluordeoxyglucose ([^{18}F]FDG)[12]

Whole-body PET images taken one to two hours after administration of [^{18}F]FDG indicate radiopharmaceutical accumulation occurs mostly in the brain, heart and urinary tract. In the postprandial state, the myocardium has a similar uptake of [^{18}F]FDG to the brain, but in prolonged fasting state (more than 12 hours), myocardial uptake of this drug is reduced because myocardial metabolism shifts to

fatty acid consumption as an energy source. [^{18}F]FDG is eliminated by renal excretion and in the absence of intense hydration, diuretics and urinary catheterization, can easily localize in the bladder and upper urinary tract [13].

Although PET after [^{18}F]FDG administration enables some anatomical structures to be visualized, the spatial resolution provided by this technique is generally inadequate to accurately localize pathology. However, combining the PET technique with a high-resolution technique such as computed tomography (CT) helps to improve anatomical identification of injured tissues[14] the spatial resolution is generally inadequate for accurate anatomic localization of pathology. However, combining the PET technique with a high-resolution technique such as computed tomography (CT) helps to improve anatomical identification of injured tissues[14].

Employing a murine model of acute MI, the main aim of this study was to evaluate [^{18}F]FDG uptake in the heart, brain and bladder using the micro PET/CT imaging technique to analyse possible alterations in the distribution of the radiopharmaceutical caused by the pathology.

Methods

Ethical Statement

This study was approved by the Research Ethics and Animal Experimentation Committee of the Universitat de València (protocol number: 2017/VSC/PEA/00106) and was performed following the guidelines of Directive 2010/63/EU.

Murine model and induction of myocardial infarction

The study was performed with C57BL/6J mice (Charles River Laboratories, Châtillon-sur-Chalaronne, France) aged 16±2 weeks and weighing 22.144±2.35 grams. The animals were bred in the animal house of the University of Valencia and maintained under specific pathogen-free conditions at a constant temperature of 22±2 °C and humidity of 60-65 % with a 12-hour light/dark cycle and with free access to a standardized diet (Teklad Global Rodent Diets, Inotiv) and autoclaved water.

The animals included in the study were randomly distributed into three groups; group I (n = 24): control (healthy group); group II (n = 10): acute infarction (1–7 days), and group III (n = 14): chronic infarction (14–21 days). Infarction in both groups (acute and chronic) was induced by permanent ligation of the left anterior descending coronary artery[15]. The knot was tightened with consequent occlusion of coronary flow. Ischemia was confirmed for all animals included, no animals were excluded, by ST-segment elevation on the electrocardiogram. The animals were kept under fasting conditions for 4–6 hours with free access to water before imaging. The main difference between groups II and III is the time elapsed from infarct provocation to imaging and subsequent animal sacrifice, which was 7 days for the acute phase infarction group (group II) and 21 days for the chronic phase (group III). Intraperitoneal buprenorphine (0.05 mg/kg, twice daily) and meloxicam (0.3 mg/kg, once daily) was administrated for 5 days after surgery.

PET/CT Protocol

PET/CT images were performed prior to euthanasia, which was 7 days for the acute infraction group and 21 days for the chronic infraction group. To have a homogeneous study, control group was also imaged at 7 and 21 days. For it, the images were obtained as explained below.

Anesthesia

Both radiopharmaceutical injection and image acquisition were performed under anesthesia induced with the aid of an anesthesia machine (Cyprante Keighleit) and flow-meter for oxygen supply. This anesthesia was administered by inhalation and consisted of two stages[16]achieving prolonged immobility for sensitive imaging modalities (magnetic resonance imaging for instance. The first phase (induction) was performed with 3–4 % isoflurane (Zoetis-IsoFLo® 100 % w/w) and 100 % from a medical oxygen

cylinder (Nippongases, Valencia). This reduces animal stress and prevents this factor from affecting subsequent uptake of the radiopharmaceutical. During the second phase (maintenance), the inhalation mixtures contained 1–2.5 % isoflurane and oxygen was maintained at 100 %.

Administration of [^{18}F]FDG

The [^{18}F]FDG used was produced and supplied by CuriumTM Life Forward, and was administered intraperitoneally [IP][17]the impact of dietary conditions, mode of anesthesia, and ambient temperature on the biodistribution of ^{18}F-FDG in mice has not been systematically studied so far. The aim of this study was to determine how these factors affect assessment of tumor glucose use by ^{18}F-FDG PET and to develop an imaging protocol that optimizes visualization of tumor xenografts. Methods: Groups of severe combined immunodeficient (SCID using a dose of between 200–300µCi in all animals included in the study. After administration of the radiopharmaceutical, the animals were kept at rest for 60 ± 10 minutes to allow complete and homogeneous distribution of the radiopharmaceutical around the body, after which the images were obtained.

Acquisition and reconstruction of images using microPET/CT

Image acquisition was performed following the Albira Acquierer protocol (New Albira Suite 3.0 - Rheinstetten, Germany) with microPET/CT equipment (Albira I, Bruker Biospin PCI GmbH Rinstetten, Germany), equipped with a spatial resolution of 70 microns for CT and 1mm for PET. Sequential whole-body images were obtained, with a duration of 7 minutes for CT and 15 min of two frames for PET.

PET and CT images were processed and reconstructed in 2D and 3D with the Albira Reconstructor program (New Albira Suite 3.0 software - Rheinstetten, Germany), using a cross OSEM algorithm and CT Rec. Alg algorithm, respectively. Visualization and fusion of both images with AMIDE version 1.0.4 software (A medical Imaging Data Examiner, Boston, MA 02111-1307-USA) enabled more precise anatomical localization of radiopharmaceutical uptake observed by PET.

Quantification of radiopharmaceutical uptake and consumption

The images obtained were used to delimit the contour or area of interest (ROI) around each specific organ. This delimited region is used to evaluate the amount of the radiopharmaceutical that accumulates in each organ by calculating the Standard Uptake Value (SUV)[18]. Both parameters allow quantification of the consumption/uptake of [^{18}F]FDG, providing information on the distribution of the radiopharmaceutical in the organism and on the physiological function of each organ. Quantification was performed with the PMOD program (Technologies® LLC, Zurich, Switzerland). The ROI and SUV values for each organ studied were analyzed in absolute and relative terms expressed as a percentage of radiopharmaceutical uptake in each organ in relation to total uptake in the organism (sum of the values obtained in the three organs studied). After digitizing the images, the quantification of all PET-CT images were performed in a dedicated laboratory by a trained observer unaware of the experimental protocol applied.

Calculation of ROI

Using CT (figure 2), the organ of interest or ROI is completely delimited, and the PET image is hybridized over this region. Next, the uptake values of [^{18}F]FDG in the total ROI expressed in kBq/cm^3 are obtained using the statistics application. To calculate the maximum ROI (ROI_M), the area of maximum radiopharmaceutical uptake is located, and several measurements are performed to obtain the value of this parameter expressed in kBq/cm^3.

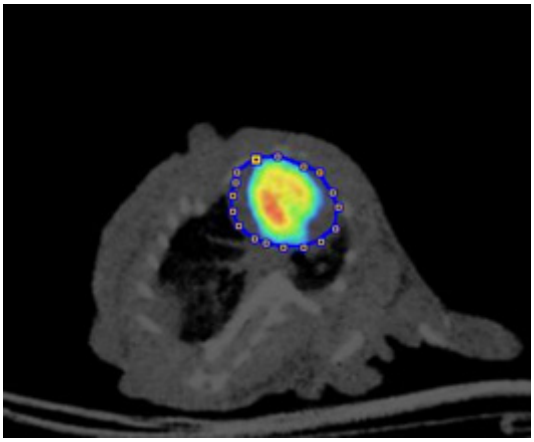

Figure 2. Region of interest (ROI) drawn on the Computed Tomography to delimit the total area of the heart (ROI_T) and the maximum uptake area (ROI_M).

SUV calculation

The total SUV (SUV_T) and maximum SUV (SUV_M) values of the heart, brain and bladder were calculated from the uptake values obtained in each ROI. The calculation was performed by dividing the ROI value by a correction factor (Activity [KBq]/Animal weight [g])[18] [equation 1]:

$$\boldsymbol{SUV} = \frac{\boldsymbol{ROI}[\boldsymbol{kBq/cm^3}]}{\boldsymbol{Activity}[\boldsymbol{kBq}]/\boldsymbol{Animal\ weight}[\boldsymbol{g}])} \quad \boldsymbol{equation\ (1)}$$

To compare [^{18}F]FDG distribution in each group studied, the two parameters (ROI and SUV) were analyzed in relative terms according to the following equation (equation 2):

$$\boldsymbol{ROI}(\%) = \frac{\boldsymbol{ROI_{Organ}} \cdot \boldsymbol{100}}{\boldsymbol{ROI_{Heart}} + \boldsymbol{ROI_{Brain}} + \boldsymbol{ROI_{Bladder}}} \quad \boldsymbol{equation\ (2)}$$

Histology study

Once the animal was sacrificed, the heart was extracted and cut into 1-mm-thick short-axis slices. Sections of the sectioned myocardium (5µm) were fixed using 4 % paraformaldehyde acid, embedded in parrafin, and mounted on double gelatin-coated glass slides. To facilitate histological analysis and quantification of infarct size, samples were stained with hematoxylin-eosin (Sigma Aldrich, St. Louis, MO) before observation with Leica DM3000 light microscope (Leica Microsystems, Wetzlar, Germany). Scoring was performed blinded on coded slides.

Statistical Analysis

Statistical analysis of the results was performed using the IBM SPSS version 25 program. We assessed normality of distribution with the Kolmogorov – Smirnov test. The parameters obtained in each organ and test group were expressed using the mean, standard deviation and 95 % confidence interval. To determine whether the study parameters showed statistically significant health status-related differences, ANOVA was performed after testing for normal distribution of the variable using the Shapiro Wilk test and homogeneity of variance (Levene's test). If the variance was not homogeneous, the Kruskal Wallis nonparametric test was used. The level of statistical significance was set at $p<0.05$.

Results

PET-CT images

Figure 3 shows hybrid images captured with PET/CT of the heart in axial, coronal and sagittal planes. The figure shows that cardiac uptake of [^{18}F]FDG differs according to the animal group studied. Thus, in group I (healthy animals, image 3a) cardiac uptake is homogeneous, whereas in acutely and chronically infarcted animals (group II, image 3b and III, image 3c, respectively) there is an absence of radiopharmaceutical uptake around infarcted tissue.

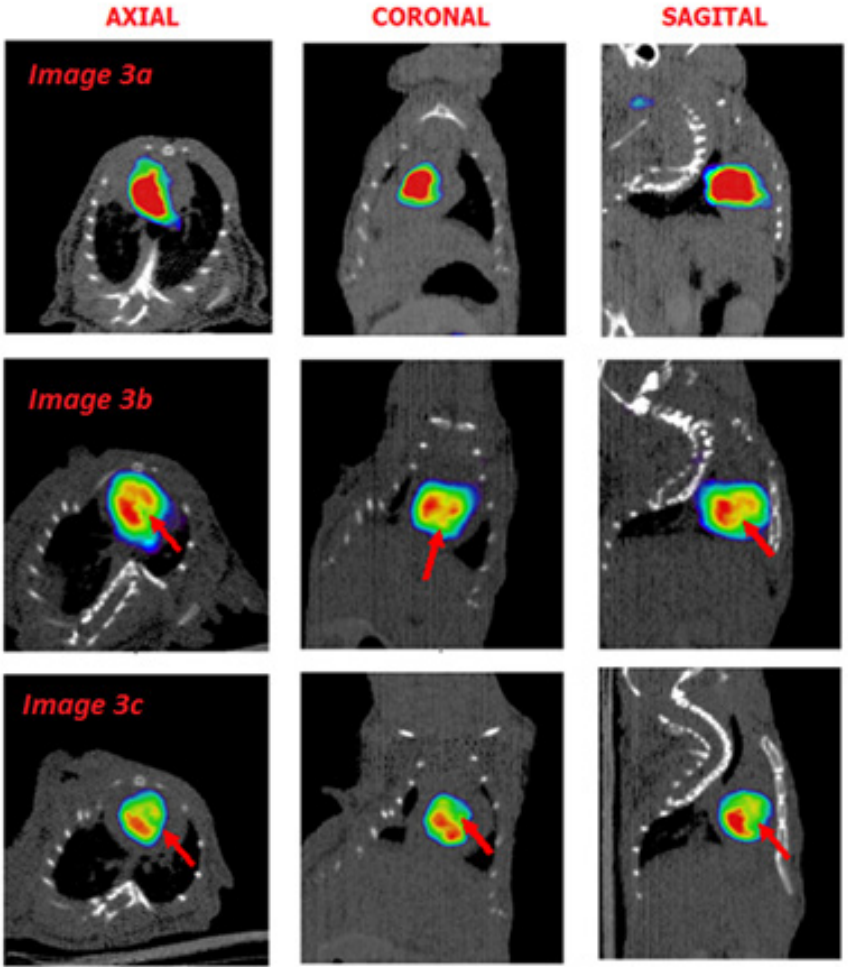

Figure 3. Cardiac images of the three axial, coronal and transverse slices. Image 3a shows a healthy mouse. Images 3b and 3c show samples from an acutely and chronically infarcted mouse, respectively (arrow: area of absence of uptake of [18F]fluordeoxyglucose ([18F]FDG)).

In figure 4, the mean values of the ROI_T and SUV_T parameters and their distribution in each organ and group tested are shown in figures 4.1 and 4.2, respectively. No statistically significant differences in [^{18}F] FDG uptake in brain and bladder were detected in ANOVA statistical analysis. However, cardiac uptake of [^{18}F]FDG showed statistically significant differences, with higher uptake in animals with acute phase infarction (group II) than in healthy animals (group I) or animals with chronic phase infarction (group III) [Kruskal-Wallis, $p<0.001$].

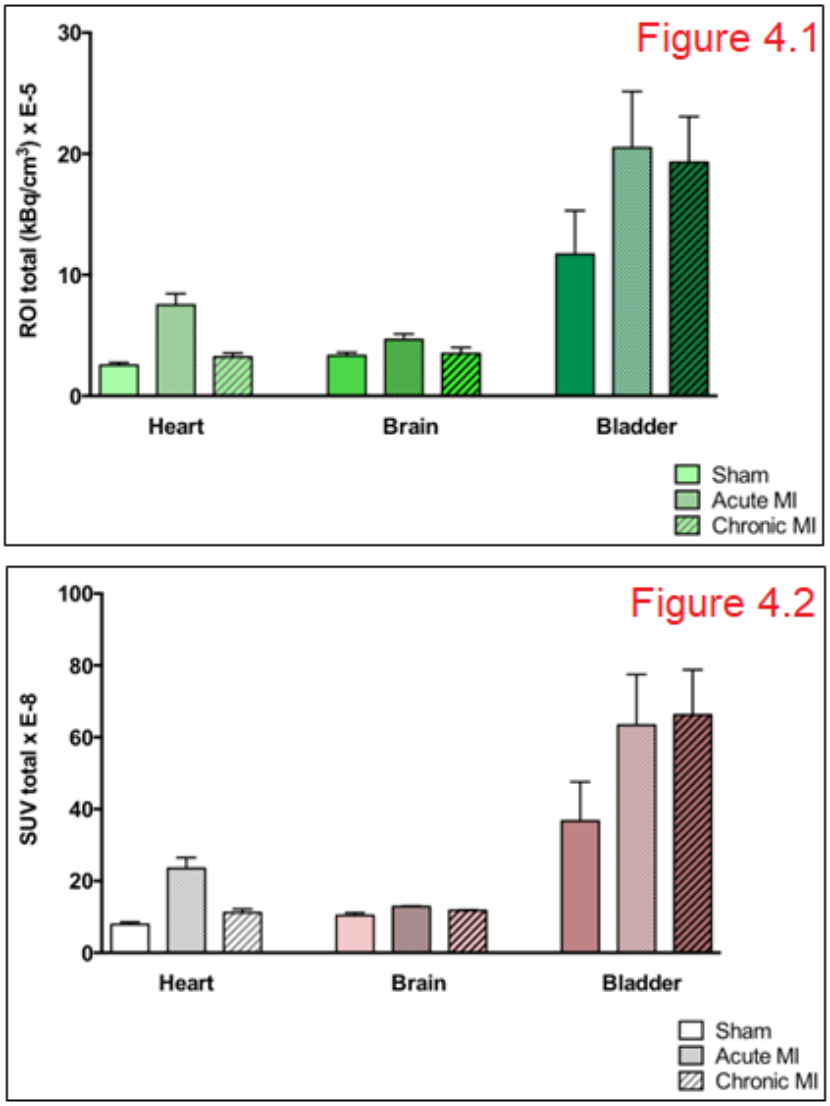

Figure 4. For each study group and organ MI, in figure 4.1, mean values and dispersion of Region of Interest (ROI_T). In figure 4.2, mean values and dispersion of Standard Uptake Value (SUV_T). MI: myocardial infarction.

The mean values of the relative ROI_T and SUV_T for each organ and group studied are shown in figure 5. As can be seen, the bladder was the organ with the highest uptake in all three of animal groups. Furthermore, in healthy animals (group I) brain uptake was slightly higher than cardiac uptake, a relationship that was inverted in animals with acute infarction (group II).

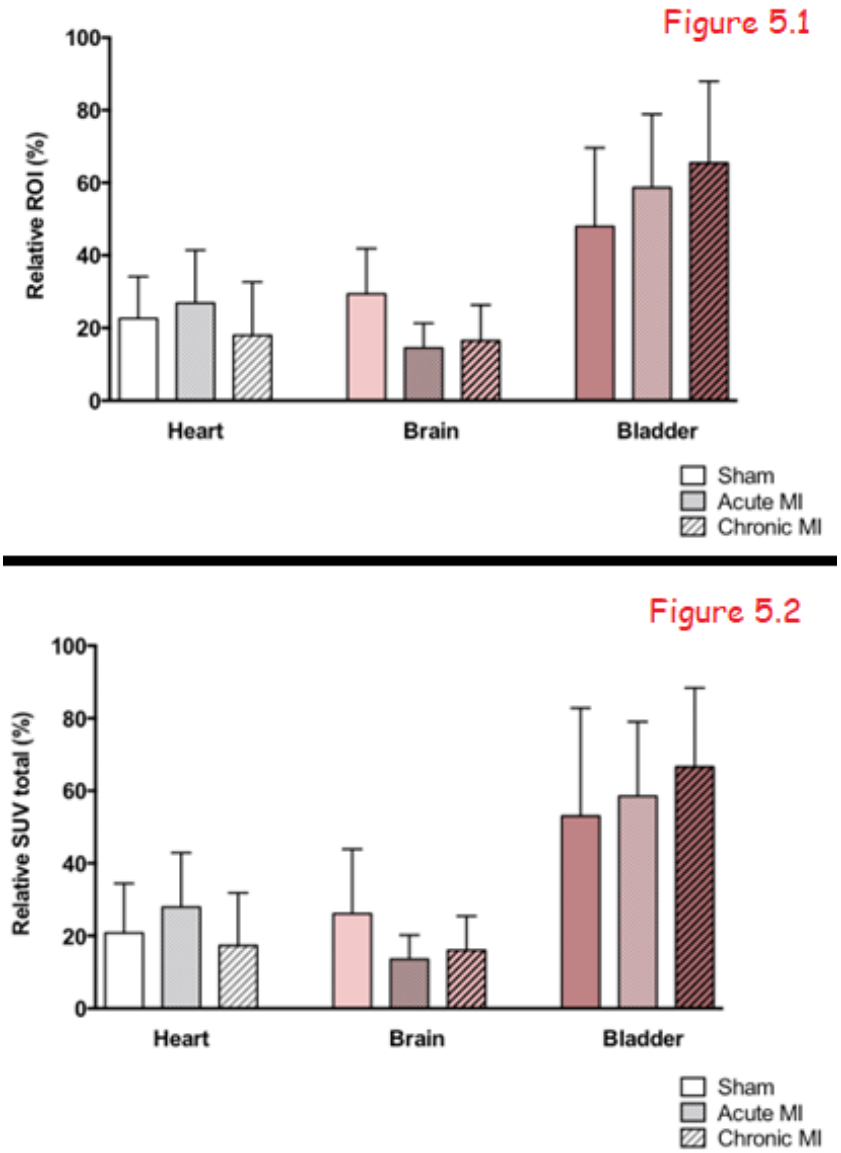

Figure 5. Figure (5.1) total Region of Interest (ROI_T) and Figure (5.2) total Standard Uptake Value (SUV_T) values in each organ expressed as a percentage in each animal group in the study. MI: myocardial infarction.

Histological study

Figure 6 shows both the histological sections of the cardiac tissue and the images taken with the Leica DM3000 optical microscope for each animal group, revealing healthy cardiomyocytes in the control group (group I), inflammatory cells in the acute phase (group II), and collagen fibers and myofibroblasts in the animals with chronic phase infarction (group III).

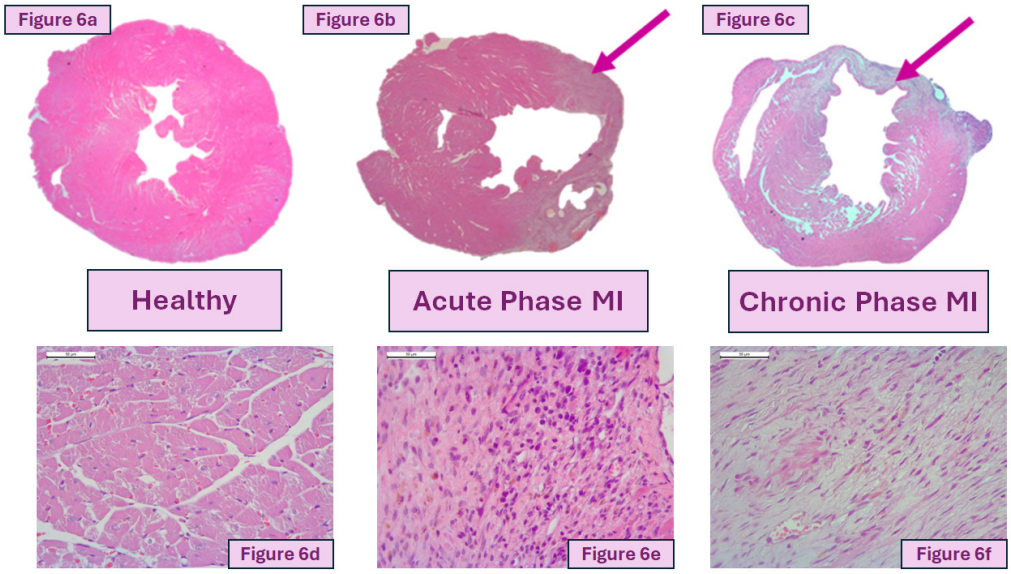

Figure 6. Histological sections of a healthy (7a), Acute phase MI (7b) and Chronic phase MI heart (7c) – Arrows shows infarction zone. Figures 7d, 7e and 7f show a microscopic image of cardiac tissue (50µm) from animals in the study groups. MI: myocardial infarction

Discussion

MI is a pathology with high incidence and is one of the leading causes of death worldwide[19,20]. Animal models aimed at understanding natural disease evolution and/or the effect of drugs to minimize cardiac injury is crucial[21]. The animal species most commonly used in models available for MI study is the mouse[22], which has a small heart and high heart rate. These models have proven useful for studying the temporal evolution of cardiac tissue after MI induction, and radiopharmaceuticals are optimal for monitoring alterations taking place in the tissue, given that radiopharmaceutical distribution and energy consumption requirements change significantly in response to MI-related damage[23].

Imaging techniques (ultrasound) for detecting MI present several limitations for studying disease evolution, including difficulty in measuring the size of the infarcted tissue and the need for acquisition angle-dependent analysis[24]. Furthermore, cardiac function and myocardial viability maintained after infarction cannot be accurately determined by these techniques. Finally, use of this technique in mice is complicated by their small heart size and high heart rate[25]. These drawbacks have prompted recourse to imaging techniques in nuclear medicine, such as PET, which require the use of radiopharmaceuticals.

Among the radiopharmaceuticals currently available that have shown to be suitable for diagnosis and localization of ischemia and/or myocardial infarction are compounds labeled with metastable tencenyl-99 (99mTc), such as 99mTc-Tetrofosmin and 99mTc-Sestamibi[26]. However, single-photon emis-

sion computed tomography (SPECT) must be used to image these compounds[27]. The limitations of this technique include the need for additional reconstruction to obtain 3D images, which only permits semi-quantitative assessment, and the lower sensitivity of SPECT compared to PET[27]. Overall, PET provides better resolution and detection sensitivity, less attenuation and fewer image artifacts than SPECT[28]. We selected the radiopharmaceutical [^{18}F]FDG for this study as it is a widely used radiopharmaceutical whose time course in the body is known[29] and which is indicated for cardiological studies[30].

PET with [^{18}F]FDG is a technique capable of detecting and differentiating damaged areas of the heart thanks to differences in radiopharmaceutical consumption and uptake by the myocardium[31] and can also be used to quantify MI magnitude by measuring the parameters of the ROI and SUV[18]. Furthermore, combining PET with CT[23] allows for a more precise anatomical localization of these uptake areas. Homogeneous [^{18}F]FDG uptake is observed in the absence of injury, whereas no uptake is observed when there is cellular damage in tissue[32] thus allowing qualitative/visual localization of tissue injury.

The results obtained in this study based on [^{18}F]FDG uptake in the heart, brain and bladder in the three groups indicated no statistically significant differences ($p>0.05$) by ANOVA in [^{18}F]FDG uptake in the brain and bladder after induction of MI. In the heart, however, the distribution and uptake of [^{18}F]FDG presented statistically significant differences ($p<0.001$) between healthy animals (group I) and those in the acute infarction phase (group II), as well as between the two infarcted animal groups (acute and chronic). That no significant differences were observed in [^{18}F]FDG uptake by the cardiac tissue of healthy and chronically infarcted animals could be explained by changes in the pathophysiology of cardiac tissue after infarction development. At around day 3–7 post-infarction, massive inflammation occurs in the myocardium to eliminate apoptotic cardiomyocytes due to lack of blood supply. Subsequently, this inflammation resolves and the process of fibrosis begins[33], consisting of the differentiation of myofibroblasts that synthesize collagen in the infarcted area to replace the dead cells removed. The images obtained at 7 and 21 days would thus correspond to the inflammatory and fibrosis phases, respectively[34].

The values of the ROI_T and SUV_T expressed in relative terms (figure 5) indicate that in healthy animals (group I) brain glucose consumption is slightly higher than cardiac consumption, which may be because to obtain energy during the fasting period undergone by all the study animals, the heart consumes more fatty acids as opposed to the brain, which requires glucose for energy. However, in animals in acute phase infarction (group II), the heart takes up more than the brain, which may be due to massive migration of macrophages that consume glucose occurring during the post-surgical inflammatory process, while in animals in chronic phase infarction (group III) radiopharmaceutical uptake is similar in the heart (17 %) and in the brain (16 %).

Histologically, the extracellular matrix of cardiac tissue is a three-dimensional network composed of fibers and ground substance that participates in providing structural support to cardiomyocytes, and in regulating survival, migration, proliferation, and inflammatory response. After acute MI, a series of changes occur at the extracellular matrix level aimed at eliminating necrotic cardiomyocytes through a controlled inflammatory response and at subsequent fibrotic scar formation. The remodelling process of the cardiac interstitium after infarction must be perfectly controlled in time and form given that its dysregulation has been associated with adverse ventricular remodelling and the appearance of heart failure.

This study confirms that the PET/CT technique using [^{18}F]FDG as a radiopharmaceutical in the murine model is highly effective in detecting cardiac lesions and quantifying the affected area. Our results are in agreement with those obtained by other authors[35] who demonstrated its utility as a noninvasive technique for determining cardiovascular function by measuring [^{18}F]FDG uptake in a murine model of permanent ligation ischemia after genetic deletion[2] Nonetheless, in order to extrapolate the results obtained in the murine animal of MI it is crucial to have a well-defined and standardized protocol in terms of anesthesia, preparation, radiopharmaceuticals, etc.[23], like the one used in this study.

Conclusion

MicroPET after [^{18}F]FDG administration provides qualitative and quantitative information on glucose consumption in the heart, brain and bladder. Combined with CT, it facilitates a more precise localization of the organs, helping to delimit the quantitative parameters (ROIs and SUVs) used to highlight the differences in distribution caused by the cardiac pathology studied, especially at the level of myocardial tissue. Micro PET/CT may therefore be of great practical interest for assessing the extent of MI-related cardiac injury, as well as for evaluating new therapies using animal experimental models.

References

1. Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, et al. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. Circulation.2022;145(8):e153-639. DOI: 10.1161/CIR.0000000000001052.

2. Zaragoza C, Gomez-Guerrero C, Martin-Ventura JL, Blanco-Colio L, Lavin B, Mallavia B, et al. Animal Models of Cardiovascular Diseases. J Biomed Biotechnol. 2011;497841. DOI: 10.1155/2011/497841.

3. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. 2018; 39(2):119-77. DOI: 10.1093/eurheartj/ehx393.

4. Frangogiannis NG. The extracellular matrix in myocardial injury, repair, and remodeling. J Clin Invest. 2017; 127(5):1600-12. DOI: 10.1172/JCI87491.

5. Hervas A, Ruiz-Sauri A, Gavara J, Monmeneu JV, de Dios E, Rios-Navarro C, et al. A Multidisciplinary Assessment of Remote Myocardial Fibrosis After Reperfused Myocardial Infarction in Swine and Patients. J Cardiovasc Transl Res. 2016; 9(4):321-33. DOI: 10.1007/s12265-016-9705-0.

6. Cesarovic N, Lipiski M, Falk V, Emmert MY. Animals in cardiovascular research: Clinical relevance and translational limitations of animal models in cardiovascular medicine. Eur Heart J. 2020; 41(2):200-3. DOI: 10.1093/eurheartj/ehz825

7. Chorro FJ, Such-Belenguer L, López-Merino V. [Animal models of cardiovascular disease]. Rev Esp Cardiol. 2009; 62(1):69-84. DOI: 10.1016/S0300-8932(09)70016-0.

8. Martin TP, MacDonald EA, Elbassioni AAM, O'Toole D, Zaeri AAI, Nicklin SA, et al. Preclinical models of myocardial infarction: from mechanism to translation. Br J Pharmacol. 2022; 179(5):770-91. DOI: 10.1111/bph.15677.

9. Fernández-Friera L, García-Álvarez A, Ibáñez B. Imagining the Future of Diagnostic Imaging. Rev Esp Cardiol. 2013; 66(2):134-43. DOI: 10.1016/j.recesp.2012.07.017.

10. Santos A, Fernández-Friera L, Villalba M, López-Melgar B, España S, Mateo J, et al. Cardiovascular imaging: what have we learned from animal models?. Front. Pharmacol . 2015; 6:227. DOI: 10.3389/fphar.2015.00227

11. Keng F. Clinical Applications of Positron Emission Tomography in Cardiology: A Review. Annals of the Academy of Medicine, Singapore. 2004; 33:175-82. DOI: 10.47102/annals-acadmedsg.V33N2p175.

12. Kunihiko Izuishi, Yuka Yamamoto, Hirohito Mori, Riko Kameyama, Shintaro Fujihara, Tsutomu Masaki, Yasuyuki Suzuki, et al. Molecular mechanisms of [18F]fluorodeoxyglucose accumulation in liver cancer. Oncology reports [Internet]. 2014; 31(2). Disponible en: https://pubmed.ncbi.nlm.nih.gov/24297035/. DOI: 10.3892/or.2013.2879.

13. Vega-González I, Roldán Valadez E. Basic concepts on 18F-FDG PET/CT: definitions and normal variants. Gac Méd Méx- 2008;144(2):137- 146.

14. Townsend DW. Positron emission tomography/computed tomography. Semin Nucl Med. 2008;38(3):152-66. DOI: 10.1053/j.semnuclmed.2008.01.003.

15. Ríos-Navarro C, Hueso L, Díaz A, Marcos-Garcés V, Bonanad C, Ruiz-Sauri A, et al. Role of antiangiogenic VEGF-A165b in angiogenesis and systolic function after reperfused myocardial infarction. Rev Esp Cardiol. 2021; 74(2):131-9. DOI: 10.1016/j.rec.2020.03.013.

16. Adams S, Pacharinsak C. Mouse Anesthesia and Analgesia. Current protocols in mouse biology. 2015; 5:51-63. DOI: 10.1002/9780470942390.mo140179.

17. Fueger BJ, Czernin J, Hildebrandt I, Tran C, Halpern BS, Stout D, et al. Impact of animal handling on the results of 18F-FDG PET Studies in Mice. J Nucl Med. 2006; 47(6):999-1006.

18. Soongsathitanon S, Masa-Ah P, Tuntawiroon M. A new Standard Uptake Values (SUV) calculation based on pixel intensity values. 2012; 6:26-33.

19. Ortega Torres YY, Armas Rojas NB, de la Noval García R, Castillo Arocha I, Suárez Medina R, Dueñas Herrera AF. Incidence of myocardial acute infarction. MEDICC Review 2011; 30(3):345-53.

20. Bahit MC, Kochar A, Granger CB. Post-Myocardial Infarction Heart Failure. JACC Heart Fail. 2018; 6(3):179-86. DOI: 10.1016/j.jchf.2017.09.015.

21. Mukherjee P, Roy S, Ghosh D, Nandi SK. Role of animal models in biomedical research: a review. Lab Anim Res. 2022;38(1):18. DOI: 10.1186/s42826-022-00128-1

22. Martin TP, MacDonald EA, Elbassioni AAM, O'Toole D, Zaeri AAI, Nicklin SA, et al. Preclinical models of myocardial infarction: from mechanism to translation. Br J Pharmacol. 2022;179(5):770-91. DOI: 10.1111/bph.15595

23. Gargiulo S, Greco A, Gramanzini M, Petretta MP, Ferro A, Larobina M, et al. PET/CT Imaging in Mouse Models of Myocardial Ischemia. J Biomed Biotechnol. 2012; 2012:541872. DOI: 10.1155/2012/541872

24. Dann MM, Clark SQ, Trzaskalski NA, Earl CC, Schepers LE, Pulente SM, et al. Quantification of murine myocardial infarct size using 2-D and 4-D high-frequency ultrasound. Am J Physiol Heart Circ Physiol. 2022; 322(3):H359-72. DOI: 10.1152/ajpheart.00600.2021

25. Pistner A, Belmonte S, Coulthard T, Blaxall B. Murine echocardiography and ultrasound imaging. J Vis Exp. 2010;(42):2100. DOI: 10.3791/2100.

26. Arbab AS, Koizumi K, Toyama K, Arai T, Araki T. Technetium-^{99m}-tetrofosmin, technetium-^{99m}-MIBI and thallium-201 uptake in rat myocardial cells. J Nucl Med. 1998; 39(2):266-71.

27. Franc BL, Acton PD, Mari C, Hasegawa BH. Small-Animal SPECT and SPECT/CT: Important Tools for Preclinical Investigation. J Nucl Med. 2008; 49(10):1651-63.

28. Houson H, Hedrick A, Awasthi V. Drug-induced cardiomyopathy: Characterization of a rat model by [18F]FDG/PET and [^{99m}Tc]MIBI/SPECT. AMEM. 2020; 3(4):295-303. DOI: 10.1002/ame2.12150.

29. Cochran BJ, Ryder WJ, Parmar A, Klaeser K, Reilhac A, Angelis GI, et al. Determining Glucose Metabolism Kinetics Using 18F-FDG Micro-PET/CT. J Vis Exp. 2017; (123):55184. DOI: 10.3791/55184.

30. Ferda J, Hromádka M, Baxa J. Imaging of the myocardium using 18F-FDG-PET/MRI.Eur J Radiol 2016;85(10):1900-8.DOI: 10.1016/j.ejrad.2016.08.001

31. Fischer M, Zacherl MJ, Weckbach L, Paintmayer L, Weinberger T, Stark K, et al. Cardiac 18F-FDG Positron Emission Tomography: An Accurate Tool to Monitor In vivo Metabolic and Functional Alterations in Murine Myocardial Infarction. Front Cardiovasc Med. 2021; 25:8:656742. DOI: 10.3389/fcvm.2021.656742.

32. Alexánderson Rosas E, Ortega López N, Ojeda Flores R, Mendoza Vázquez G, Adame Ocampo G, Meave González A, et al. A method designed for the assessment of myocardial metabolism in rats with 18F–FDG using small–animal–PET. Initial experience in Mexico. 2008; 78(1):11-8. DOI: 10.1016/S1405-9940(14)70625-0.

33. Vasudevan P, Gäbel R, Stenzel J, Förster J, Kurth J, Vollmar B, et al. 18F-FDG PET-Based Imaging of Myocardial Inflammation Following Acute Myocardial Infarction in a Mouse Model. Int J Mol Sci. 2020; 21(9):3340. DOI: 10.3390/ijms21093340.

34. Hervas A, Ruiz-Sauri A, Gavara J, Monmeneu JV, de Dios E, Rios-Navarro C, et al. A Multidisciplinary Assessment of Remote Myocardial Fibrosis After Reperfused Myocardial Infarction in Swine and Patients. J Cardiovasc Transl Res. 2016; 9(4):321-33. DOI: 10.1007/s12265-016-9705-0.

35. Alexánderson E, Mendoza RG, Ricalde A, Romero JL. Experiencia en México de la utilización de la tomografía por emisión de positrones (PET). Gac Med Mex. 2006; 142(4). DOI: 10.24875/GMM.M07000094.

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.32202
Artículos originales

The *Albizia julibrissin* and *Caesalpinia decapetala* extracts induces potential enzymes and cell growth inhibition via anti-acetylcholinesterase, anti-lipase, anti-glycation and cytotoxicity activity

Los extractos de *Albizia julibrissin* y *Caesalpinia decapetala* inducen la inhibición potencial de enzimas y el crecimiento celular a través de la actividad antiacetilcolinesterasa, antilipasa, antiglicación y citotoxicidad

Iram Fatima[1,2]
Naila Safdar[1]
Razia Kalsoom[2]
Wasim Akhtar[3]
Syed Hussain Imam Abidi[4]
Gul-e-Saba Chaudry[5] 0000-0002-4555-8572
Samia Akmal[1]

[1]Department of Biotechnology, Fatima Jinnah Women University, Rawalpindi.

[2]PCSIR Labs Complex, Plot no. 16, Sector H-9, Islamabad-44000, Pakistan.

[3]Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad-13100, Pakistan.

[4]Pakistan Council of Scientific and Industrial Research (PCSIR), Head Office, 1-Constitution Avenue, G-5/2 Islamabad-44000, Pakistan.

[5]Institute of Climate Adaptation and marine Biotechnology, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Malaysia

Correspondence

Gul-e-Saba Chaudry, Naila Safdar
gul.saba@umt.edu.my, nailahussain@fjwu.edu.pk

Received: 10.12.2024
Accepted: 23.03.2025
Published: 20.06.2025

Funding

No funding was received for conducting this study

Conflict of interests

The authors declare no conflict of interest.

Resumen

Introducción: Las plantas medicinales son una fuente dinámica de salud humana debido a su potencial terapéutico en el tratamiento de diversas dolencias. Este estudio tuvo como objetivo determinar las actividades antiacetilcolinesterasa, antilipasa, antiglicación y anticancerígena de *Albizia julibrissin* y *Caesalpinia decapetala*. (family Fabaceae).

Métodos: Los extractos de hojas se prepararon para investigar sus capacidades inhibitorias contra la acetilcolinesterasa, la lipasa y los productos de glicación. El potencial anticancerígeno se evaluó contra las líneas celulares HeLa, PC3 y 3T3 utilizando el ensayo MTT y se realizó un análisis HPLC para cuantificar seis compuestos.

Resultados: Los resultados indicaron la mayor actividad anti-acetilcolinesterasa (IC50 2,391 µg/ml) en el extracto metanólico de *A. julibrissin*, mientras que la mayor actividad anti-lipasa (114,9 µg/ml) y anti-glicación (43,69 µg/ml) se registró en el extracto metanólico de *C. decapetala*. El mayor potencial citotóxico también se observó en *C. decapetala* contra las líneas celulares PC3, 3T3 y HeLa. (144,3 ppm, 201,0 ppm and 236,0 ppm). Al final, la HPLC mostró que *A. julibrissin* exhibe la mayor concentración de ácido clorogénico (56,06 ppm) y dihidrato de quercetina (15,71 ppm), mientras que los extractos de hojas de *C. decapetala* poseen la mayor concentración de ácido gálico. (73,55 ppm).

Conclusiones: Los resultados sugieren que estos extractos inhiben significativamente las actividades enzimáticas, los productos de glicación y el crecimiento de células citotóxicas y, por lo tanto, pueden ser utilizados como nuevos compuestos farmacológicos para tratar diversas enfermedades.

Palabras clave: Cáncer; Enzimas; lipasa; Glucosilación; Cromatografía líquida de alta resolución; Productos naturales

Abstract

Introduction: Medicinal plants are dynamic source of human health because of their therapeutic potential in treating various ailments. This study aimed to determine the anti-acetylcholinesterase, anti-lipase, anti-glycation and anti-cancer activities of *Albizia julibrissin* and *Caesalpinia decapetala* (family Fabaceae).

Methods: The leaf extracts were prepared to investigate their inhibitory capacities against acetylcholinesterase, lipase and glycation products. The anti-cancer potential was evaluated against HeLa, PC3 and 3T3 cell lines using MTT assay and HPLC analysis was done to quantify six compounds.

Results: Results indicated highest anti-acetylcholinesterase (IC_{50} 2.391 µg/ml) activity in *A. julibrissin* methanolic extract while highest anti-lipase (114.9 µg/ml) and anti-glycation (43.69 µg/ml) activity was recorded in *C. decapetala* methanolic extract. Highest cytotoxic potential was also observed in *C. decapetala* against PC3, 3T3 and HeLa cell lines (144.3 ppm, 201.0 ppm and 236.0 ppm). In the end, HPLC showed that *A. julibrissin* exhibit the highest concentration of chlorogenic acid (56.06 ppm) and quercetin dihydrate (15.71 ppm) while *C. decapetala* leaves extracts possess highest concentration of gallic acid (73.55 ppm).

Conclusions: The results suggest that these extracts significantly inhibit the enzymatic activities, glycation products and the growth of cytotoxic cells and thus, can be used as novel pharmacological leads to treat various diseases.

Keywords: Cancer; Enzymes; lipase; High-performance Liquid Chromatography; Medicinal Plants

Highlights

• Medicinal plants, including *Albizia julibrissin* and *Caesalpinia decapetala* from the Fabaceae family, have significant therapeutic potential in managing various health conditions. This study aimed to investigate their ability to inhibit acetylcholinesterase, lipase, glycation, and cancer cell growth.

• The methanolic extract of *A. julibrissin* exhibited the strongest acetylcholinesterase inhibition with an IC50 of 2.391 µg/ml.The methanolic extract of *C. decapetala* showed the most potent lipase inhibition (114.9 µg/ml) and anti-glycation activity (43.69 µg/ml). *C. decapetala* extract demonstrated significant cytotoxic effects on PC3 (144.3 ppm), 3T3 (201.0 ppm), and HeLa (236.0 ppm) cell lines.

• HPLC analysis revealed that *A. julibrissin* contained high levels of chlorogenic acid (56.06 ppm) and quercetin dihydrate (15.71 ppm), while *C. decapetala* was rich in gallic acid (73.55 ppm).

Introduction

Oxidative stress produces excessive free radicals that damage cell components *viz.* lipids, proteins, carbohydrates and nucleic acid; resulting in neurodegenerative and metabolic disorders such as Alzheimer disease (AD), cancer and diabetes[1]. According to an estimate, about 55 million people were diagnosed with AD in 2020 and this number may increase to 78 million in 2030[2]. One of the most useful approaches to treat AD includes increasing acetylcholine (ACh) levels in the brain[3]. AChE is the major enzyme in the pathogenesis of Alzheimer's disease and its suppression reduces the Alzheimer's disease[4]. The synthetic drugs adverse reactions have prompted researchers to screen alternative natural plant-based products to treat AD[5,9]. Similarly, obesity and inactive lifestyles have caused diabetes in many individuals by increasing blood glucose levels in the body. The International Diabetes Federation (IDF) observed 537 million diabetic individuals in 2021[6]. Due to the increased insulin resistance, hyperglycemic condition leads to the protein glycation of proteins that produces advanced glycation end products (AGEs)[7-8]. Moreover, cancer is another critical life-threatening condition and the cervical cancer accounts for 15.9 % of the total cancer with 300,000 million cases every year[10].

Previously, some studies have explored the biological properties of Fabaceae species. For instance, *Albizia julibrissin* is traditionally used to treat ulcers, fractures, anxiety, bruises and hemorrhoids[11-12]. *Caesalpinia decapetala* is used as antiseptic, astringent, analgesic, anti-pyretic and to treat burns[13-14]. Due to the multiple health benefits of these species, present studies were designed to investigate the acetylcholinesterase, porcine pancreatic lipase, non-enzymatic glycation and cytotoxic inhibitory potential of *A. julibrissin* and *C. decapetala*. Additionally, the concentration of six compounds was evaluated using HPLC.

Methods

Extracts preparation

Fresh leaves of *A. julibrissin* subsp. Julibrissin and *C. decapetala* (Roth) Alston were collected and their accession numbers were obtained from the Herbarium of National Agriculture Research Center (NARC), Islamabad. The weight of extracts was measured to ascertain extract yield as shown in Table 1.

Table 1. List of selected species, their accession numbers and extract yield.

Plant species	Accession Numbers	Extracts	Extract weight (g)	Extract yield (%)
A. julibrissin	RAW101502	Methanol	5.32	26.60
		Meth-DMSO	5.80	29.00
C. decapetala	RAW101504	Methanol	7.53	37.65
		Meth-DMSO	8.07	40.35

*Meth: Methanol; DMSO: Dimethyl sulfoxide; the weight of extract was taken in grams (g) and the extract yield was measured in percentage (%).

Acetylcholinesterase inhibitory assay

The acetylcholinesterase inhibition potential was assessed using 140 µL of 0.1 M sodium phosphate buffer (pH 8) was added in 20 µL of plant extract, 15 µL of AChE enzyme solution (0.2 units/ml) and 10 µL of 15 mM DTNB followed by the incubation for 10 min at room temperature. The absorbance of the final product was measured at 412 nm and galantamine (100, 500 and 1000 µg/ml) was measured as a positive control. The enzyme inhibition was determined as

$$Enzyme\ Inhibition\ \% = 100 - percent\ enzyme\ activity$$
$$Percent\ Enzyme\ Activity\ \% = 100\ x\ V/Vmax$$

Where V_{max} is an enzyme activity without inhibitor.

Lipase inhibitory assay

Briefly, 5 mg/ml lipase from porcine pancreatic type II (Sigma) was added to 50 mM Tris-HCl buffer (pH 8.0) and then centrifuged for 5 min at 5500 rpm. Then, 100 µL of lipase solution was added to 50 µL of plant extract (100, 500 and 1000 µg/ml prepared in DMSO) and left for incubation on ice and the absorbance was noted at 405 nm. Orlistat was measured as a standard and the inhibition potential was calculated [15].

$$Inhibition\ \% = \left(1 - \frac{Abssample}{Abscontrol}\right) x\ 100$$

Anti-glycation assay

The AGE inhibition in plant extracts was determined using the method[16]. The fluorescence was measured at the emission wavelength of 440 nm and the percentage inhibition of AGE formation was calculated as:

$$\%\ Anti - glycation = Abscontrol - Abssample\ /\ Abscontrol\ x\ 100$$

Cytotoxicity assay against various cell lines

MTT assay

The cytotoxic potential of selected extracts was evaluated using standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay[17-18]. The absorbance was measured at 490 nm and the percentage inhibition and IC_{50} values were calculated.

HPLC analysis

The plant extracts (1 mg) were dissolved in 5 ml of 10 % methanol and then filtered using membrane filters of 0.45 nm size. An Agilent 1260 HPLC system having a quaternary pump, auto-sampler and C18 column was run at 30 °C and the mobile phase was degassed before injecting into the HPLC. The separation was achieved using 0.2 % H3PO4, methanol and acetonitrile and flow rate was 1 ml/min. The mobile phase was increased from 0 to 15 %, 50 %, 70 %, 100 % and then kept isocratic for additional 5 min. The injected volume was 10 µL and the wavelength was set at 210 nm. The standards were measured at 10, 20, 30, 40, 50 and 70 ppm concentrations to derive the calibration equation. Total four phenolic compounds and two flavonoid compounds were identified based on their retention times and UV spectras'. The concentration of compounds was determined using peak area of samples versus the analyte concentration obtained from the calibration curve [19].

Statistics

All experiments were conducted in duplicate. Results were interpreted with mean ±SD and Least Significant Difference (LSD) was evaluated using Statistix 8.1. Moreover, the IC_{50} values in all biological assays were determined using GraphPad Prism 5 software.

Results and Discussion

Anti-acetylcholinesterase assay

Plants are the potential source of compounds that can prevent or treat neurodegenerative diseases by inhibiting acetylcholinesterase[20]. The present study revealed highest acetylcholinesterase inhibitory activity in *A. julibrissin* methanolic extract (IC_{50} 2.391 µg/ml) followed by *A. julibrissin* methanol-DMSO extract (IC_{50} 10.16 µg/ml) and *C. decapetala* methanolic extract (IC_{50} 22.79 µg/ml). However, *C. decapetala* methanol-DMSO extract showed lowest anti-acetylcholinesterase activity by displaying IC_{50} value of 30.24 µg/ml (Table 2).

Table 2: Anti-acetylcholinesterase activity of extracts

Plant species	Extracts	Percentage Inhibition at Different Doses (µg/ml)			IC_{50} values
		100	500	1000	
A. julibrissin	Methanol	71.4	74.4	83	2.391
	Methanol-DMSO	75	78.7	94.6	10.16
C. decapetala	Methanol	63.5	70.0	82	22.79
	Methanol-DMSO	65.3	69	90.86	30.24
Standard*		78.0	84.5	93	5.99

*Galantamine was used as a standard and IC_{50} values indicates half maximal inhibitory concentration and is measured in µg/ml

The AChE inhibition potential of these extracts could be attributed to the existing phenolic and flavonoid compounds[21]. A previous report [22] indicated anti-acetylcholinesterase activity in the ethanolic leaves extracts of *Albizia lucidor* (IC_{50} 24.89 ± 1.60 µg/ml) and *Albizia procera* (IC_{50} 43.50 ± 2.10 µg/ml). In another study, [23] demonstrated highest inhibition potential (10.20 mg galantamine equivalent/g) of *C. decapetala* leaves extracts against butyrylcholinesterase. However, the acetylcholinesterase inhibitory activity of selected species has been examined for the first time. It can be inferred that the variations within the same plant species could be due to the different phyto-constituents of the plants that are grown in various geographical areas in different seasons of the year. The phyto-constituents may vary depending on the soil, water, stage of plant and the time of collection[24]. It can be suggested that *A. julibrissin* and *C. decapetala* leaves may serve as an ideal candidate for designing new AChE inhibitors to treat neurodegenerative disorders. However, detailed *in vivo* tests are needed to validate the efficiency of these species for medicinal use.

Anti-lipase and anti-glycation assays

The inhibition of pancreatic lipase (a lipolytic enzyme) and glycation products is a highly effective method that protects against fat absorption in individuals with obesity and hyperglycemia[25]. In this study, pancreatic anti-lipase and anti-glycation potential of two species have been investigated and results are presented in Figure 1a and b. The highest lipase and glycation inhibitory potential was observed in *C. decapetala* methanolic extract (IC_{50} 114.9 µg/ml and 43.69 µg/ml) followed by the methanol-DMSO extract of *A. julibrissin* (IC_{50} 138.7 µg/ml and 74.06 µg/ml). However, weak anti-lipase and anti-glycation activity was recorded in *A. julibrissin* methanolic extract (IC_{50} 155.2 µg/ml and 182.5 µg/ml) and *C. decapetala* methanol-DMSO extract (IC_{50} 168.4 µg/ml and 247.3 µg/ml) respectively (Table 2). The remarkable inhibitory activity of selected extracts indicates their potential use as a powerful source of anti-obesity and anti-glycation agents.

Previously, researchers have investigated biological potential of different species of *Albizia* and *Ceasalpinia* grown in different regions of world. For instance, [26] demonstrated pancreatic lipase inhibition potential in *Caesalpinia sappan* commonly grown in Thailand. Contrarily, [27] observed no lipase inhibitory potential in *Albizia lebbeck* that was grown in Thailand. It can be inferred that species belonging to the same family, irrespective to different climatic conditions, exhibit potent anti-lipase and anti-glycation activities. The relative bio-efficacy can be ascribed to the presence of phenolic and flavonoid compounds as suggested in previous literature [28-29]. However, the plant extract is a mixture of bioactive compounds that contribute to the bioactivities altogether. This suggests that the composition of active compounds, structural features and other potential factors in plant extract may have also affected pancreatic lipase and hyperglycemic conditions. Therefore, the exact relationship between the active compounds and biological activities needs to be further investigated.

Table 2: Percentage inhibition and IC_{50} values observed in anti-lipase and anti-glycation assays.

Plant species	Extracts	Anti-lipase assay (µg/ml)				Anti-glycation assay (µg/ml)			
		Percentage Inhibition			IC_{50} values	Percentage inhibition			IC_{50} values
		100	500	1000		100	500	1000	
A. julibrissin	Methanol	45.89 ± 3.35	60.64 ± 1.06	67.85 ± 4.87	155.2	43.90 ± 4.10	54.25 ± 1.76	82.95 ± 1.34	182.5
C. decapetala	Meth-DMSO	46.60 ± 4.12	62.90 ± 1.95	77.49 ± 3.81	138.7	56.00 ± 2.12	70.60 ± 5.65	89.10 ± 1.41	74.06
	Methanol	48.30 ± 5.72	68.40 ± 0.43	77.50 ± 1.85	114.9	58.50 ± 4.94	69.50 ± 0.70	78.85 ± 3.04	43.69
	Meth-DMSO	41.52 ± 2.83	67.10 ± 3.45	76.56 ± 3.53	168.4	43.00 ± 2.82	53.95 ± 0.07	63.05 ± 1.34	247.3
Control		49.00 ± 1.41	59.00 ± 1.41	81.00 ± 1.41	127.5	69.00 ± 2.82	77.70 ± 0.84	88.50 ± 1.27	17.33

Meth. stands for methanol and DMSO stands for dimethyl sulfoxide; Values in tables are presented as mean ± SD (n=3); IC_{50} value indicates half maximal inhibitory concentration.

Orlistat was used as a control in anti-lipase assay and rutin was used as a control in anti-glycation assay.

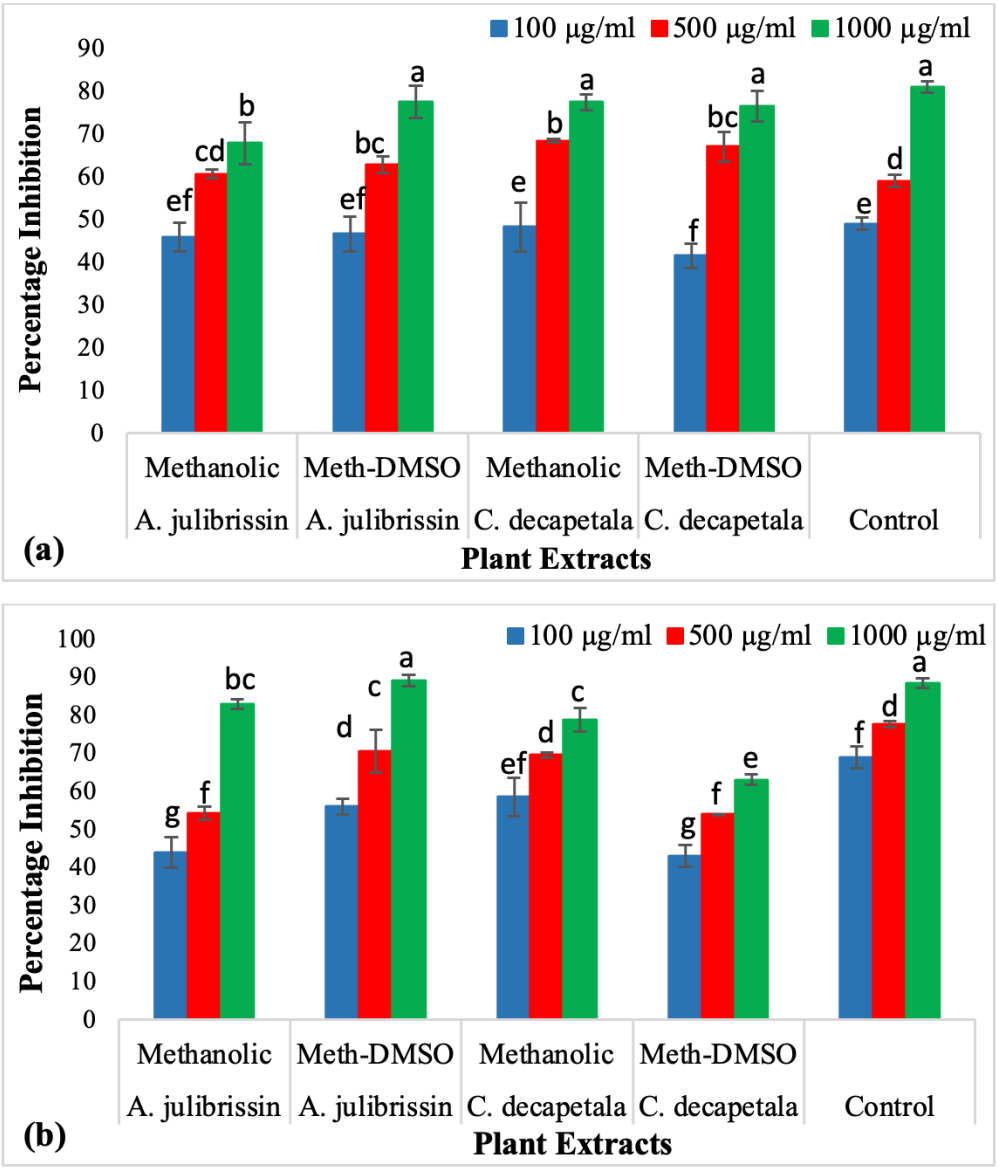

Figure 1: Anti-lipase and anti-glycation activity of leaves extracts of selected species (a) Anti-lipase assay (b) Anti-glycation assay. *Data indicates mean ± SD (3n) and each alphabetical letter (a-g) indicates a significance difference at $P < 0.05$. *Orlistat was used as a control in anti-lipase assay and rutin was used as a control in anti-glycation assay.

Cytotoxic activity

As the methanolic extracts displayed significant biological activities, hence were further tested to observe there *in vitro* cytotoxic potential against three different cell lines. Among different plant extracts, *C. decapetala* showed highest cytotoxic potential against PC3 and 3T3 cell lines by showing IC_{50} value of 144.3 ppm and 201.0 ppm. Contrarily, the lowest activity was recorded in *A. julibrissin* methanolic extract against PC3 cell lines (IC_{50} 459.8 ppm) and 3T3 cell lines (IC_{50} 392.6 ppm). However, the moderate inhibitory potential was observed in both species when tested against HeLa cell lines. Overall, *C. decapetala* leaf extract displayed the highest cytotoxic potential compared to *A. julibrissin* (Table 3).

Table 3: The cytotoxicity effects observed in selected species against HeLa, PC3 and 3T3 cells.

Plant species	Cell lines	Percentage Inhibition at Different Concentrations (ppm)					IC_{50} (ppm)
		30	60	90	120	150	
A. julibrissin	HeLa	0	1.2	4.9	9.7	14.7	285.4
	PC3	0	1.2	2.7	4.3	6.1	459.8
	3T3	-2.8	0	2.8	5.1	7.3	392.6
C. decapetala	HeLa	0	1.1	5.5	11.6	19.6	236.0
	PC3	10.6	21.2	31.8	42.4	53	144.3
	3T3	1.7	3.3	6.2	7.4	23.8	201.0

IC_{50}: Half-maximal inhibitory concentration.

Previously, [30] isolated emodin, baicalein and apigenin from *C. decapetala* roots and confirmed their anti-tumor activities against human gastric carcinoma cell line MGC-803 cell line with IC_{50} values of 15.6, 16.3 and 13.2 µmol/L using MTT assay. Our studies also corroborate the earlier findings [23] who reported highest cytotoxicity (*i.e.* 46.08 µg/ml CC_{50}) for the bark methanol extract of *C. decapetala* on the HeLa cells. Moreover, previous researcher [31] isolated oleanane-type saponins, julibrosides, from the stem bark of *A. julibrissin* and examined their cytotoxic effects against HCT-116, BGC-823, HepG2 and A549 cell lines. Earlier studies [32,33] revealed anti-cancer activity of another specie of *Albizia* genus *i.e. Albizia lebbeck* using MCF-7 (human breast cancer), HeLa and A549 cell lines. However, *A. julibrissin* leaves extracts have been examined for the first time against selected cell lines. The anti-carcinogenic potential of examined species could be attributed to the active compounds such as Julibroside saponins present in *A. julibrissin*[34]. In general, leaves of selected species showed promising results as a cytotoxic agent due to the relatively high toxicity on selected cells. Hence, it can be inferred that the exact mechanism of apoptosis should be investigated using flow cytometry and microscopy techniques.

HPLC analysis

In current study, HPLC method was used to quantify six compounds present in the leaves of selected species. The obtained HPLC chromatograms are presented in Figure 2 (a to d) which indicated that *A. julibrissin* possesses highest concentration of chlorogenic acid (56.06 ppm) and quercetin dihydrate (15.71 ppm) while *C. decapetala* leaves extracts exhibit the highest concentration of gallic acid (73.55 ppm). However, all other compounds were detected in lower concentrations (*i.e.* below 12 ppm) in these species (Table 4).

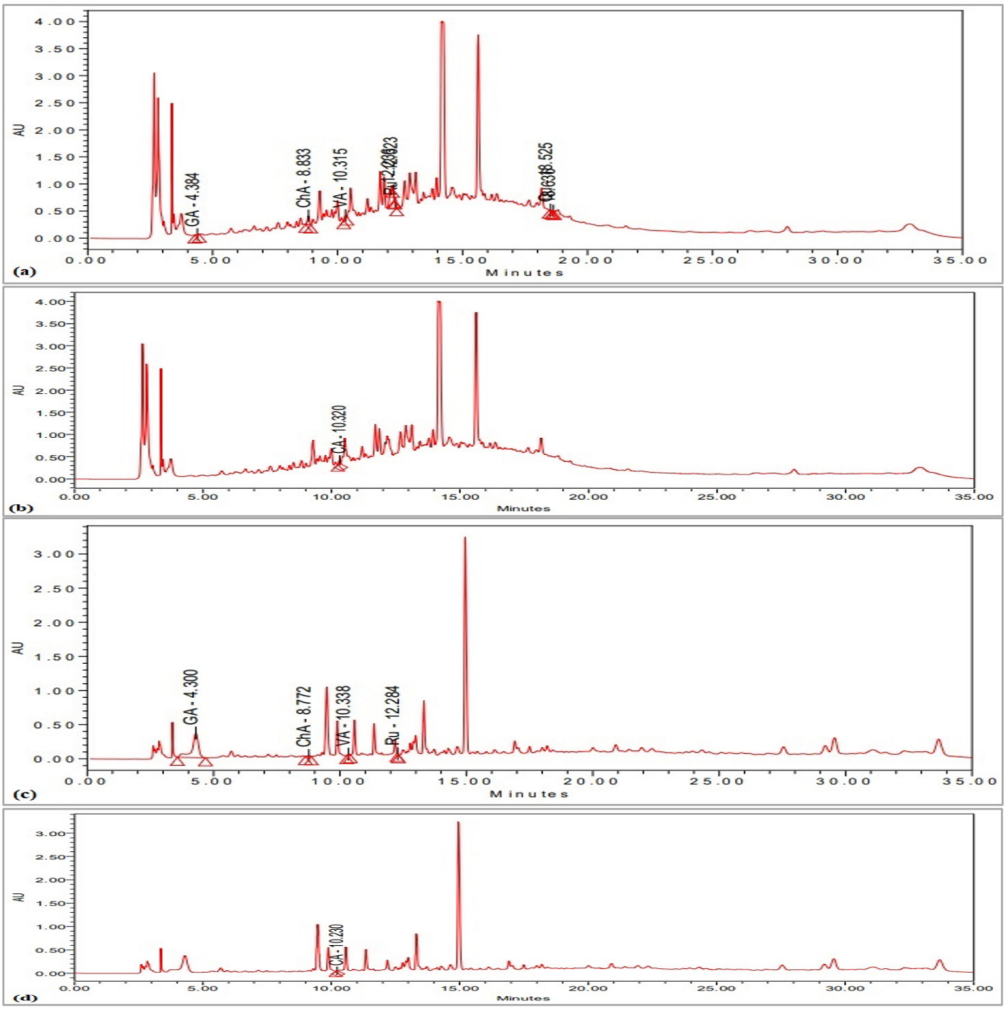

Figure 2: HPLC chromatograms indicating six phenolic and flavonoid compounds at respective retention times in selected species (a) Gallic acid, vanillic acid, chlorogenic acid, rutin trihydrate and quercetin dihydrate in *A. julibrissin* (b) Caffeic acid in *A. julibrissin* (c) Gallic acid, vanillic acid, chlorogenic acid, rutin trihydrate and quercetin dihydrate in *C. decapetala* (d) Caffeic acid in *C. decapetala*.

Table 4. Concentration of six compounds observed in selected species using HPLC.

Plant species	Concentration of compounds (ppm)					
	Gallic acid	Caffeic acid	Vanillic acid	Chlorogenic acid	Rutin trihydrate	Quercetin dihydrate
A. julibrissin	1.75	11.81	6.61	56.06	0.11	15.71
C. decapetala	73.55	0.29	4.92	8.89	1.02	ND

*ND: Not detected

Previously, scientists [13] showed the presence of gallic acid, quercetin, catechin, 4-hydroxybenoic acid and *p*-coumaric acid as the main phenolic compounds in *C. decapetala* extracts using the HPLC technique which has been confirmed in the current study. Similarly, [35] reported eight compounds in *C. decapetala* using UPLC-MS/MS. Furthermore, [36-37] indicated the presence of lignans, triterpenoids saponins and some phenolic compounds in the stem bark of *A. julibrissin*. Besides, cassane diterpenoid, caesaldecan, squalene, lupeol, resveratrol, quercetin, stigmasterol and astragalin, the main phytochemicals observed in *C. decapetala* are terpenoids, flavonoids and tannins[38]. It can be inferred that the selected species possesses a valuable reservoir of polyphenolic compounds of pharmacological significance and thus needs to be isolated and investigated for food and industrial application.

Conclusion

In the light our findings, it can be concluded that the methanolic extracts exhibited the most pronounced biological activities compared to the methanol-DSMO extracts of two selected species. *A. julibrissin* methanolic extract showed highest acetylcholinesterase inhibitory activity while *C. decapetala* methanolic extract displayed highest anti-lipase, anti-glycation and cytotoxic potential. HPLC indicated that *A. julibrissin* possesses highest concentration of chlorogenic acid and quercetin dihydrate while *C. decapetala* leaves extracts exhibited highest concentration of gallic acid. Therefore, the results of this study reinforce the potential therapeutic benefits of *A. julibrissin* and *C. decapetala* methanolic extracts. Further research and clinical trials are required to validate these findings and thereby uncover more evidence of its biological activities.

Bibliography

1. Wojtunik-Kulesza KA, Oniszczuk A, Oniszczuk T, Waksmundzka-Hajnos M. The influence of common free radicals and antioxidants on development of Alzheimer's Disease. Biomed Pharmacother. 2016;78:39–49. doi:10.1016/j.biopha.2015.12.024.

2. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and meta-analysis. Alzheimers Dement. 2013; 9(1):63–75. doi:10.1016/j.jalz.2012.11.007.

3. Yiannopoulou KG, Papageorgiou SG. Current and future treatments in Alzheimer disease: an update. J Cent Nerv Syst Dis. 2020; 12:1179573520907397. doi:10.1177/1179573520907397.

4. Köse LP, Gülçin İ, Gören AC, Namiesnik J, Martinez-Ayala AL, Gorinstein S. LC–MS/MS analysis, antioxidant and anticholinergic properties of galanga (Alpinia officinarum Hance) rhizomes. Ind Crops Prod. 2015; 74:712–21. doi:10.1016/j.indcrop.2015.05.034.

5. Chu YC, Chang CH, Liao HR, Fu SL, Chen JJ. Anti-cancer and anti-inflammatory activities of three new chromone derivatives from the marine-derived *Penicillium citrinum*. Mar Drugs. 2021; 19(8):408. doi:10.3390/md19080408.

6. Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health Sci Rep. 2024; 7(3):e2004. doi:10.1002/hsr2.2004.

7. Mata-Torres G, Andrade-Cetto A, Espinoza-Hernandez F. Approaches to decrease hyperglycemia by targeting impaired hepatic glucose homeostasis using medicinal plants. Front Pharmacol. 2021; 12:809994. doi:10.3389/fphar.2021.809994.

8. Ramsay RR, Tipton KF. Assessment of enzyme inhibition: a review with examples from the development of monoamine oxidase and cholinesterase inhibitory drugs. Molecules. 2017; 22(7):1192. doi:10.3390/molecules22071192.

9. Xu-Dong H, Li-Lin S, Yun-Feng C, Yi-Nan W, Qi Z, Sheng-Quan F, et al. Pancreatic lipase inhibitory constituents from *Fructus Psoraleae*. Chin J Nat Med. 2020; 18(5):369–78. doi:10.1016/S1875-5364(20)30043-1.

10. de Sanjose S, Tsu VD. Prevention of cervical and breast cancer mortality in low-and middle-income countries: a window of opportunity. Int J Womens Health. 2019; 11:381–6. doi:10.2147/IJWH.S197115.

11. Kang J, Huo CH, Li Z, Li ZP. New ceramides from the flower of *Albizia julibrissin*. Chin Chem Lett. 2007; 18(2):181–4. doi:10.1016/j.cclet.2006.12.042.

12. Ikeda T, Fujiwara S, Araki K, Kinjo J, Nohara T, Miyoshi T. Cytotoxic glycosides from *Albizia julibrissin*. J Nat Prod. 1997; 60(2):102–7. doi:10.1021/np960556t.

13. Gallego MG, Skowyra M, Gordon MH, Azman NAM, Almajano MP. Effect of leaves of *Caesalpinia decapetala* on oxidative stability of oil-in-water emulsions. Antioxidants. 2017; 6(1):19. doi:10.3390/antiox6010019.

14. Parveen A, Akash MS, Rehman K, Mahmood Q, Qadir MI. Analgesic, anti-inflammatory and anti-pyretic activities of *Caesalpinia decapetala*. Bioimpacts. 2014; 4(1):43. doi:10.5681/bi.2014.013.

15. Chen Z, Zhang D, Guo JJ, Tao W, Gong RX, Yao L, et al. Active components, antioxidant, inhibition on metabolic syndrome-related enzymes, and monthly variations in mature leaf hawk tea. Molecules. 2019; 24(4):657. doi:10.3390/molecules24040657.

16. Franco RR, Zabisky LF, de Lima Júnior JP, Alves VH, Justino AB, Saraiva AL, et al. Antidiabetic effects of *Syzygium cumini* leaves: A non-hemolytic plant with potential against processes of oxidation, glycation, inflammation, and digestive enzymes catalysis. J Ethnopharmacol. 2020; 261:113132. doi:10.1016/j.jep.2020.113132.

17. Masood S, et al. Zn(II) and Cd(II) pincer complexes bearing meta alkylated pyridinium amidates; synthesis & preliminary anticancer studies. New J Chem. 2023;47(47):21845–53.

18. Mehmood R, Sadiq A, Alsantali RI, Mughal EU, Alsharif MA, Naeem N, et al. Synthesis and evaluation of 1,3,5-triaryl-2-pyrazoline derivatives as potent dual inhibitors of urease and α-glucosidase together with their cytotoxic, molecular modeling, and drug-likeness studies. ACS Omega. 2022; 7(4):3775–95. doi:10.1021/acsomega.1c06694.

19. Abdelkhalek A, Salem MZ, Kordy AM, Salem AZ, Behiry SI. Antiviral, antifungal, and insecticidal activities of *Eucalyptus* bark extract: HPLC analysis of polyphenolic compounds. Microb Pathog. 2020; 147:104383. doi:10.1016/j.micpath.2020.104383.

20. Tuzimski T, Petruczynik A. Determination of anti-Alzheimer's disease activity of selected plant ingredients. Molecules. 2022; 27(10):3222. doi:10.3390/molecules27103222.

21. Ferreira J, Santos S, Pereira H. In vitro screening for acetylcholinesterase inhibition and antioxidant activity of *Quercus suber* cork and corkback extracts. Evid Based Complement Alternat Med. 2020;2020:3825629. doi:10.1155/2020/3825629.

22. Hussein ME, Mohamed OG, El-Fishawy AM, El-Askary HI, Hamed AA, Abdel-Aziz MM, et al. Anticholinesterase activity of budmunchiamine alkaloids revealed by comparative chemical profiling of two *Albizia* spp., molecular docking, and dynamic studies. Plants. 2022; 11(23):3286. doi:10.3390/plants11233286.

23. Zengin G, Mahomoodally MF, Picot-Allain MCN, Sinan KI, Ak G, Etienne OK, et al. Chemical composition, biological properties and bioinformatics analysis of two Caesalpina species: A new light in the road from nature to pharmacy shelf. J Pharm Biomed Anal. 2021; *198*, 114018. doi: 10.1016/j.jpba.2021.114018.

24. Gomes AF, Almeida MP, Leite MF, Schwaiger S, Stuppner H, Halabalaki M, et al. Seasonal variation in the chemical composition of two chemotypes of *Lippia alba*. Food Chem. 2019; 273:186–93. doi:10.1016/j.foodchem.2017.11.089.

25. Herrera T, Del Hierro JN, Fornari T, Reglero G, Martin D. Inhibitory effect of quinoa and fenugreek extracts on pancreatic lipase and α-amylase under in vitro traditional conditions or intestinal simulated conditions. Food Chem. 2019; 270:509–17. doi:10.1016/j.foodchem.2018.07.145.

26. Ruangaram W, Kato E. Selection of Thai medicinal plants with anti-obesogenic potential via in vitro methods. Pharmaceuticals. 2020; 13(4):56. doi:10.3390/ph13040056.

27. Sirichai P, Kittibunchakul S, Thangsiri S, On-Nom N, Chupeerach C, Temviriyanukul P, et al. Impact of drying processes on phenolics and in vitro health-related activities of indigenous plants in Thailand. Plants. 2022; 11(3):294. doi:10.3390/plants11030294.

28. Wang R, Wang L, Zhang L, Wan S, Li C, Liu S. Solvents effect on phenolics, iridoids, antioxidant activity, antibacterial activity, and pancreatic lipase inhibition activity of noni (*Morinda citrifolia* L.) fruit extract. Food Chem. 2022; 377:131989. doi:10.1016/j.foodchem.2021.131989.

29. Zeng SL, Li SZ, Wei MY, Chen BZ, Li P, Zheng GD, et al. Evaluation of anti-lipase activity and bioactive flavonoids in the *Citri Reticulatae Pericarpium* from different harvest times. Phytomedicine. 2018; 43:103–9. doi:10.1016/j.phymed.2018.04.008.

30. Wei XH, Yang SJ, Liang N, Hu DY, Jin LH, Xue W, et al. Chemical constituents of *Caesalpinia decapetala* (Roth) alston. Molecules. 2013; 18(1):1325–36. doi:10.3390/molecules18011325.

31. Han Q, Qian Y, Wang X, Zhang Q, Cui J, Tu P, et al. Oleanane-type saponins and prosapogenins from *Albizia julibrissin* and their cytotoxic activities. Phytochemistry. 2021; 185:112674. doi:10.1016/j.phytochem.2021.112674.

32. Kavitha CN, Raja KD, Rao SK. Antitumor activity of Albizia lebbeck L. against Ehrlich ascites carcinoma in vivo and HeLa and A549 cell lines in vitro. J Cancer Res Ther. 2021; *17*(2), 491-498. doi: 10.4103/jcrt.JCRT_454_9.

33. Desai TH, Joshi SV. Anticancer activity of saponin isolated from Albizia lebbeck using various in vitro models. J Ethnopharm. 2019; *231*, 494-502. doi: 10.016/j.jep.2018.11.004.

34. Han Q, Qian Y, Wang X, Zhang Q, Cui J, Tu P, et al. Cytotoxic oleanane triterpenoid saponins from *Albizia julibrissin*. Fitoterapia. 2017; 121:183–93. doi:10.1016/j.fitote.2017.07.015.

35. Ghavidel A, Bak M, Hofmann T, Hosseinpourpia R, Vasilache V, Sandu I. Comparison of chemical compositions in wood and bark of Persian silk tree (*Albizia julibrissin* Durazz.). Wood Mater Sci Eng. 2022; 17(6):759–70. doi:10.1080/17480272.2021.1953141.

36. Li YT, Liu H, Meng WS, Zhou T, Gong ZP, Huang Y, Zheng L. Simultaneous determination of content of eight components in Caesalpinia decapetala by UPLC-MS/MS. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi*= China J Chin Mater Med. 2022; 47(3): 692-700. doi: 10.19540/j.cnki.cjcmm.20211011.201.

37. Li W, Yang HJ. Isolation and identification of lignans and other phenolic constituents from the stem bark of *Albizia julibrissin* Durazz and evaluation of their nitric oxide inhibitory activity. Molecules. 2020; 25(9):2065. doi:10.3390/molecules25092065.

38. Van Kiem P, Van Minh C, Huong HT, Lee JJ, Kim YH. Caesaldecan, a cassane diterpenoid from the leaves of *Caesalpinia decapetala*. Chem Pharm Bull. 2005;53(4):428–30. doi:10.1248/cpb.53.428.

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.33054
Artículos originales

Validez y fiabilidad de la versión en español del cuestionario SKILLD en una población panameña

Validity and Reliability of the Spanish version of the SKILLD questionnaire in a Panamanian population

Jose Pedro García-Corpas[1] 0000-0002-1250-5300
Hermes Rodríguez Battikh[2] 0000-0003-0729-9861
Eric Conte Valdes[3] 0000-0001-8169-6713
Sara Itzela Gómez Castillo[4] 0009-0006-7609-3639
Fernando Martínez Martínez[1,2] 0000-0002-2747-6960

[1]Grupo de Investigación en Atención Farmacéutica. Universidad de Granada. (CTS-131) (España).
[2]Cátedra María José Faus Dáder de Atención Farmacéutica. Universidad de Granada (España).
[3]Instituto Commemorativo Gorjas de Estudios de la Salud (ICGES) (Panamá).
[4]Ministerio Salud Pública (MINSA) (Panamá).

Correspondencia

José Pedro García Corpas
jpcorpas@ugr.es

Recibido: 10.03.2025
Aceptado: 29.05.2025
Publicado: 20.06.2025

Financiación

Este proyecto ha sido beneficiario de la convocatoria pública de Fomento I+D para el desarrollo sostenible (IDDS) 2022 de la Secretaría Nacional de Ciencia, Tecnología e Innovación de Panamá (SENACYT). Número de contrato 206-2022.

Conflicto de intereses

Los autores declaran que no que tienen ningún tipo de conflicto de interés.

Resumen

Introducción: El objetivo fue estudiar la validez y la fiabilidad de una versión en español del SKILLD (Spoken Knowledge in Low Literacy in Diabetes Scale) en pacientes con Diabetes Mellitus tipo 2 (DM2) y estimar el conocimiento que tienen sobre esta enfermedad.

Método: El estudio se llevó a cabo en 4 centros de salud de Veraguas (Panamá). Los entrevistados completaron el SKILLD y se midió el porcentaje de hemoglobina glicosilada (% HbA1C). Se evaluaron la validez de constructo (análisis factorial de componentes principales) y de criterio, así como la fiabilidad (alfa de Cronbach y test-retest).

Resultados: 175 pacientes participaron en el estudio. 115 (68 %) fueron mujeres. La edad media fue 60,0 (DE: 12,0) años. Según el índice de masa corporal (IMC), sólo el 29,1 % (51) tenía normopeso. El 67,4 % de los pacientes no tenían controlada la DM2. El % HbA1C medio fue 8,6 (DE: 2,3). La puntuación total media del SKILLD fue de 4,1 (DE: 2,1) sobre 10. Respecto del SKILLD, la fiabilidad medida mediante consistencia interna fue α = 0,559 y mediante estabilidad temporal fue ICC = 0,803 (IC 95 %=0,670-0,882). En análisis factorial de componentes principales extrajo 4 factores que explicaban el 55,3 % de la varianza total.

Conclusiones: El conocimiento que tienen los pacientes de la región de Veraguas (Panamá) sobre la diabetes tipo 2 que padecen es bajo, independientemente de que sus valores de %HbA1C estén dentro de los valores recomendados por la (American Diabetes Association) ADA o no. El cuestionario SKILLD ha demostrado ser una herramienta útil, fiable y valida, aunque son necesarios más estudios que refuercen estos datos.

Palabras clave: Diabetes Mellitus Tipo 2; Conocimientos, Actitudes y Práctica en Salud; Encuestas y Cuestionarios; Reproducibilidad de los Resultados.

Abstract

Introduction: The objective was to study the validity and reliability of a Spanish version of the SKILLD (Spoken Knowledge in Low Literacy in Diabetes Scale) in patients with T2D in Veraguas (Panama) and to assess their knowledge about this disease.

Method: The study was conducted in four health centers in Veraguas (Panama). Participants completed the SKILLD questionnaire, and their HbA1C was measured. Construct validity (principal component factor analysis) and criterion validity were assessed, as well as reliability (Cronbach's alpha and test-retest).

Results: A total of 175 patients participated in the study, of whom 115 (68 %) were women. The mean age was 60.0 years (SD: 12.0). According to BMI, only 29.1 % (51) had a normal weight. The 67.4 % of patients had uncontrolled T2D. The mean HbA1C was 8.6 % (SD: 2.3). The mean SKILLD score was 4.1 (SD: 2.1). Regarding SKILLD, reliability measured by internal consistency was α = 0.559, and temporal stability was ICC = 0.803 (95 % CI = 0.670-0.882). The principal component factor analysis extracted four factors that explained 55.3 % of the total variance.

Conclusions: Patients in the Veraguas region (Panama) have low knowledge about their type 2 diabetes, regardless of whether their HbA1C values are within the ADA recommended range or not. The SKILLD questionnaire has proven to be a useful, reliable, and valid tool, although further studies are needed to reinforce these findings.

Keywords: Diabetes Mellitus Type 2; Health Knowledge, Attitudes, Practice; Surveys and Questionnaires; Reproducibility of Results.

Puntos clave

Para poder autogestionar la DM2, el paciente debe tener conocimientos sobre ella. Para hacer una educación terapéutica adecuada es importante cuantificar el conocimiento del paciente. El cuestionario SKILLD se diseñó para ello pero no ha mostrado su validez y fiabilidad en poblaciones panameñas.

Se aportan datos de validez y fiabilidad del SKILLD en una muestra de pacientes con DM2 Veraguas (Panamá) y se mide el conocimiento que tienen sobre esta enfermedad.

El conocimiento de los pacientes con DM2 es bajo. Es necesario establecer un plan de educación terapéutica que permita la autogestión de la enfermedad. Esto puede prevenir complicaciones a largo plazo y mejorar la calidad de vida del paciente. El cuestionario es válido y fiable en esta muestra, aunque son convenientes más estudios al respecto.

Introducción

La Diabetes Mellitus (DM) es un grave problema de salud pública en todo el mundo, tanto por su morbi-mortalidad como por los costes que genera a los sistemas de salud [1]. De todos los casos de DM aproximadamente el 90 % es de Diabetes Mellitus tipo 2 (DM2). El último informe de International Diabetes Federation (IDF) muestra que la prevalencia en el mundo de la DM ha llegado al 10,5 % y aumenta con la edad y con el hecho de vivir en zonas urbanas. En Panamá, la prevalencia llega al 12,4 % y solo 62,6 % ha sido diagnosticada [2].

Mantener las cifras de glucemia y el porcentaje de hemoglobina glicosilada (% HbA1c) en rangos propuestos por las guías clínicas disminuye la probabilidad de aparición de complicaciones asociadas a la DM2, por tanto, mejora la calidad de vida del paciente y disminuye los costes asociados a esta enfermedad [3,4]. En Panamá, apenas el 39,6 % de los pacientes consigue tener sus valores de glucemia dentro de estos rangos [2].

Para que le paciente pueda adoptar las medidas necesarias para un adecuado autocontrol es necesario que tenga conocimiento sobre la enfermedad en cuestión y sobre el tratamiento farmacológico y no farmacológico. Saber cuánto sabe el paciente sobre estas cuestiones se fundamental para que los profesionales de la salud puedan diseñar intervenciones educativas adecuadas, y posteriormente evaluar el impacto que han tenido en los pacientes.

Se han diseñado varias herramientas para medir el conocimiento que tiene el paciente sobre su enfermedad. Varias de ellas pueden ser consideradas obsoletas debido a que se diseñaron en los años 80 del siglo XX. Otras son largas y complicadas. En estas escalas se incluyen preguntas que se centran en el conocimiento de la fisiopatología de la diabetes, que es difícil que sean conocidas por personas que no sean sanitarios. Además, pueden no ser buenos marcadores del conocimiento práctico de la diabetes de los pacientes [5].

En 2005 en EE. UU. se desarrolló el SKILLD (Spoken Knowledge in Low Literacy in Diabetes Scale) que buscaba evaluar el conocimiento que tenía el paciente sobre el autocuidado de la diabetes [5]. Posteriormente este cuestionario fue traducido y testado en varias ocasiones para comprobar su validez y fiabilidad en otras poblaciones de habla hispana [6-8], pero hasta donde sabemos, nunca ha sido estudiado en la población panameña. En consecuencia, el objetivo de este trabajo fue estudiar la validez y la fiabilidad de la versión en español traducida por García et al. [7] en pacientes con DM2 en Veraguas (Panamá) y estimar el conocimiento que tienen de la esta enfermedad.

Métodos

Diseño

Este trabajo analiza las propiedades psicométricas y los datos obtenidos por el SKILLD de los datos que se recopilaron como parte de un estudio cuasiexperimetal ante-después en el que se utilizó para medir el conocimiento que tenía el paciente sobre DM2.

Población de estudio

El estudio se llevó a cabo en los centros de salud (CS) del Ministerio de Salud de Panamá (MINSA) en las provincias de Veraguas (CS de Montijo, CS de Atalaya, CS de Santiago y el MINSA-CAPSI la Mata).

Se incluyeron pacientes mayores de 18 años diagnosticados con DM2 que hayan estado en tratamiento durante al menos 6 meses antes de la fecha de inclusión en el estudio y que no necesiten ayuda para tomar su mediación (no necesiten cuidador). Se excluyeron aquellos pacientes: a) incapaces de responder cuestionarios por si solos o que padezcan de alguna discapacidad visual, auditiva o cognitiva que les dificulte llevar a cabo una entrevista; b) que tengan dificultades para entender o hablar el idioma español; c) mujeres embarazadas; d) personas que estén participando en algún programa de salud que esté relacionado con la DM2 o con la adherencia a cualquier tipo de medicamento.

La captación de pacientes se realizó mediante 3 formas: a) Se informó a los médicos de los CS participantes sobre el estudio, se les hizo entrega de folletos informativos para entregar a los pacientes que considerasen que se podrían beneficiar de este trabajo y se pidió que les explicaran el estudio brevemente; b) se colocaron carteles publicitarios en murales informativos y en salas de espera de los CS informando de la existencia de este trabajo y la posibilidad de participar en él; c) se preparó una web con toda información sobre este trabajo. Esta web disponía de una "landing page" que permitió al paciente solicitar que un investigador le llame por teléfono para concertar una entrevista donde sería totalmente informado. Posteriormente se comprobó cuáles de los pacientes interesados cumplieron los criterios de inclusión y exclusión.

El tamaño de la muestra fue calculado en base a la proporción de control de la DM2 proporcionado por Quintana et al. (39,6 %) [2]. Con un error de estimación del 10 % y una significación estadística de 0,05 serían necesarios 92 pacientes. Se aumentó un 25 % este tamaño para prevenir posibles pérdidas, por tanto, fueron necesarios al menos 115 pacientes.

Variables principales

Control de la DM2: Un paciente se considerará controlado si tiene un porcentaje adecuado de (%HbA1c). Ésta se midió con el equipo A1cNow (PTS Diagnostics) [9] en una pequeña muestra de sangre capilar. Se obtuvo variable continua que se categorizó en "Controlado" o "No controlado" en función de los puntos de corte indicados según la guía clínica 2024 de la American Diabetes Association (ADA) [10] (tabla 1).

Tabla 1. Objetivos de hemoglobina glicosilada propuestos por la ADA.

Población	Objetivos de HbA1c
Adultos	<7,0 % (53 mmol/mol)
Adultos complicados (historial de hipoglicemias severas, esperanza de vida limitada, complicaciones microvasculares y macrovasculares avanzadas, o presencia de muchas comorbilidades)	<8,0 % (64 mmol/mol)
Adultos mayores de 65 años saludables (< 3 enfermedades crónicas coexistentes*, estado cognitivo y funcional intacto)	<7-7,5 % (53-58 mmol/mol)
Adultos mayores de 65 años complicados (≥ 3 enfermedades crónicas coexistentes o discapacidad para realizar 2 actividades cotidianas complejas** o discapacidad cognitiva leve a moderada)	<8,0 % (64 mmol/mol)
Adultos mayores de 65 años muy complicados (fase terminal de una enfermedad crónica† o discapacidad cognitiva moderada a severa o dependencia para realizar actividades cotidianas***)	Evitar depender de la HbA1c; El objetivo del tratamiento debe centrarse en evitar hipoglicemias y hiperglicemias sintomáticas

*Enfermedades crónicas coexistentes son enfermedades suficientemente serias para necesitar tratamiento farmacológico y cambios en el estilo de vida como por ejemplo artritis, cáncer, insuficiencia cardiaca congestiva, depresión, enfisema, hipertensión, incontinencia, enfermedad renal crónica en fase 3 o peor, infarto de miocardio.
**Actividades cotidianas complejas: incluye actividades como preparar comidas, administrar su medicación, administrar sus finanzas, comunicarse con otras personas.
***Actividades cotidianas: actividades básicas como lo son la higiene personal, alimentarse, deambular de manera independiente, vestirse.
†La presencia de una enfermedad crónica en fase terminal como fase 3-4 insuficiencia cardiaca congestiva, enfermedad pulmonar dependiente de oxígeno, enfermedad crónica de riñón que requiere diálisis o cáncer metastásico descontrolado, que pueden causar síntomas significativos o discapacidad del estado funcional y reducir significativamente la esperanza de vida.

El cuestionario SKILLD [7]: Este cuestionario está compuesto por 10 preguntas abiertas referentes a niveles de glucosa y actividades que eviten la aparición de complicaciones a largo plazo en pacientes que tuviesen bajo nivel de alfabetización. Cada pregunta tiene una puntuación de cero (respuesta incorrecta) o uno (respuesta correcta). La puntuación total de la escala es la suma de todas las preguntas

y por tanto, tiene un rango de 0 a 10, donde una puntuación más alta indica un conocimiento mayor de la enfermedad. Las preguntas se consideraron correctas si la respuesta verbal era consistente con respuestas aceptables (Tabla 2).

Tabla 2. Cuestionario SKILLD. Preguntas principales, alternativas y respuestas de control.

SKILLD
1. ¿Cuáles son los signos y síntomas del azúcar alto en la sangre? (al menos dos) Alternativa: ¿Cómo se siente cuando tiene alto su nivel de azúcar o cuando le diagnosticaron que lo tiene alto? La respuesta debe contener 2 de: Sed extrema, micción frecuente, beber o comer, visión borrosa, somnolencia, fatiga.
2. ¿Cuáles son los signos o síntomas del azúcar bajo en la sangre? (al menos dos) Alternativa: ¿Cómo se siente cuando tiene muy bajo su nivel de azúcar? La respuesta debe contener 2: hambre, nerviosismo, inquietud, cambios de humor, irritabilidad, confusión, sudoración, frecuencia cardíaca rápida mareos, aturdimiento, debilidad
3. ¿Cómo trata el nivel bajo de azúcar? Alternativa: ¿Qué debería hacer si tiene bajo su nivel de azúcar? ¿Cómo puede usted subir su nivel de azúcar si está muy bajo? La respuesta debe ser clara sobre la acción: beber zumo, comer dulces, beber leche, comer azúcar o dulces, beber refrescos azucarados o al menos 15 gramos de carbohidratos.
4. ¿Con qué frecuencia una persona con diabetes debe revisarse sus pies? Alternativa: ¿Una vez al día, una vez a la semana o una vez al mes? La respuesta no puede variar: diariamente
5. ¿Por qué son importantes los exámenes de los pies en las personas con diabetes? Alternativa: ¿Por qué es importante que se revise los pies? ¿Qué busca cuando se los revisa? La respuesta debe ser clara acerca de la acción. Los siguientes son ejemplos. Las respuestas pueden variar, pero deben ser claras acerca de las consecuencias. Los pies se dañan, revisa si hay llagas, revisa si hay heridas, la sensibilidad/sensación cambia o empeora.
6. ¿Con qué frecuencia debería consultar a un oftalmólogo (oculista) y por qué es importante? Alternativa: ¿con qué frecuencia? ¿Por qué? La respuesta debe contener una respuesta de dos partes: Las visitas son anuales. Las razones pueden incluir la verificación de daños en los ojos, la diabetes causa problemas en los ojos, la ceguera puede ocurrir, los ojos pueden dañarse, el glaucoma o la verificación de cambios en los ojos.
7. ¿Cuál es un nivel normal de glucosa o de azúcar en la sangre en ayunas? Alternativa: ¿Cuándo se levanta temprano en la mañana y revisa su nivel de azúcar antes de comer o tomar alguna medicina, qué nivel de azúcar debería tener? ¿Cuáles son los 2 números nivel más alto y más bajo) que debería tener? La respuesta debe incluir ambos números [rango]: 70 o 80 a 120.
8. ¿Cuál es un nivel normal de HbA1c (hemoglobina A1C) o “prueba promedio de azúcar en la sangre”? Alternativa: ¿Cuándo le sacan sangre de su brazo y obtienen una lectura promedio de azúcar en la sangre, ¿cuál debería ser la lectura promedio? La respuesta debe contener cualquier número <7
9. ¿Cuántas veces a la semana debería hacer ejercicio alguien con diabetes y por cuánto tiempo? Alternativa: ¿Cuántas veces a la semana? ¿Cuánto tiempo por día? La respuesta debe contener una respuesta de dos partes. Los números reportados deben estar dentro del rango: 3 a 5 veces por semana y 30 a 45 minutos
10. ¿Cuáles son algunas de las complicaciones a largo plazo de la diabetes sin control? Alternativa: ¿Sabe usted de alguien que tenga diabetes y que le haya sucedido “cosas malas”? ¿Cuáles son algunas de esas “cosas malas”? Las respuestas deben contener 2 de las consecuencias: ceguera, problemas de visión, daño renal, diálisis, amputación, heridas, infecciones, neuropatía, impotencia, problemas estomacales, problemas cardíacos, problemas en los pies o presión arterial alta

En la validación original de Rothman et al. [5] en 2005 las respuestas que se usarían como referencia para comparar con las que daba el paciente estaban redactadas en terminología médica. García et al. modificaron estas referencias a un argot coloquial de forma que fuese más fácil la comparación con la respuesta que daba el paciente. Por otro lado, este cuestionario consta de una pregunta principal y una alternativa que se pregunta en caso del que el paciente no comprenda la pregunta principal. Es decir, al paciente se le lee la pregunta principal y se le da de 10 a 15 segundos para responder. Si el paciente no puede responder a la pregunta principal, se hace la pregunta alternativa y se asignan otros 10 a 15 segundos para que responda el paciente [5]. Se formó a los administradores de la prueba para que hicieran las preguntas a cada sujeto de la misma manera y para que dieran la máxima puntuación solo a las respuestas completas.

Además, se recogieron variables sociodemográficas (sexo, edad, vive solo o acompañado, nivel de estudios y hábito tabáquico), datos relacionados con la medicación (número de medicamentos totales prescritos y número de medicamentos para tratar la DM2), comorbilidades (hipertensión arterial (HTA), dislipemias, depresión, arritmias…), complicaciones asociadas a la DM2 (retinopatías, nefropatías y neuropatías (Tabla 2).

Recogida de datos

Los pacientes que cumplieron los criterios de inclusión fueron informados de forma oral y por escrito, y firmaron un consentimiento informado. Fueron citados y entrevistados por farmacéuticos entrenados para ello.

Este estudio fue aprobado por el Comité de Bioética de la Investigación de la Universidad Metropolitana de Educación, Ciencia y Tecnología (UMECIT) de Panamá (CBIU_5-001).

Análisis Estadístico

Se realizó un análisis descriptivo de la muestra. Para las variables cuantitativas se estudió la tendencia central (media) y dispersión (desviación estándar) y para las variables cualitativas se realizó un análisis de frecuencias.

Se estudiaron las frecuencias de endose (análisis de frecuencia de respuesta) y la correlación ítem-total de la escala (aceptables valores ≥ 0,3) para comprobar la capacidad discriminante de los ítems.

La fiabilidad del cuestionario se estudió mediante homogeneidad y mediante estabilidad temporal. Para la homogeneidad se estudió se utilizó el estadístico alfa de Cronbach (α). Por su parte, la estabilidad temporal se estudió mediante la prueba de test-retest. Esta prueba evalúa el acuerdo entre dos mediciones repetidas separadas durante algún tiempo (tres meses en este caso). Se utilizó el coeficiente de correlación intraclase (aceptable ICC ≥ 0,8.)

Para la evaluación de la validez de constructo se llevó a cabo un análisis factorial de componentes principales (AFCP) con rotación Varimax. Se extrajeron los factores con autovalores mayores que 1. Se utilizó el test de esfericidad de Bartlett garantizar que este método era adecuado y el test de Kaiser-Meyer-Oklin (KMO) para comprobar que el tamaño de muestra era suficiente. Se consideraron los siguientes intervalos: KMO > 0,90 Excelente; 0,80 ≥ KMO<0,90 Buena; 0,70 ≥ KMO<0,80 Normal; 0,60 ≥ KMO < 0,70 Medio; KMO < 0,60 Insuficiente.

La validez de criterio predictiva se estudió mediante la sensibilidad (S), especificidad (E), valor predictivo positivo (VPP) y valor predictivo negativo (VPN).

Resultados

De los 175 pacientes incluidos en el estudio, 115 (68 %) fueron mujeres. La edad media fue 60,0 (DE: 12,0) años. El 84,6 % (148) vivía acompañado, el 54,9 % (96) eran sin estudios o con estudios primarios y 130 pacientes (74,3 %) nunca fumó. Según el IMC, sólo el 29,1 % (51) tenía normopeso y, respecto a la DM2, el 67,4 % de los pacientes no tenían controlada su enfermedad. El %HbA1C medio fue 8,6 (DE: 2,3) (Tabla 3).

Tabla 3. Caracterización de la muestra.

	Total N= 175	DM2		p-valor
		Controlada; n (%) 57 (32,6)	No Controlada; n (%) 118 (67,4)	
%HbA1C; media (DE)	8,6 (2,3)	6,2 (0,6)	9,8 (1,8)	<0,001
Centro; n (%)				
Montijo	53 (30,3)	18 (31,6)	35 (39,7)	
Atalaya	31 (17,7)	11 (19,3)	20 (16,9)	
Santiago	61 (34,7)	20 (35,1)	41 (34,7)	
La Mata	30 (17,1)	8 (14,0)	22 (18,6)	0,886
Sexo; n (%)				
Hombre	56 (32,0)	23 (40,4)	33 (28,8)	
Mujer	119 (68,0)	34 (59,6)	85 (72,0)	0,100
Edad (años); media (DE)	60,0 (12,0)	63,4 (10,7)	58,3 (12,2)	0,080
Estudios; n (%)				
Sin Estudios	22 (12,6)	7 (12,3)	15 (12,7)	
Primarios	74 (42,3)	26 (45,6)	48 (40,7)	
FP/Bachillerato	49 (28,0)	12 (21,1)	37 (21,4)	
Universitarios	30 (17,1)	12 (21,1)	18 (15,3)	0,487
Vive acompañado; n (%)				
No	27 (15,4)	14 (24,6)	13 (11,0)	
Si	148 (84,6)	43 (75,4)	105 (89,0)	0,020
Tabaco; n (%)				
Nunca Fumo	130 (74,3)	39 (68,4)	91 (77,1)	
Ex≤1 año	42 (24,0)	16 (28,1)	26 (22,0)	
Ex>1 año	1 (0,6)	1 (1,8)	-	
Fumador	2 (1,1)	1 (1,8)	1 (0,8)	0,345
Peso (kg); media (DE)	72,8 (15,1)	71,8 (15,6))	73,3 (15,1)	0,544
Talla (m); media (DE)	1,6 (0,1)	1,6 (0,1)	1,6(0,1)	0,495
IMC (kg/m^2); media (DE)	28,7 (5,5)	28,1 (5,5)	29,0 (5,4)	0,703
Obesidad; n (%)				
Normopeso	51 (29,1)	17 (29,8)	34 (28,8)	
Sobrepeso	65 (37,1)	24 (42,1)	41 (34,7)	
Obesidad grado I	34 (19,4)	9 (15,8)	25 (21,2)	
Obesidad grado II	19 (10,9)	5 (8,8)	14 (11,9)	
Obesidad grado III	6 (3,4)	2 (3,5)	4 (3,4)	0,828
PC (cm); media (DE)	100,2 (11,9)	97,6 (10,7)	101,5 (12,2)	0,045
P. Cadera (cm); media (DE)	107,4 (12,2)	105,6 (9,9)	108,2 (13,0)	0,176
ITC (cm); media (DE)	0,63 (0,1)	0,61 (0,1)	0,64 (0,1)	0,021
ICC (cm); media (DE)	0,94 (0,1)	0,93 (0,1)	0,94 (0,1)	0,210
ICC adecuado				
Sí	35 (20,0)	15 (26,3)	20 (16,9)	
No	140 (80,0)	42 (73,7)	98 (83,1)	0,147
HTA; n (%)				
No	56 (32,0)	12 (21,1)	44 (37,3)	
Sí	119 (68,0)	45 (78,9)	74 (62,7)	0,031
IAP; n (%)				
No	174 (99,4)	57 (100,0)	117 (99,2)	
Sí	1 (0,6)	-	1 (0,8)	0,486
Var/Hemor; n (%)				
No	173 (98,9)	56 (98,2)	117 (99,2)	
Sí	2 (1,1)	1 (1,8)	1 (0,8)	0,597

	Total N= 175	DM2		p-valor
		Controlada; n (%) 57 (32,6)	No Controlada; n (%) 118 (67,4)	
Dislipemias; n (%)				
No	146 (83,4)	45 (78,9)	101 (85,6)	
Sí	29 (16,6)	12 (21,1)	17 (14,4)	0,268
IAM; n (%)				
No	170(97,1)	55 (96,5)	115 (97,5)	
Sí	5 (2,9)	2 (3,5)	3 (2,5)	0,719
AP; n (%)				
No	175 (100,0)	57 (100,0)	118 (100,0)	
Sí	-	-	-	-
IC; n (%)				
No	172 (98,3)	55 (96,5)	117 (99,2)	
Sí	3 (1,7)	2 (3,5)	1 (0,8)	0,204
Arritmias; n (%)				
No	168 (96,0)	53 (93,0)	115 (97,5)	
Sí	7 (4,0)	4 (7,0)	3 (2,5)	0,157
Ictus; n (%)				
No	174 (99,4)	57 (100,0)	117 (99,2)	
Sí	1 (0,6)	-	1 (0,8)	0,486
IRenal; n (%)				
No	173 (98,9)	56 (98,2)	117 (99,2)	
Sí	2 (1,1)	1 (1,8)	1 (0,8)	0,597
Ansiedad; n (%)				
No	171 (97,7)	56 (98,2)	115 (97,5)	
Sí	4 (2,3)	1 (1,8)	3 (2,5)	0,744
Depresión; n (%)				
No	171 (97,7)	56 (98,2)	115 (97,5)	
Sí	4 (2,3)	1 (1,8)	3 (2,5)	0,744
Asma; n (%)				
No	167 (95,4)	54 (94,7)	113 (95,8)	
Sí	8 (4,6)	3 (5,3)	5 (4,2)	0,761
EPOC; n (%)				
No	176 (100,0)	57 (100,0)	118 (100,0)	
Sí	-	-	-	-
Hipertiroidismo; n (%)				
No	174 (99,4)	57 (100,0)	117 (99,2)	
Sí	1 (0,6)	-	1 (0,8)	0,486
Hipotiroidismo; n (%)				
No	170 (97,1)	54 (94,7)	116 (98,3)	
Sí	5 (2,9)	3 (5,3)	2 (1,7)	0,184
Retinopatía; n (%)				
No	168 (96,0)	55 (96,5)	113 (95,8)	
Sí	7 (4,0)	2 (3,5)	5 (4,2)	0,818
Neuropatía; n (%)				
No	169 (96,6)	57 (100,0)	112 (94,9)	
Sí	6 (3,4)	-	6 (5,1)	0,083
Pie Diabético; n (%)				
No	167 (95,4)	54 (94,7)	113 (95,8)	
Sí	8 (4,6)	3 (5,3)	5 (4,2)	0,761
Años DM2; media (DE)	11,6 (9,9)	10,6 (8,6)	12,0 (8,6)	0,401
NMT; media (DE)	3,4 (1,8)	3,7 (2,0)	3,3 (1,6)	0,081

	Total N= 175	DM2		p-valor
		Controlada; n (%) 57 (32,6)	No Controlada; n (%) 118 (67,4)	
NMT; n (%)				
1	21 (12)	5 (8,8)	16 (13,6)	
2	36 (20,6)	10 (17,5)	26 (22,0)	
3	45 (25,7)	15 (26,3)	30 (25,4)	
>3	73 (41,7)	27 (47,4)	46 (39,0)	0,621
NAD; media (DE)	3,4 (1,8)	1,4 (0,6)	1,7 (0,6)	0,026
NAD; n (%)				
1	86 (49,1)	36 (63,2)	50 (42,4)	
2	74 (42,3)	17 (29,8)	57 (48,3)	
3	15 (8,6)	4 (7,0)	11 (9,3)	0,035
Actividad Física; n (%)				
Inactivo	108 (61,7)	32 (56,1)	76 (64,4)	
Moderada	48 (27,4)	16 (28,1)	32 (27,1)	
Intensa	19 (10,9)	9 (15,8)	10 (8,5)	0,312

%HbA1c: porcentaje de hemoglobina glicosilada; AP: angina de pecho; DM2: diabetes Mellitus tipo 2; EPOC: enfermedad pulmonar obstructiva crónica; HTA: hipertensión arterial; IAM: Infarto de miocardio; IAP: insuficiencia arterial periférica; IC: Insuficiencia cardiaca; ICC: índice cintura cadera; ICT: índice cintura talla; IMC: índice de masa corporal; IRenal: insuficiencia Renal; NAD: número de Antidiabéticos; NMT: número de medicamentos en total; PC: perímetro de cintura; P cadera: perímetro de cadera; Var/Hemor: varices o hemorroides.

La puntuación total media del SKILLD fue de 4,1 (DE: 2,1). Por su parte, en base al punto de corte establecido para clasificar a los conocedores de la DM2 (5 > puntos) y no conocedores (≤ 5 puntos), sólo 44 pacientes (25,1 %) fueron conocedores, y solo el 20 % tubo una puntuación entre 6 y 7. Ninguno supero esa puntuación. Si se estudia en función de control de la DM2, no hay diferencia estadísticamente significativa entre los pacientes con control de la DM2 (4,2; DE: 2,1) y sin control de la enfermedad (4,1; DE: 2,0).

Las frecuencias de endose muestran que todos los resultados se encuentran entre el 20 % y el 80 % de respuesta, a excepción de la pregunta 9 (13,1 %). La correlación ítem-total de la escala fue moderada en las preguntas 3, 5 y 7, en el resto fue baja (Tabla 4).

Tabla 4. Frecuencias de endose y correlación ítem-total.

	Sí; n (%)	Correlación ítem-Total
1. Signos y síntomas del azúcar alto en la sangre	50 (28,6)	0,246
2. Signos y síntomas del azúcar bajo en la sangre	50 (28,6)	0,176
3. Tratamiento de nivel bajo de azúcar	103 (58,6)	0,304
4. Frecuencia revisión de los pies	108 (61,7)	0,197
5. Importancia de examen de pies	124 (70,9)	0,374
6. Frecuencia de consulta al oftalmólogo	43 (24,6)	0,184
7. Nivel de azúcar en sangre en ayunas	55 (31,4)	0,307
8. Nivel normal de HbA1C	60 (34,3)	0,210
9. Frecuencia de ejercicio físico	23 (13,1)	0,172
10. Conocimiento de complicaciones de la DM2	100 (57,1)	0,281

HbA1c: hemoglobina glicosilada; DM2: diabetes Mellitus tipo 2.

La fiabilidad medida mediante consistencia interna fue $\alpha = 0,559$ y mediante estabilidad temporal fue ICC = 0,803 (IC 95 % = 0,670 – 0,882).

En el estudio de la validez, el AFCP arrojó 4 factores y la varianza total explicada (VTE) fue 55,3 %. El primero incluyó a las preguntas 1, 3, 7 y 10 (varianza explicada = 20,60 %) y podría tener que ver con la glucemia elevada en sangre y sus consecuencias, el segundo incluyó las preguntas 2, 4 y 5 (varianza explicada = 12,81 %) y tendría que ver con la hipoglucemia y el cuidado de los pies, el tercero agrupó las preguntas 6 y 8 (11,21 %) y tendría que ver con el cuidado de los ojos, y el cuarto factor solo incluyó la pregunta 9 (10,65 %) referente a la actividad física. El KMO fue 0,649 y la prueba de esfericidad de Bartlett fue estadísticamente significativa.

Respecto a la validez de criterio, la S fue del 76,2 %, la E fue del 28,1 %; el VPP fue del 68,7 %; VPN fue del 36,4 %. (Tabla 5).

Tabla 5. Distribución del conocimiento que tiene el paciente sobre la DM2 en función del control de la DM2.

		Control DM2		Total
		No	Sí	
SKIILD	No conoce (≤ 5 puntos)	90	41	131
	Conoce (≤ 5 puntos)	28	16	44
Total		118	57	175

DM2: diabetes Mellitus tipo 2; SKILLD: Spoken Knowledge in Low Literacy in Diabetes Scale

Discusión

Este estudió evaluó el conocimiento que tiene una muestra de pacientes con DM2 de la región de Veraguas (Panamá) y estudió la fiabilidad y la validez de herramienta utilizada para medir dicho conocimiento (SKILLD). Esto permitiría conocer hasta qué punto se puede hacer una extrapolación de los resultados obtenidos al total de muestra estudiada [11,12]. Así pues, para estudiar la fiabilidad de la herramienta se utilizó la consistencia interna (α) que llegó a ser de 0,559. Este valor es un poco más bajo de lo que obtuvieron García. et al. (0,64) [7] y Rothman et al. (0,72) [5], pero un poco más alto del que obtuvieron Jappesen et al. (0,54) [6]. Por tanto, la consistencia interna parece estar influenciada por el tamaño y selección de la muestra a la que se entrevista, más que por las propias preguntas en sí mismas. Desde este punto de vista, podría decirse que en este estudio la fiabilidad ha sido poco menos que aceptable. La estabilidad temporal por su parte fue aceptable (CCI = 0,803), un valor adecuado que no se pudo comparar con otros estudios similares porque no aportaron este dato.

Respecto a la validez, el constructo estuvo compuesto por 4 factores. Algunos más de lo deseable en un cuestionario con tan solo 10 preguntas. La pregunta 9 referente a la actividad física ocupa un único factor. En ciertas condiciones, por esta razón y por el bajo endose que aportó, esta pregunta sería candidata a ser eliminada del cuestionario [13], sin embargo, fue recomendable mantenerla para no alterar la validez de contenido y porque consigue explicar por sí misma un 10,65 % de la VTE [11,12]. Por otra parte, es interesante remarcar que tal y como se han agrupado las cargas factoriales de las preguntas hace difícil nombrar a los distintos factores. Lo que hace pensar que, o bien el constructo teórico, o bien las preguntas o la escala de puntuación debería ser revisados. Este tipo de análisis tampoco fue realizado en los otros estudios donde se realizó la validación del SKILLD. Solo se hizo algo similar en el estudio de Hu et. [8] donde encontraron un único factor, pero el tipo de AFCP no es comparable al que se hizo en este estudio.

Desde el punto de vista de la validez de criterio se obtuvo una S aceptable (76,2 %), por lo tanto, la probabilidad de acertar sería bastante elevada para un paciente de esta muestra, cuando se afirma que si no tiene conocimiento sobre la DM2 no tendría controlada la enfermedad. El VPP es un poco más bajo (68,7 %), pero también interesante, ya que casi con un 70 % de probabilidad acertaríamos al predecir que un paciente que no tiene conocimientos sobre la DM2 no tendría controlada su DM2. La E y el VPN son muy bajos, por lo que sería muy fácil fallar al indicar que cuando una persona conoce

su enfermedad tendría controlada la enfermedad. Por tanto, desde el punto de vista clínico, este cuestionario podría ayudar a detectar personas que por no conocer su enfermedad podrían tener la DM2 no controlada, útil bien para seleccionarlos para determinados tipos de estudios donde se investiguen algunos tipos intervenciones (educativas, farmacológicas…), bien con el fin de confirmar el estado real de su enfermedad.

Respecto al conocimiento que tienen los pacientes sobre la DM2 fue bastante bajo, de hecho, la media de toda la muestra apenas llegó a 4,1 (DE: 2,1), y aunque era de esperar que aquellas personas que tenían controlada su DM2 supiesen algo más sobre la enfermedad, esto no fue así. Esto encontraría explicación en el hecho de que los pacientes podrían obedecer las instrucciones del médico sin informarse del porqué de dichas instrucciones. Esto dificulta enormemente la posibilidad de autocontrol de la enfermedad por parte del paciente porque no sabe cómo actuar en casos de síntomas de hiperglucemia, hipoglucemias o incluso de síntomas en los pies o en los ojos. Puesto que la evidencia actual confirma la importancia de que el paciente conozca su enfermedad [14], se hace evidente la necesidad de educación del paciente por parte de los profesionales sanitarios [15]. De esta forma el paciente podría ayudar al profesional de la salud en la toma de decisiones respecto a su enfermedad, mejorar su adherencia a los tratamientos, facilitar la resolución de problemas y colaborar de forma activa con dichos profesionales para mejorar los resultados clínicos, su estado de salud y su calidad de vida [16].

Este estudio tiene algunas limitaciones que deben ser consideradas. Puesto que la muestra no se seleccionó de forma aleatoria, es posible que los pacientes que hayan decidido participar en este estudio fuesen los que, de alguna forma, se encontrasen peor y pensasen que les vendría bien participar. Esto podría llevar consigo un sesgo de selección que sobreestimase la falta de conocimiento.

Conclusión

El conocimiento que tienen los pacientes de la región de Veraguas (Panamá) sobre la diabetes tipo 2 que padecen es bajo, independientemente de que sus valores de %HbA1C estén dentro de los valores recomendados por la ADA o no. El cuestionario SKILLD ha demostrado ser una herramienta útil, fiable y valida, aunque son necesarios más estudios que refuercen estos datos.

Bibliografía

1. International Diabetes Federation (IDF) Diabetes Atlas. 10th edition. [monografía en internet]. International Diabetes Federation; 2021[citado 10 de marzo de 2025] disponible en: https://fmdiabetes.org/wp-content/uploads/2022/01/IDF_Atlas_10th_Edition_2021-comprimido.pdf

2. Quintana HK, Moreno Velásquez I, Montenegro Mendoza R, Niño Hall C, Motta J, Roa R. Diabetes mellitus, its prevalence, awareness, and control in Panama: Data from ENSPA 2019, a national cross-sectional study. Medicine (Baltimore). 2023;102(32):e34600. DOI: 10.1097/MD.0000000000034600

3. Díaz MS. Priorizar el control glucémico temprano para reducir las complicaciones micro y macrovasculares en personas con diabetes tipo 2 [Internet]. RedGDPS 2022 [citado 21 de febrero de 2025]. Disponible en: https://redgedaps.blogspot.com/2022/06/

4. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes. New England Journal of Medicine. 2008;359(15):1577-89. DOI: 10.1056/NEJMoa0806470

5. Rothman RL, Malone R, Bryant B, Wolfe C, Padgett P, DeWalt DA, et al. The Spoken Knowledge in Low Literacy in Diabetes scale: a diabetes knowledge scale for vulnerable patients. Diabetes Educ. 2005;31(2):215-24. DOI: 10.1177/0145721705275002

6. Jeppesen KM, Hull BP, Raines M, Miser WF. A validation study of the spoken knowledge in low literacy in diabetes scale (SKILLD). J Gen Intern Med. 2012;27(2):207-12. DOI: 10.1007/s11606-011-1900-9

7. Garcia AA, Zuniga J, Reynolds R, Cairampoma L, Sumlin L. Evaluation of the spoken knowledge in low literacy in diabetes scale for use with Mexican Americans. J Transcult Nurs. 2015;26(3):279-86. DOI: 10.1177/1043659614524246

8. Hu J, Amirehsani KA, McCoy TP, Wallace DC, Coley SL, Zhan F. Reliability and Validity of the Spoken Knowledge in Low Literacy in Diabetes in Measuring Diabetes Knowledge Among Hispanics With Type 2 Diabetes. Diabetes Educ. 2020;46(5):465-74. DOI: 10.1177/0145721720941409

9. A1CNow+ System [Internet]. PTS Diagnostics. [citado 10 de marzo de 2025]. Disponible en: https://ptsdiagnostics.com/a1cnow-plus-system/

10. American Diabetes Association. Glycemic Goals and Hypoglycemia: Standards of Care in Diabetes-Diabetes Care 2024 [Internet]. 2024 [citado 10 de marzo de 2025]. Disponible en: https://diabetesjournals.org/care/article/47/Supplement_1/S111/153951/6-Glycemic-Goals-and-Hypoglycemia-Standards-of

11. Strainer DL, Norman GR, Cairney J. Health measurement scales. A practical guide to their development and use. 5th ed. Oxford: Oxford University Press; 2015.

12. de DeVellis, RF. Scale Development: Theory and Applications (Applied Social Research Methods). 3rd Edition. SAGE Publications, Inc; 2011.

13. Martínez González M, Sánchez-Villegas A, Faulín Fajardo J. Bioestadística amigable. 2a ed. España: Diaz de Santos; 2006.

14. Lim PC, Rajah R, Lee CY, Wong TY, Tan SSA, Karim SA. Systematic Review and Meta-Analysis of Diabetes Knowledge among Type 2 Diabetes Patients in Southeast Asia. Rev Diabet Stud. 2021;17(2):82-9. DOI: 10.1900/RDS.2021.17.82

15. Nguyen TH, Tran TTT, Nguyen NK, Diep HG, Vo SD, Taxis K, et al. A randomized controlled trial of a pharmacist-led intervention to enhance knowledge of Vietnamese patients with type 2 diabetes mellitus. Int J Pharm Pract. 2022;30(5):449-56. DOI: 10.1093/ijpp/riac030

16. Sociedad Española de Diabetes. Programa de educación terapéutica para personas con diabestes tipo 2 y/o familiares y/o cuidadores [Internet]. 2019. [citado 10 de marzo de 2025] disponible en: https://d2q8uh6bd0ohj9.cloudfront.net/wp-content/uploads/2019/05/27152144/educacion-terapeutica-tipo2.pdf

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.33139
Artículos especiales

Protocolo de revisión sistemática y metaanálisis de los tratamientos biológicos y sintéticos en artritis psoriásica refractaria

Protocol for systematic review and meta-analysis of biologic and synthetic treatments in refractory psoriatic arthritis

Carmen María Domínguez-Santana[1] 0009-0005-0179-6295
Silvia Fénix-Caballero[2] 0000-0002-3063-494X
Gala Cano-Martínez[2]
Emilio Jesús Alegre-del-Rey[2] 0000-0003-0041-2024
Eva Martín-Ruiz [3,4] 0000-0003-1509-1198
Antonio Olry de Labry-Lima[5,6,4] 0000-0001-5448-1370

[1] Farmacia Hospitalaria. Hospital Ribera Povisa. Vigo, España.

[2] Farmacia Hospitalaria. Hospital Universitario de Puerto Real: Puerto Real, Andalucía, España

[3] Universidad de Sevilla, Facultad de Enfermería, Fisioterapia y podología. Departamento de Enfermería. Sevilla, España.

[4] Escuela Andaluza de Salud Pública. Granada. España.

[5] Centro Andaluz de Documentación e Información de Medicamentos (CADIME). Escuela Andaluza de Salud Pública. Granada. España.

[6] CIBER en Epidemiología y Salud Pública (CIBERESP), Madrid. España.

[7] Instituto de Investigación Biosanitaria, Hospitales Universitarios de Granda/ Universidad de Granada, España.

Correspondencia

Eva Martin Ruiz
emruiz@us.es

Recibido: 18.03.2025
Aceptado: 15.05.2025
Publicado: 20.06.2025

Agradecimientos

Agradecemos la participación de Camila Higueras, responsable de biblioteca EASP.

Financiación

Este trabajo se ha realizado sin financiación.

Conflicto de intereses

Los autores del presente artículo declaran que no están sujetos a ningún conflicto de interés relacionado con el tema tratado que pueda afectar al diseño, el análisis o la presentación de resultados.

Otras declaraciones

Este Trabajo ha recibido el Premio Extraordinario de Grado y Máster Universitario de la Universidad Miguel Hernández, curso 2023-2024.

Resumen

Introducción: La artritis psoriásica es una enfermedad inflamatoria crónica con variedad de manifestaciones en articulaciones, piel y otros tejidos. En los últimos años, se han comercializado diferentes terapias para pacientes refractarios; no se dispone de comparaciones directas entre la mayoría de los tratamientos, lo cual dificulta la toma de decisiones clínicas para la elección del mismo. Por ello, se plantea comparar la eficacia y seguridad de los distintos tratamientos para la artritis psoriásica activa y analizar posibles diferencias por subgrupos según tratamiento biológico previo.

Método: Se realizará una revisión sistemática y un metaanálisis en red. La información de los ensayos clínicos en fase II o III relevantes se recuperará de las bases de datos, incluidas PubMed, Cochrane Library, Embase y ClinicalTrials. Se construirá una búsqueda con términos libres y controlados (MeSH y Emtree). La búsqueda manual tratará de encontrar otros informes no publicados y datos complementarios. Los datos se extraerán mediante una hoja de volcado de datos diseñada ad hoc. Se utilizará la herramienta de riesgo de sesgo avaladas por Cochrane de los estudios incluidos. Se realizará un metaanálisis en red para comparar la proporción de pacientes que alcanzaron una tasa de mejora del 50 % en la valoración del American College of Rheumatology (ACR50). La seguridad se evaluará mediante la incidencia de eventos adversos.

Conclusiones: Los resultados de este metaanálisis contribuirán a la toma de decisiones clínica en torno al uso de los fármacos en el tratamiento de la artritis psoriásica.

Palabras clave: artritis psoriásica; tratamiento biológico; tratamiento sintético dirigido.

Abstract

Introduction: Psoriatic arthritis (PsA) is a chronic inflammatory disease with a variety of manifestations in joints, skin and other tissues. In recent years, different therapies have been marketed, but direct comparisons between most treatments are not available, which makes clinical decision making difficult in refractory patients, generating high uncertainty. The objective is to compare the efficacy and safety of different treatments for active psoriatic arthritis and to analyze possible differences according to previous treatment.

Methods: a systematic review and network meta-analysis will be performed following the PRISMA recommendations and their extensions. Information from relevant phase II or III clinical trials will be retrieved from major databases including PubMed, Cochrane Library, Embase, ClinicalTrials.gov and Google Scholar. A search was constructed using both free and controlled terms (MeSH and Emtree), which has been validated by a librarian with extensive experience. Google and manual search will find other unpublished reports and supplementary data. Data will be extracted using an ad hoc designed data dump sheet. The Cochrane risk of bias tool will be used to assess the quality of clinical trials. Finally, a network meta-analysis will be performed to compare the proportion of patients who achieved a 50 % improvement rate on the American College of Rheumatology (ACR50) assessment. Safety will be assessed by the incidence of adverse events.

Conclusions: The results of this meta-analysis may be important for health personnel and health policy makers regarding the use of drugs in the treatment of psoriatic arthritis

Keywords: Psoriatic arthritis; biological treatment; targeted synthetic treatment.

Puntos clave

La artritis psoriásica es una enfermedad inflamatoria sistémica crónica, donde los fármacos antirreumáticos modificadores de la enfermedad, biológicos y sintéticos dirigidos, forman parte también del arsenal terapéutico junto a otros tratamientos de primera línea.

Actualmente no existen comparaciones directas entre los tratamientos farmacológicos disponibles, lo cual dificulta la toma de decisiones en la selección del fármaco más adecuado. Este estudio aportará una síntesis y análisis de la evidencia disponible que compare todos los tratamientos disponibles.

Se plantea la realización de una revisión sistemática y metaanálisis en red independiente que compare la eficacia y seguridad de los tratamientos disponibles para la artritis psoriásica activa refractaria frente a convencional mediante metaanálisis en red, contribuyendo así a la toma de decisiones clínicas.

Introducción

La artritis psoriásica (AP) es una enfermedad inflamatoria sistémica crónica, con una presentación heterogénea, que presenta diversidad de manifestaciones músculo-esqueléticas y extraarticulares y asociada a diversas comorbilidades. Se caracteriza por la presencia de inflamación, deformidad y destrucción articular y pertenece al grupo de las espondiloartritis periféricas, que involucran a múltiples tejidos y dominios clínicos como la artritis, espondilitis, entesitis y dactilitis.[1]

No está definida su etiopatogenia, se relaciona con factores genéticos, desencadenantes ambientales, factores locales según la ubicación de la enfermedad (articulaciones, piel, columna vertebral/entesis) y la interacción con respuestas inmunes innatas y adaptativas.[2]

En la población general, la prevalencia de AP oscila entre 0,1 % y 1 %, y llega al 20 % entre los pacientes con psoriasis. Esta disparidad puede justificarse por heterogeneidad geográfica o metodológica. En las diferencias territoriales intervienen a factores genéticos, ambientales (clima e infecciones), estilos de vida y/o hábitos dietéticos y diferencias metodológicas o en la definición de la patología.[3] Una revisión sistemática con metaanálisis estimó la tasa de prevalencia en la población general de 133 casos/100.000 habitantes (0,13 %) y una incidencia de 83 casos/100.000 habitantes-año.[4] En España, la prevalencia de AP en la población general se estimó en 0,58 % (IC95 % 0,38-0,87).[5]

En cuanto al tratamiento farmacológico, son fármacos de primera línea los fármacos antiinflamatorios no esteroideos (AINEs), inyecciones locales de corticosteroides para los síntomas musculoesqueléticos, terapias tópicas para la psoriasis y fármacos antirreumáticos modificadores de la enfermedad (FAME) sintéticos convencionales (FAMEsc) como metotrexato (MTX). En segunda línea de tratamiento, para los pacientes no respondedores o intolerantes al tratamiento inicial, se recomienda el empleo de terapias biológicas (FAMEb) y FAME sintéticos dirigidos (FAMEsd),[6,7] en monoterapia o en combinación con FAMEsc.

Se ha descrito que la eficacia y seguridad de las terapias biológicas se puede modificar según la experiencia previa a terapias biológicas, características de pacientes o a su uso combinado con FAMEsc o en monoterapia. Diferentes asociaciones profesionales proponen estrategias para la optimización del tratamiento.[7-9] No obstante, en la actualidad no existen comparaciones directas entre la mayoría de los tratamientos farmacológicos disponibles, lo cual dificulta la toma de decisiones clínicas en la selección del fármaco más adecuado.

En la literatura científica publicada, se localizan cuatro revisiones sistemáticas con metaanálisis en red sobre los tratamientos para la artritis psoriásica refractaria. Estos trabajos presentan algunas diferencias respecto a este protocolo que pudieran modificar el enfoque y detalle de los resultados. En la mayoría de esas revisiones no se incluyen todos los tratamientos disponibles,[10-12] las financian la industria farmacéutica[10,11,13] y sus autores no realizan un análisis de sensibilidad mediante un metaanálisis por metodología frecuentista para evaluar los resultados.[10-13]

Por todo ello, el presente estudio pretende comparar la eficacia y seguridad de los tratamientos disponibles para la artritis psoriásica activa refractaria a tratamiento convencional mediante un metaanálisis en red y analizar posibles diferencias de subgrupos de pacientes en función del tratamiento previo recibido.

Métodos

El presente protocolo se desarrolló conforme a las directrices de PRISMA-P (Preferred reporting items for systematic review and meta-analysis protocols) así como sus extensiones para meta-análisis en red (PRISMA-NMA).[14-15] Se ha registrado en PROSPERO (International prospective register of systematic reviews, CRD 420250620748 http://www.crd.york.ac.uk). La tabla 1 muestra los criterios de inclusión y exclusión contemplados.

En el Anexo (tabla A) se proporciona la evaluación realizada siguiendo la lista de verificación PRISMA-P completa. En el Anexo tabla suplementaria S1 se recogen los criterios de inclusión utilizados siguiendo el esquema PICO de la pregunta de investigación.

Tabla 1. Criterios de inclusión y exclusión.

Criterios de inclusión
Ensayos clínicos controlados aleatorizados fase II o III, que proporcionen resultados sobre la respuesta del ACR (ACR50, ACR20 o ACR70) entre las semanas 16 y 24. Pacientes adultos diagnosticados de artritis psoriásica activa según los criterios CASPAR, tratados con FAMEb o FAMEsd. Uso de placebo, FAMEsc, FAMEb o FAMEsd como comparador. Si el estudio incluía también a pacientes que solo presentasen psoriasis, se requirió que los resultados se presentasen claramente diferenciados por patología.
Criterios de exclusión
Estudios realizados en menores de 18 años.

ACR: American College of Rheumatology.

Criterios CASPAR (Criterios de Clasificación Para la Artritis Psoriásica); FAMEb: fármacos antirreumáticos modificadores de la enfermedad terapias biológicas; FAMEsc: fármacos antirreumáticos modificadores de la enfermedad sintéticos convencionales; FAMEsc: fármacos antirreumáticos modificadores de la enfermedad sintéticos dirigidos

Se realizará una búsqueda en las bases de datos de PubMed, Cochrane Library, EMBASE y Clinicaltrials (Anexo tabla suplementaria S2) así como en fuentes complementarias para identificar literatura gris. Se ha definido una estrategia de búsqueda, que se adaptará a las diferentes bases de datos, validada por una bibliotecaria con amplia experiencia, utilizando términos libres y controlados (MeSH y Emtree).

Extracción de datos y síntesis de resultados

Tras eliminar los duplicados, se realizará un cribado por título y resumen de todas las referencias identificadas para verificar que cumplen los criterios de inclusión establecidos. Dos revisores independientes revisarán y evaluarán a texto completo los artículos seleccionados. A lo largo del proceso, cualquier desacuerdo entre los revisores será resuelto mediante la intervención de un tercer revisor. Todo este proceso se realizará con el programa Rayyan QCRI.[17]

Se ha diseñado ad hoc una hoja de volcado de datos que incluye las siguientes variables: características del ensayo (nombre del estudio, autor principal, año, países de realización, diseño, cegamiento, número total de pacientes, presencia de resultados por subgrupos y duración del estudio), brazos de tratamiento, tiempo de evaluación de las variables, características de pacientes (sexo, edad, duración de la enfermedad), proporción de pacientes que recibe FAMEb previos, proporción de pacientes con

MTX concomitante, variables del tratamiento (fármaco, dosis y posología), y características de los eventos adversos.

Síntesis cualitativa: Se realizará una síntesis cualitativa de la eficacia y seguridad de los diferentes tratamientos, con el foco en la comparación descriptiva de las principales características y resultados reportados en los estudios incluidos. Síntesis cuantitativa:

Síntesis cuantitativa: Se realizará un metaanálisis mediante modelo de efectos fijos o aleatorios, dependiendo del nivel de heterogeneidad presente. La heterogeneidad se evaluará mediante la prueba Q de Cochrane (considerando $p < 0,10$ como indicativa de heterogeneidad) y el índice I^2 (con un valor > 50 % indicativo de heterogeneidad sustancial). Además, se utilizará el criterio de información de desviación (DIC: Deviance Information Criterion), para evaluar la complejidad y el ajuste del modelo. Adicionalmente, se realizará un análisis de inconsistencia para determinar la concordancia entre comparaciones directas e indirectas.

Posteriormente, se ejecutará un metaanálisis en red para comparar de forma indirecta y simultánea los tratamientos incluidos en la revisión, aplicando metodología bayesiana (paquete GEMTC del programa R-Statistics®).[16] Los resultados se analizarán por subgrupos en función a la exposición previa a FAMEb/sd y según el uso concomitante del FAMEb/sd con FAMEsc o en monoterapia.

Para determinar si las diferencias identificadas en el metaanálisis tienen relevancia clínica significativa, se aplicará la metodología descrita en la guía de alternativas terapéuticas equivalentes (ATE) del grupo GÉNESIS de la SEFH[18]. Para la evaluación del sesgo de publicación se procederá a la realización de un gráfico de embudo (funnel plot), analizando la asimetría de los resultados como indicador de posibles sesgos.

Riesgo de sesgo

La evaluación del riesgo de sesgo se realizará por dos investigadores de forma independiente mediante la utilización de la herramienta Cochrane RoB-2 para ensayos aleatorios.[19] Este instrumento tiene 6 dominios: sesgo en la generación de la secuencia de aleatorización (selección), desviaciones del protocolo (realización), cegamiento de los evaluadores de resultados (detección), manejo incompleto de los datos (desgaste), sesgo en la selección de resultados reportados (notificación), y otros posibles sesgos. En caso de desacuerdo entre revisores se procederá a una reevaluación para llegar a un consenso, recurriendo a un tercer revisor para resolver las posibles discrepancias.

Discusión

En la actualidad, los sistemas sanitarios se enfrentan a desafíos significativos impulsados por factores demográficos y económicos. España presenta una de las tasas de fecundidad más bajas de la Unión Europea que, junto con una elevada esperanza de vida y una edad avanzada para la maternidad, ha dado lugar a un envejecimiento poblacional progresivo. Este cambio demográfico incrementa la demanda de servicios sanitarios, especialmente para el tratamiento de enfermedades crónicas, lo cual ejerce presión sobre los recursos del sistema de salud y genera un gran impacto sobre el sistema sanitario.[20-21]

Las cifras muestran un aumento constante en el número de pacientes tratados, favorecido por los avances en el diagnóstico y en la investigación de nuevas terapias dirigidas. Estas terapias permiten una identificación más temprana y precisa de las patologías, ofreciendo un abanico más amplio de opciones de tratamiento. En los últimos años se incrementó el estudio y creación de medicamentos de síntesis biológica, los cuales, aunque altamente efectivos y con terapias cada vez más específicas, se caracterizan por sus elevados costes. Este fenómeno contribuye al crecimiento exponencial del gasto farmacéutico hospitalario, que aumentó de 2.324 millones de euros en 2003 a 9.606 millones de euros en 2023. Las proyecciones sugieren que esta tendencia de crecimiento continuará en el futuro, lo cual plantea serios desafíos de sostenibilidad para el sistema sanitario.[22] Además, es importante resaltar la disparidad en el manejo de las distintas líneas de tratamiento disponibles y la dificultad para seleccionar la terapia más apropiada, dada la falta de comparaciones directas entre los fármacos disponibles.

En este contexto, la Ley 29/2006 de garantías y uso racional de los medicamentos y productos sanitarios establece la importancia de una selección adecuada de medicamentos basada en su eficacia, seguridad y coste-beneficio.[23] Esta legislación promueve la racionalización de los recursos mediante la selección de medicamentos que ofrezcan beneficios clínicos sustanciales a costes razonables, facilitando a profesionales de la salud la elección del fármaco más apropiado en cada situación clínica. En el ámbito de la artritis psoriásica, donde los tratamientos disponibles representan un elevado coste y abarcan una amplia variedad de familias farmacológicas, la falta de comparaciones directas entre fármacos, subraya la necesidad de una evaluación que orienten las decisiones clínicas del tratamiento más adecuado.

Los resultados de este proyecto permitirán la realización de una evaluación clínica comparativa de la eficacia y seguridad de los distintos fármacos disponibles complementada por un análisis económico que aportará una valiosa información adicional para la priorización y selección de los medicamentos para el tratamiento de la AP. La evaluación económica comprende un conjunto de metodologías que, debido a la progresiva limitación de recursos y la necesidad de establecer prioridades en el gasto sanitario, la han situado como una información imprescindible para la toma de decisiones en el campo de la salud.

Este estudio presenta varias limitaciones que deben tenerse en cuenta para la interpretación de los resultados. Es posible que algunos estudios mezclen pacientes naive con aquellos pretratados, en diferentes proporciones. Estos dos subtipos de pacientes pueden presentar diferencias relevantes en sus resultados, ya que los pacientes pretratados suelen tener mayor refractariedad al tratamiento debido al fracaso de terapias previas con FAMEb. Por ello, sería ideal realizar un análisis separado para pacientes naive y pretratados. Sin embargo, en muchos casos, no se dispone de resultados desagregados para todos los tratamientos evaluados, lo que limita la posibilidad de realizar análisis por subgrupos para todas las comparaciones.

En consecuencia, solo se puede recurrir a un análisis conjunto para todos los tratamientos en algunos casos, señalando la limitación que eso supone, y explicitando el porcentaje de pacientes naive de cada estudio. Así será posible valorar el sesgo potencial esperable de cada comparación indirecta, que favorecerá a los fármacos probados en una mayor proporción de pacientes naive. Por otro lado, se llevará a cabo un análisis por subgrupos únicamente con los estudios que presenten resultados diferenciados, lo que permitirá obtener conclusiones más precisas dentro de las limitaciones metodológicas.

Conclusión

Este protocolo establece una base sólida para llevar a cabo una evaluación rigurosa y exhaustiva de los tratamientos disponibles para la artritis psoriásica refractaria, que contribuirá al desarrollo de estrategias terapéuticas más eficaces, sostenibles y basadas en la evidencia, con el objetivo de mejorar los resultados clínicos y optimizar el uso de los recursos sanitarios.

Bibliografía

1. Azuaga AB, Ramírez J, Cañete JD. Psoriatic Arthritis: Pathogenesis and Targeted Therapies. Int J Mol Sci. 2023;24(5):4901. DOI: 10.3390/ijms24054901

2. Stober C. Pathogenesis of psoriatic arthritis. Best Pract Res Clin Rheumatol. 2021;35(2):101694. DOI: 10.1016/j.berh.2021.101694

3. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H. Classification criteria for psoriatic arthritis: Development of new criteria from a large international study. Arthritis Rheum. 2006;54(8):2665-73. DOI: 10.1002/art.21972

4. Scotti L, Franchi M, Marchesoni A, Corrao G. Prevalence and incidence of psoriatic arthritis: A systematic review and meta-analysis. Semin Arthritis Rheum. 2018;48(1):28-34. DOI: 10.1016/j.semarthrit.2018.01.003

5. Romero Pérez A, Queiro R, Seoane-Mato D, Graell E, Chamizo E, Chaves Chaparro L, et al. Higher prevalence of psoriatic arthritis in the adult population in Spain? A population-based cross-sectional study. PLoS ONE. 2020;15(6):e0234556. DOI: 10.1371/journal.pone.0234556

6. Torre Alonso JC, Díaz del Campo Fontecha P, Almodóvar R, Cañete JD, Montilla Morales C, Moreno M, et al. Recomendaciones de la Sociedad Española de Reumatología sobre el tratamiento y uso de terapias sistémicas biológicas y no biológicas en artritis psoriásica. Reumatol Clínica. 2018;14(5):254-68. DOI: 10.1016/j.reuma.2017.08.007

7. Coates LC, Soriano ER, Corp N, Bertheussen H, Callis Duffin K, Campanholo CB, et al. Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA): updated treatment recommendations for psoriatic arthritis 2021. Nat Rev Rheumatol. 2022;18(8):465-79. DOI: 10.1038/s41584-022-00798-0

8. Gossec L, Kerschbaumer A, Ferreira RJO, Aletaha D, Baraliakos X, Bertheussen H, et al. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2023 update. Ann Rheum Dis 2024. DOI: 10.1136/ard-2024-225531 Disponible en: https://ard.bmj.com/content/early/2024/03/18/ard-2024-225531

9. Sociedad Española de Reumatología. Grupo de trabajo ESPOGUIA. Guía de Práctica Clínica para el Tratamiento de la Espondiloartritis Axial y la Artritis Psoriásica. Actualización. Sociedad Española de Reumatología, Madrid. 2024.

10. Mease PJ, McInnes IB, Tam LS, Rajalingam R, Peterson S, Hassan F, et al. Comparative effectiveness of guselkumab in psoriatic arthritis: updates to a systematic literature review and network meta-analysis. Rheumatology. 2023;62(4):1417-25. DOI: 10.1093/rheumatology/keac500

11. Mease PJ, Reddy S, Ross S, Lisse JR, Reis P, Griffing K, et al. Evaluating the efficacy of biologics with and without methotrexate in the treatment of psoriatic arthritis: a net-work meta-analysis. RMD Open. 2024;10(1):e003423. DOI: 10.1136/rmdopen-2023-003423

12. Montezuma T, Probst LF, Almeida MO. Effectiveness and safety of biological and target synthetic drugs treatment for psoriatic arthritis: a systematic review with network meta-analysis. Adv Rheumatol. 2024;64(1):21. DOI: 10.1186/s42358-024-00361-3

13. Mease PJ, Gladman DD, Merola JF, Nash P, Grieve S, Laliman-Khara V, Willems D, Taieb V, Prickett AR, Coates LC. Comparative efficacy and safety of bimekizumab in psoriatic arthritis: a systematic literature review and network meta-analysis. Rheumatology (Oxford). 2024;63(7):1779-1789. DOI: 10.1093/rheumatology/kead705.

14. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015;349:g7647. DOI: 10.1136/bmj.g7647

15. Haddaway NR, Page MJ, Pritchard CC, McGuinness LA. PRISMA*2020:* An R package and Shiny app for producing PRISMA 2020 - compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis. Campbell Syst Rev. 2022;18(2):e1230. DOI: 10.1002/cl2.1230

16. van Valkenhoef G, Kuiper J. gemtc: Network Meta-Analysis Using Bayesian Methods [Internet]. 2023 [citado 11 de septiembre de 2024]. p. 1.0-2. Disponible en: https://CRAN.R-project.org/package=gemtc

17. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid, A, 2016. Rayyan-a web and mobile app for systematic reviews. Syst. Rev. 2016;5: 1–10. DOI:10.1186/s13643-016-0384-4

18. Alegre Del Rey EJ, Fénix Caballero S, Castaño Lara R, Sierra García F. Evaluación y posicionamiento de medicamentos como alternativas terapéuticas equivalentes. Med Clínica. 2014;143(2):85-90.

19. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019 366:l4898. doi: 10.1136/bmj.l4898. PMID: 31462531.

20. Conde-Ruiz JI, Gonzalez CI. El proceso de envejecimiento en España. FEDEA, Estudios sobre la Economía Española, 2021; 2021-07, Madrid.

21. Salvar vidas, reducir el gasto: Una respuesta estratégica a las enfermedades no transmisibles. Ginebra (Suiza), Organización Mundial de la Salud, 2018 (WHO/NMH/NVI/18.8). Licencia: CC BY-NC-SA 3.0 IGO.

22. Autoridad Independiente de Reponsabilidad Fiscal. Evaluación del Gasto Público 2019. Gasto hospitalario del sistema nacional de salud: farmacia e inversión en bienes de equipo. Madrid, AIReF, 2020. [Internet]. [citado 12 de noviembre de 2024]. Disponible en: https://www.airef.es/wp-content/uploads/2020/10/SANIDAD/PDF-WEB-Gasto-hospitalario-del-SNS.pdf

23. Garjón Parra J. Evaluación y selección de medicamentos. Farm Aten Primaria. 2011;9(3):89-94.

ANEXO A Lista de verificación PRISMA-P 2015

Section/topic	#	Checklist item	Information reported		Reported on page #
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1[a]	Identify the report as a protocol of a systematic review			1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such			
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract			1
Authors					
Contact	3[a]	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author			
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review			
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments			
Support					
Sources	5[a]	Indicate sources of financial or other support for the review			16
Sponsor	5b	Provide name for the review funder and/or sponsor			16
Role of sponsor/ funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol			16
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known			8-10
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)			10, 26
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review			11, 26
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage			11, 27
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated			27

Section/topic	#	Checklist item	Information reported		Reported on page #
			Yes	No	
STUDY RECORDS					
Data management	11[a]	Describe the mechanism(s) that will be used to manage records and data throughout the review			13
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)			13
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators			13
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications			13
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale			12
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis			14
DATA					
Synthesis	15[a]	Describe criteria under which study data will be quantitatively synthesized			14
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)			15
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)			15
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned			
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)			
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)			16

Esta lista de verificación se ha adaptado para su uso con envíos de protocolos a revisiones sistemáticas de la Tabla 3 en Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Reviews 2015 4:1.

ANEXO 2

Tabla suplementaria S1. Criterios de inclusión según pregunta de investigación en formato PICO

Población	**Adultos con artritis psoriásica activa refractaria a tratamiento convencional. Subgrupos: Naive a tratamiento previo con iTNF vs. tratamiento previo con iTNF. Con/sin tratamiento concomitante con FAMEsc (ej. metotrexato).**		
Intervención	iTNF SC Adalimumab Golimumab Etanercept Certolizumabpegol iTNF IV Golimumab Infliximab iPDE-4 Apremilast	CTLA-4-Ig Abatacept iIL17 Ixekizumab Secukinumab Brodalumab ABT-122 (Remtolumab) iIL-17A/17F Bimekizumab	iIL-23 Guselkumab Risankizumab Tildrakizumab iIL-12/23 Ustekinumab iJAK Tofacitinib Upadacitinib Deucravacitinib
Comparador(es)	Placebo, FAMEb o FAMEsd.		
Resultados	EFICACIA (en semana 16 o próxima) Principal: ACR50. Secundarias: PASI75 ACR20 ACR70	SEGURIDAD EA EAG	
Diseño del estudio	Incluidos Ensayos clínicos aleatorizados controlados. Fase 2 o 3. Pacientes adultos diagnosticados de artritis psoriásica activa según los criterios CASPAR, tratados con FAMEb o FAMEsd. Uso de placebo, FAMEsc u otro FAMEb como comparador. Estudios que proporcionen resultados para, al menos, la variable principal de eficacia: tasa de mejora del 50 % del American College of Rheumatology (ACR50) en la semana 16 o próxima calculada en relación con el valor inicial o para ACR20 o ACR70. Sin restricción de idioma. Si el estudio incluía pacientes con psoriasis, se requirió que los resultados se presentasen claramente diferenciados por patología. Excluidos Estudios que incluyan únicamente a pacientes menores de 18 años. Investigaciones que no proporcionen datos disponibles para su extracción. Trabajos que no sean originales (cartas, editoriales, etc.). Resúmenes presentados en congresos sin información detallada disponible.		
Restricciones	Publicaciones originales, excluyendo resúmenes a congresos.		

ACR50: respuesta del American College of Rheumatology para una mejora del 50 %. EA: eventos adversos. EAG: eventos adversos graves. FAME: Fármacos anti-artríticos modificadores de la enfermedad. FAMEsc: FAME sintéticos convencionales. FAMEb: FAME biológicos. FAMEsd: FAME sintéticos dirigidos. PASI: índice de gravedad y área de la psoriasis. SC: subcutáneo. IV: intravenoso.

Tabla suplementaria S2. Detalles de la estrategia de búsqueda.

PubMed
('psoriatic arthritis'/exp OR 'psoriatic arthritis') AND (('Antirheumatic Agents' OR 'Biological Products' OR 'Biosimilar Pharmaceuticals' OR 'Tumor Necrosis Factor-alpha' OR 'interleukin 1 receptor antagonist protein' OR 'interleukin-17' OR 'Interleukin-23' OR 'Intekeukin-12/23' OR 'Janus Kinase Inhibitors' OR 'abatacept'/exp OR abatacept) OR ('adalimumab'/exp OR adalimumab) OR ('apremilast'/exp OR apremilast) OR ('bimekizumab'/exp OR bimekizumab) OR ('brepocitinib'/exp OR brepocitinib) OR ('brodalumab'/exp OR brodalumab) OR ('certolizumab'/exp OR certolizumab) OR ('deucravacitinib'/exp OR deucravacitinib) OR ('etanercept'/exp OR etanercept) OR ('filgotinib'/exp OR filgotinib) OR ('golimumab'/exp OR golimumab) OR ('guselkumab'/exp OR guselkumab) OR ('infliximab'/exp OR infliximab) OR ('ixekizumab'/exp OR ixekizumab) OR ('risankizumab'/exp OR risankizumab) OR ('secukinumab'/exp OR secukinumab) OR ('tildrakizumab'/exp OR tildrakizumab) OR ('tofacitinib'/exp OR tofacitinib) OR ('upadacitinib'/exp OR upadacitinib) OR ('ustekinumab'/exp OR ustekinumab)) AND 'psoriatic arthritis'/dm AND 'randomized controlled trial'/de AND ([adult]/lim OR [aged]/lim OR [middle aged]/lim OR [very elderly]/lim OR [young adult]/lim) AND ('article'/it OR 'article in press'/it)
EMBASE
('psoriatic arthritis'/exp OR 'psoriatic arthritis') AND (('Antirheumatic Agents' OR 'Biological Products' OR 'Biosimilar Pharmaceuticals' OR 'Tumor Necrosis Factor-alpha' OR 'interleukin 1 receptor antagonist protein' OR 'interleukin-17' OR 'Interleukin-23' OR 'Intekeukin-12/23' OR 'Janus Kinase Inhibitors') OR ('abatacept'/exp OR abatacept) OR ('adalimumab'/exp OR adalimumab) OR ('bimekizumab'/exp OR bimekizumab) OR ('brepocitinib'/exp OR brepocitinib) OR ('brodalumab'/exp OR brodalumab) OR ('certolizumab'/exp OR certolizumab) OR ('deucravacitinib'/exp OR deucravacitinib) OR ('etanercept'/exp OR etanercept) OR ('filgotinib'/exp OR filgotinib) OR ('golimumab'/exp OR golimumab) OR ('guselkumab'/exp OR guselkumab) OR ('infliximab'/exp OR infliximab) OR ('ixekizumab'/exp OR ixekizumab) OR ('risankizumab'/exp OR risankizumab) OR ('secukinumab'/exp OR secukinumab) OR ('tildrakizumab'/exp OR tildrakizumab) OR ('tofacitinib'/exp OR tofacitinib) OR ('upadacitinib'/exp OR upadacitinib) OR ('ustekinumab'/exp OR ustekinumab)) AND 'psoriatic arthritis'/dm AND 'randomized controlled trial'/de AND ([adult]/lim OR [aged]/lim OR [middle aged]/lim OR [very elderly]/lim OR [young adult]/lim) AND ('article'/it OR 'article in press'/it OR 'review'/it)
COCHRANE LIBRARY
IDSearch #1. MeSH descriptor: [Adult] explode all trees #2. MeSH descriptor: [Arthritis, Psoriatic] explode all trees #3. MeSH descriptor: [Antirheumatic Agents] explode all trees #4. MeSH descriptor: [Biological Products] explode all trees #5. MeSH descriptor: [Biosimilar Pharmaceuticals] explode all trees #6. MeSH descriptor: [Tumor Necrosis Factor-alpha] explode all trees #7. MeSH descriptor: [Interleukin 1 Receptor Antagonist Protein] explode all trees #8. MeSH descriptor: [Interleukin-17] explode all trees #9. MeSH descriptor: [Interleukin-23] explode all trees #10. MeSH descriptor: [Interleukin-12] explode all trees #11. MeSH descriptor: [Interleukin-23] explode all trees #12. MeSH descriptor: [Janus Kinase Inhibitors] explode all trees #13. #1 AND #2 AND (#3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12) in Trials
CLINICALTRIALS
Criterios básicos: Contiene todos estos términos: "Psoriatic arthritis".

Ars Pharmaceutica
https://revistaseug.ugr.es/index.php/ars
E-ISSN: 2340-9894 ISSN: 0004-2927

doi: 10.30827/ars.v66i3.32081
Artículos de revisión

Caloric Restriction Mimetics: A comparative analysis of natural and synthetic agents in autophagy modulation

Miméticos de la Restricción Energética: Un Análisis Comparativo de Agentes Naturales y Sintéticos en la Modulación de la Autofagia

Antonio Fernando Murillo-Cancho[1] 0000-0002-2436-3856
Bruno José Nievas-Soriano[1] 0000-0002-5917-7404
David Lozano-Paniagua[1] 0000-0002-1046-6483

[1]Universidad de Almería, Facultad de Ciencias de la Salud, Departamento de Enfermería, Fisioterapia y Medicina, Almería, España.

Correspondencia

David Lozano-Paniagua
dlozano@ual.es

Recibido: 03.12.2024
Aceptado: 23.03.2025
Publicado: 20.06.2025

Financiación

Sin financiación

Conflicto de intereses

Ninguno

Otras declaraciones

Todos los autores son responsables de la investigación informada, y todos, han participado en el concepto y diseño, análisis e interpretación de los datos, redacción o revisión del manuscrito, y han aprobado el manuscrito tal como fue presentado. Asimismo, revisaron críticamente el trabajo, aprobaron su versión final y están de acuerdo con su publicación en Ars Pharmaceutica.

Resumen

Introducción: La restricción calórica (CR) ha demostrado ser efectiva en prolongar la longevidad y prevenir enfermedades relacionadas con la edad. Sin embargo, su implementación en humanos presenta desafíos como efectos adversos y baja adherencia. Los miméticos de la restricción calórica (CRM) surgen como alternativas prometedoras, replicando los beneficios de la CR mediante la modulación de vías metabólicas clave como AMPK, mTOR y SIRT1.

Método: Se realizó una revisión narrativa basada en estudios desde 2004 hasta octubre de 2024, utilizando términos clave relacionados con la CR y CRM. La selección incluyó estudios en modelos animales y humanos, evaluando agentes naturales como el resveratrol y la espermidina, y sintéticos como la metformina y la rapamicina.

Resultados: Los CRM naturales, como el resveratrol y la espermidina, promueven la autofagia a través de la activación de SIRT1 y la regulación de mTOR, mejorando la homeostasis celular y reduciendo la inflamación crónica. Los agentes sintéticos, como la metformina y la rapamicina, ofrecen una inducción más potente de la autofagia, aunque con riesgos asociados como inmunosupresión. Los estudios en animales muestran mejoras en longevidad y salud metabólica, mientras que en humanos, los beneficios requieren más validación.

Conclusiones: Los CRM representan una opción innovadora para prevenir enfermedades relacionadas con la edad y mejorar la calidad de vida. La combinación de agentes naturales y sintéticos podría optimizar su efectividad, pero son necesarios estudios longitudinales para garantizar su seguridad y aplicación clínica.

Palabras clave: Miméticos de la restricción calórica; autofagia; AMPK; mTOR, SIRT1; longevidad; envejecimiento.

Abstract

Introduction: Caloric restriction (CR) has been proven effective in extending longevity and preventing age-related diseases. However, its implementation in humans faces challenges, such as adverse effects and low adherence. Caloric restriction mimetics (CRMs) are emerging as promising alternatives, replicating the benefits of CR by modulating key metabolic pathways like AMPK, mTOR, and SIRT1.

Method: A narrative review was conducted based on studies from 2004 2004 to October 2024 using key terms related to CR and CRMs. The selection included studies on animal models and humans, evaluating natural agents such as resveratrol and spermidine, and synthetic compounds like metformin and rapamycin.

Results: Natural CRMs, such as resveratrol and spermidine, promote autophagy through SIRT1 activation and mTOR regulation, improving cellular homeostasis and reducing chronic inflammation. Synthetic agents, like metformin and rapamycin, induce more potent autophagy, though with associated risks such as immunosuppression. Animal studies show improvements in longevity and metabolic health, while human benefits require further validation.

Conclusions: CRMs represent an innovative option to prevent age-related diseases and enhance quality of life. Combining natural and synthetic agents could optimize their effectiveness, but longitudinal studies are essential to ensure their safety and clinical applicability.

Keywords: Caloric restriction mimetics; autophagy; AMPK; mTOR; SIRT1; longevity; aging.

Highlights

Caloric restriction is an effective intervention to extend longevity and reduce age-related diseases. However, its adherence is challenging, prompting the development of mimetics that replicate its benefits without reducing caloric intake.

This study compares natural and synthetic agents, analyzing their effectiveness in inducing autophagy and their potential to prevent age-related diseases in human and animal models.

Caloric restriction mimetics could revolutionize the prevention of age-related diseases, enhancing quality of life through personalized and sustainable strategies in anti-aging medicine.

Introduction

Caloric restriction (CR) is one of the most effective interventions to extend lifespan and improve health in animal models[1]. It optimizes metabolic functions, reduces cumulative cellular damage, and preserves homeostasis through reduced energy intake[2,3]. Aging, characterized by the progressive deterioration of cellular function and loss of homeostasis, increases susceptibility to chronic diseases such as type 2 diabetes, cardiovascular diseases, cancer, and neurodegeneration[4,5]. CR delays the onset of these pathologies by modulating metabolic pathways related to cellular stress and inflammation[3,6].

During CR, AMPK activation and mTOR inhibition enhance autophagy, a critical process for the elimination of damaged proteins and organelles[7,8]. This contributes to energy efficiency, reduces oxidative stress, and mitigates chronic inflammation, which are key factors in aging and its associated diseases[6-9]. In animal models, CR has been shown to increase lifespan by 30-50 %, an effect attributed to improvements in mitochondrial efficiency, immune function, and genetic expression related to longevity, such as the activation of sirtuins (SIRT1) and the preservation of telomeres[1,3,8-12].

However, adherence to CR in humans faces significant challenges, such as psychological and social barriers. This has driven the search for alternatives that emulate its benefits without its limitations or adverse effects, such as sarcopenia and reduced bone density in older adults[10,16]. Natural and synthetic caloric restriction mimetics (CRM) have emerged as promising solutions in this regard[13-16].

Mechanisms of anti-aging action: Autophagy

Autophagy is a key process for degrading and recycling dysfunctional cellular components through the lysosomal system[17-19]. This mechanism is essential for maintaining cellular homeostasis, as it removes misfolded proteins and damaged organelles that accumulate over time[20,21]. However, with aging, the efficiency of autophagy declines, contributing to cellular deterioration and the development of chronic diseases such as diabetes, cardiovascular diseases, and neurodegenerative conditions[6,9,13,22].

The induction of autophagy is associated with increased longevity in organisms such as yeast, nematodes, and mammals, improving the clearance of toxic proteins, oxidative stress, and metabolic efficiency[3,16,23]. This process is regulated by AMPK and mTOR, whose complementary roles control autophagy activation in response to cellular energy conditions[7,8]. Additionally, sirtuins, such as SIRT1, also play an important regulatory role[8,11].

Autophagy dysfunction is linked to chronic diseases such as Alzheimer's, where toxic proteins accumulate, and sarcopenia, which affects muscle mass in older adults[22-24]. Promoting autophagy through CR or CRMs has shown significant improvements in cellular function and longevity[5,21]. Natural compounds have demonstrated the ability to induce autophagy, reduce inflammation, and maintain cellular homeostasis[13,25]

Given its central role in aging and related diseases, there is growing interest in developing therapies to optimize autophagic activity in older individuals[3,26,27]. However, modulating autophagy requires caution, as excessive activation can trigger apoptosis in healthy cells[28]. CRMs represent a promising alternative for this purpose, and their analysis will be the focus of this study.

The role of Caloric Restriction Mimetics (CRMs)Caloric Restriction Mimetics (CRMs)

The concept of CRMs has gained relevance due to the demonstrated benefits of CR in longevity and the reduction of age-related diseases[1,3]. Functionally, CRMs aim to replicate the positive effects of CR without reducing energy intake, making them a viable alternative for individuals who struggle to adhere to prolonged CR[3,5]. They represent a promising preventive and therapeutic option with a more accessible clinical application.

CRMs act by modulating key metabolic pathways, such as activating AMPK and inhibiting mTOR, which are fundamental mechanisms underlying CR benefits[8]. AMPK stimulates autophagy, enhances mitochondrial efficiency, and improves insulin sensitivity, while mTOR inhibition reduces protein synthesis and cell growth, promoting longevity and resilience to cellular stress[22].

CRMs also increase NAD+ levels, activating sirtuins that are essential for autophagy regulation and protection against oxidative damage[8,11]. This promotes cellular longevity by maintaining homeostasis and reducing chronic inflammation[3,9].

Challenges and Justification

With these physiological effects known, attempts have been made to clinically apply some of these CRMs, as their activity induces a potential anti-aging effect. There is growing interest in CRM research and development due to the increasing need to address population aging and the associated chronic diseases, which pose a significant burden on healthcare systems[25]. In a context of rising life expectancy, the primary challenge is not only to extend lifespan but also to improve its quality by reducing the incidence of aging-related diseases[3,9].

Although CR has proven effective in delaying aging and chronic diseases, its long-term adherence is challenging, and it may result in undesirable side effects such as muscle loss and decreased bone density[10,16]. Therefore, CRMs offer a more practical and sustainable solution, replicating CR benefits without its drawbacks[3,15] Therefore, they could replace it in clinical practice.

This work analyzes both natural and synthetic agents, providing a comprehensive perspective that identifies effective therapeutic approaches to prevent and treat age-related diseases[27]. It also encourages further research into developing treatments based on the modulation of autophagy and cellular homeostasis[15,22]. In the context of high morbidity and mortality from chronic diseases, CRMs could transform medical approaches toward preventive strategies, reducing the socioeconomic burden of aging[6,9].

The primary objective of this study is to conduct a comparative analysis of CRM effects on autophagy modulation and their impact on molecular pathways involved in cellular aging as an alternative to classic CR. After examining the mechanisms of action of various CRMs, their efficacy in improving cellular longevity, reducing oxidative damage, and mitigating chronic inflammation—key factors in preventing age-related diseases—is evaluated[22].

The study also assesses limitations related to bioavailability and safety in clinical applications[15,25], exploring their therapeutic potential as viable alternatives to CR, with a focus on anti-aging interventions that improve quality of life[3].

Although there is currently extensive knowledge about CRM substances, especially those of natural origin (14), this study has been limited to spermidine and resveratrol as they are the most well-known, abundant in foods, and likely possess the most versatile mechanistic profile, making them ideal candidates for evaluation.

Similarly, metformin and rapamycin were chosen as synthetic CRMs due to the extensive clinical experience with the former and the latter being a model compound for studying this mimetic activity.

The comparison between these two types of CRMs is appropriate since natural compounds are perceived as safe due to their dietary origin, although their bioavailability and clinical efficacy may be limited. On the other hand, synthetic CRMs are designed drugs that often show greater potency, although their use can be associated with side effects or toxicity at certain doses. Evaluating these differences will help identify safer and more effective options.

Additionally, a significant gap exists in the literature regarding how these two types of CRMs could complement each other or be used in combination to maximize clinical benefits. This integrated approach could transform strategies for healthy aging and open new avenues for personalized interventions based on individual metabolic needs.

Methods

This narrative review aims to analyze the molecular mechanisms and therapeutic applications of caloric restriction mimetics (CRMs) in longevity and healthy aging.

The bibliographic search for documents used in this narrative review employed MeSH terms and keywords such as “caloric restriction mimetics,” “spermidine,” “resveratrol,” “autophagy,” and “aging,” combined with Boolean operators (AND, OR) to retrieve the maximum number of relevant articles. The search strategy covered the period from 2004 to October 2024, focusing on studies explaining mechanisms, clinical applications, and effects on longevity. Articles were selected from PubMed/Medline, Scopus, and Google Scholar, including all studies ranging from laboratory and animal models to clinical trials written in English and Spanish. Priority was given to studies published in peer-reviewed journals, particularly those describing mechanisms of action, comparisons between natural and synthetic mimetics, and therapeutic applications in the context of aging.

After removing duplicates, the titles and abstracts of the retrieved studies were reviewed. Articles deemed irrelevant, with weak designs, or unrelated to CRMs, as well as conference abstracts, letters to the editor, viewpoints, and editorials, were excluded. For potentially relevant studies, full texts were reviewed before making the final selection. To describe and explain the similarities and differences between various CRMs, a descriptive comparative approach was used. This involved selecting elements of interest, analyzing similarities, and describing the selected elements in their respective contexts. The selected studies were critically analyzed in terms of methodological design, consistency of results, and recognized limitations.

Results

To understand the rationale for investigating and developing CRMs, it is essential to explore the mechanisms by which CR promotes longevity and improves metabolic health. At the cellular and molecular levels, limited energy availability triggers adaptations that enhance homeostasis and stress resistance, reducing the risk of chronic diseases associated with aging [1,3]. These adaptations are mediated by the regulation of key metabolic pathways, including AMPK activation, mTOR inhibition, and sirtuin modulation[7,8].

AMPK activation, mTOR inhibition

This pathway plays a central role in energy metabolism and cellular aging. AMPK acts as an energy sensor, activated under energy-deprived conditions such as CR, fasting, or intense exercise[1,7]. By detecting low ATP levels, AMPK promotes cellular responses that increase energy production and decrease consumption, supporting cell survival under stress[7,8].

AMPK activation stimulates fatty acid oxidation, enhances glucose uptake, and drives autophagy—a process essential for clearing damaged proteins and dysfunctional organelles[15,22]. This facilitates cellular homeostasis and prevents cumulative damage associated with aging[13,22].

In contrast, mTOR regulates cell growth in response to nutrients and energy availability. Under abundant conditions, mTOR activation stimulates protein synthesis and cell proliferation while suppressing autophagy[8,21]. AMPK activation inhibits mTOR, promoting autophagy, recycling damaged cellular components, and improving cellular function[7,8]. This also reduces low-grade chronic inflammation, a key factor in aging and the progression of chronic diseases[6,9].

Beyond regulating autophagy, AMPK/mTOR modulation enhances cellular adaptation to stress, such as oxidative damage[15,16]. The interaction between these pathways is crucial for maintaining cellular quality and preventing premature aging[13,22].

Sirtuin modulation

Sirtuins, particularly SIRT1, are pivotal for longevity and cellular homeostasis. These NAD^+-dependent enzymes regulate processes including autophagy, stress responses, and inflammation[8,11]. During CR, increased NAD^+ levels activate SIRT1, which deacetylates specific proteins to enhance the formation and function of autophagosomes[13,22].

SIRT1 also deacetylates FOXO3, a transcription factor that upregulates genes involved in antioxidant defense and autophagy[8,11], and modulates p53 activity, reducing cellular senescence and promoting cellular longevity[11,22]. Furthermore, it activates PGC-1α, enhancing mitochondrial biogenesis and the capacity to handle oxidative stress[8,15,16]. Lastly, SIRT1 deacetylates NF-κB, reducing systemic inflammation[6]. These mechanisms collectively contribute to a healthy cellular environment, extending organismal lifespan[9,13].

Additional Processes Modulated by CR

CR influences diverse biological processes beyond mere caloric intake reduction. It triggers molecular and cellular adaptations that enhance metabolic health, reduce oxidative stress, lower inflammation, and optimize mitochondrial metabolism[1,3].

• Oxidative Stress Reduction: One of CR's most significant effects is the reduction of reactive oxygen species (ROS) production, which damages DNA, proteins, and lipids, contributing to cellular decline and chronic diseases[6,16]. CR increases the expression of antioxidant enzymes, such as superoxide dismutase and catalase, which neutralize ROS and protect against oxidative damage[15].

• Nrf2 Pathway Activation: CR activates Nrf2, a master regulator of antioxidant responses, increasing the expression of detoxifying and antioxidant genes. This maintains cellular redox balance and protects against oxidative stress, particularly important in preventing neurodegenerative and cardiovascular diseases[6,13].

• Chronic Inflammation Reduction: CR decreases pro-inflammatory cytokines such as IL-6, TNF-α, and C-Reactive Protein[6,9] and reduces NF-κB activation, a key mediator of chronic inflammation. Additionally, it promotes the clearance of senescent cells, which are a major source of inflammatory mediators[9,12].

• Mitochondrial Function Enhancement: With age, mitochondria deteriorate, reducing energy production capacity and increasing ROS generation. CR stimulates mitochondrial biogenesis, a process regulated by PGC-1α, enhancing ATP production efficiency and enabling mitochondria to better manage oxidative stress[15,16].

• Metabolic Shift: CR promotes a metabolic shift towards greater reliance on fatty acid oxidation and ketogenesis, improving energy efficiency and reducing lipid accumulation. This positively impacts insulin sensitivity and lowers the risk of metabolic diseases like type 2 diabetes[5,8,15]. CR also elevates NAD^+ levels, facilitating DNA repair, autophagy, and mitochondrial biogenesis[8,11].

• The mechanisms activated by CR provide pathways to prevent age-related chronic diseases and offer a promising foundation for developing therapeutic strategies in anti-aging medicine[3,27]. In this context, CRMs, by targeting some or all these biological mechanisms, emerge as potential therapeutic alternatives with practical applicability.

Natural Caloric Restriction Mimetics

Resveratrol

Resveratrol is a natural polyphenol found in foods such as the skin of red grapes, blueberries, blackberries, and peanuts[13,25]. It has drawn researchers' attention due to its antioxidant, anti-inflammatory, and cardioprotective properties[5,13].

It exhibits excellent ability to emulate some benefits of CR, making it an attractive option, particularly because it can stimulate autophagy through the following mechanisms:

• It increases NAD^+ levels, facilitating the activation of SIRT1, promoting the deacetylation of proteins that regulate autophagy[13,25], and inhibiting mTOR[8,15], encouraging cellular maintenance and recycling processes rather than growth. This helps eliminate damaged proteins and dysfunctional organelles, preventing the accumulation of cellular damage associated with aging[8,21].

• It activates the Nrf2 pathway, strengthening the antioxidant response by increasing the expression of enzymes like SOD, catalase, and glutathione peroxidase, reducing oxidative stress and inflammation, and protecting cells from ROS-induced damage[6,15]. This improves cellular health and contributes to better quality of life by reducing factors associated with the development of aging-related diseases.

Studies in animal models have shown that resveratrol improves mitochondrial function, reduces inflammation, and protects against metabolic diseases such as obesity and insulin resistance[8,13]. In mice fed a high-fat diet, it improved insulin sensitivity and reduced liver fat accumulation, demonstrating a protective effect against metabolic syndrome[13,15].

Preliminary clinical studies in humans indicate that it may improve endothelial function, reduce inflammation, and increase insulin sensitivity in patients with type 2 diabetes[5,15]. Although further research is needed to confirm these effects in larger populations over the long term, these findings suggest significant potential as a therapeutic agent to promote healthy aging and prevent associated chronic diseases[3,27].

Spermidine

Spermidine is a natural polyamine present in foods such as wheat germ, soy, mushrooms, and fermented products[5,25]. Polyamines are essential for cell proliferation, DNA stabilization, and the regulation of multiple biological processes, including autophagy and proteostasis[5,15].

Dietary intake of spermidine stimulates its action at the intestinal level; however, endogenous levels decrease with age, which has been associated with increased cellular deterioration and the development of aging-related diseases[13,25].

Its classification as a CRM is based on its actions through the following mechanisms:

• It inhibits acetyltransferases, promoting protein deacetylation, an essential step for autophagy activation, which facilitates the formation of autophagosomes and the degradation of damaged proteins and dysfunctional organelles[15,25].

• It regulates the mTOR pathway, reducing protein synthesis and favoring metabolic efficiency, like other CRMs[5,15].

• It contributes to maintaining protein stability, essential for preventing the accumulation of misfolded proteins associated with aging and neurodegenerative diseases[22,25]. It also improves mitochondrial function by reducing ROS production and promoting the elimination of dysfunctional mitochondria through mitophagy, thereby improving long-term cellular health[15,16].

The latest findings have suggested that spermidine is likely to promote an increase in telomerase activity[14], which strengthens its favorable status as an anti-aging agent.

In animal studies, supplementation has been shown to extend lifespan by inducing autophagy, reducing oxidative stress, and improving cognitive function[15,25].

In humans, higher intake of spermidine-rich foods is associated with lower cardiovascular mortality, better cognitive performance, and lower incidence of aging-related diseases, suggesting a positive impact on longevity and quality of life[5,13,15,25].

In the Table 1, resveratrol and spermidine are compared, considering their characteristics, mechanisms of action, biological effects, and available experimental evidence.

Table 1. Comparing the differences and similarities between resveratrol and spermidine. (Own elaboration).

Characteristic Resveratrol Spermidine
Origin Polyphenol found in grapes, red fruits, and peanuts Natural polyamine found in wheat germ, soy, mushrooms, and fermented foods
Main Mechanism of Action Activation of SIRT1 Inhibition of acetyltransferases
Activated Signaling Pathways Activation of SIRT1 and Nrf2 Inhibition of mTOR, stabilization of proteostasis
Impact on Autophagy Inhibits mTOR through SIRT1 activation, enhances autophagy Induces autophagy by inhibiting acetyltransferases
Antioxidant Effects Increases expression of antioxidant genes via the Nrf2 pathway Promotes antioxidant response and reduces oxidative stress
Reduction of Inflammation Inhibits NF-κB, reducing chronic inflammation Decreases inflammation by enhancing autophagy and removing damaged proteins
Mitochondrial Protection Improves mitochondrial function and reduces ROS production Optimizes mitochondrial function and promotes mitophagy
Evidence in Animal Models Extends lifespan and improves metabolic health in mice Prolongs lifespan in flies, nematodes, and mice; enhances cognitive function
Evidence in Humans Improves endothelial function and insulin sensitivity in type 2 diabetes patients Associated with lower cardiovascular mortality and better cognitive function in older adults
Bioavailability Limited, requires formulations to enhance absorption High bioavailability through dietary intake
Safety and Adverse Effects Well-tolerated in moderate doses, but bioavailability is a challenge Considered safe, associated with positive effects on cognition and cardiovascular health

Synthetic Caloric Restriction Mimetics

Metformin

Metformin is a widely used drug for the treatment of type 2 diabetes due to its ability to improve insulin sensitivity and reduce blood glucose levels[7]. It has pleiotropic effects that may influence aging and longevity[3]. Studies suggest that metformin could reduce the risk of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative conditions[5,6].

It has a well-established safety profile, making it an attractive option for use in older adults to improve metabolic health and potentially extend longevity[5,27].

Its potential as a CRM lies in its ability to activate AMPK, which leads to mTOR inhibition[7]. This promotes autophagy, reduces oxidative stress, and mitigates metabolic dysfunction—key factors in aging[15,22].

Metformin also activates the SKN-1/Nrf2 pathway, which regulates genes that increase the expression of antioxidant and detoxifying enzymes[6], protecting cells from oxidative damage and reducing chronic inflammation[6,13].

In animal models, metformin prolongs lifespan, improves mitochondrial function, and reduces systemic inflammation[5,22].

In humans, observational studies indicate that diabetic patients treated with metformin have a lower incidence of age-related diseases and greater life expectancy compared to patients treated with other antidiabetic drugs[5,6]. One study found that diabetic patients taking metformin had a longer life expectancy than even non-diabetic individuals not treated with metformin[3,5].

Ongoing clinical trials are investigating its potential to extend lifespan in non-diabetic older adults, exploring its impact on the prevention of aging-related diseases[5,27].

Rapamycin

Also known as sirolimus, rapamycin is a macrolide isolated from a soil bacterium found on Easter Island. Originally developed as an immunosuppressant to prevent organ transplant rejection, it has shown potential as an anti-aging agent due to its ability to extend lifespan in animal models[15,21].

Its potential activity as a CRM focuses on the inhibition of mTORC1, which promotes autophagy independently of sirtuins[5,8]. This process suppresses protein synthesis, redirecting cellular resources toward repair and maintenance, facilitating the clearance of damaged proteins and dysfunctional organelles[5,21].

Additionally, it modulates inflammatory pathways associated with aging and chronic diseases[5,6]. It reduces NF-κB activity, decreasing systemic inflammation[6]. Its capacity to inhibit mTOR also contributes to regulating cellular apoptosis, limiting tissue damage associated with aging[5,8].

Animal studies show that rapamycin extends lifespan, improves insulin sensitivity and metabolic health, and reduces the incidence of tumors and neurodegenerative diseases[15,21].

In humans, preclinical trials suggest similar benefits, although its use requires further investigation to evaluate long-term safety due to its immunosuppressive effects[15,27].

A detailed comparison of the characteristics, mechanisms of action, and experimental evidence between metformin and rapamycin is presented in Table 2.

Table 2. Comparing the differences and similarities between metformin and rapamycin. (Own elaboration)

Characteristics **Metformin** **Rapamycin**
Clinical Use Antidiabetic drug used to treat type 2 diabetes Immunosuppressant used to prevent organ transplant rejection
Primary Mechanism of Action Activation of AMPK Inhibition of mTOR
Signaling Pathways Targeted Activation of AMPK, Inhibition of mTOR Direct inhibition of mTORC1
Impact on Autophagy Promotes autophagy by activating AMPK and inhibiting mTOR Strongly induces autophagy by inhibiting mTOR independently of SIRT1
Effects on Inflammation Reduces inflammation by activating Nrf2 and inhibiting NF-κB Reduces chronic inflammation and apoptosis

Characteristics Metformin Rapamycin
Mitochondrial Protection Improves mitochondrial function, reduces ROS Enhances mitophagy, reduces mitochondrial damage
Evidence in Animal Models Extends lifespan, improves metabolic health in mice Prolongs lifespan, reduces tumors, and protects against neurodegeneration
Evidence in Human Studies Associated with reduced incidence of age-related diseases in diabetics Currently under investigation for age-related health benefits
Potential Side Effects Generally safe, but may cause gastrointestinal issues Potential immunosuppressive effects, increased risk of infections

Discussion

The principal CRMs were compared, categorized into natural and synthetic agents, with a focus on their activity as autophagy modulators—considered the primary anti-aging mechanism promoted by CR—and their therapeutic utility for delaying aging and treating associated chronic diseases.

This analysis considered various characteristics to evaluate the different compounds reviewed:

1. Efficacy in Autophagy Modulation

Autophagy, as an essential cellular process for recycling and maintaining homeostasis, is critical in aging and chronic diseases[13,22]. Both natural and synthetic agents effectively induce autophagy, though they differ in mechanisms of action and efficacy.

Natural agents induce autophagy through distinct pathways. Resveratrol activates SIRT1 and Nrf2, enhancing the antioxidant response and reducing inflammation[8,13]. Spermidine inhibits acetyltransferases and modulates mTOR, promoting the clearance of damaged proteins and improving proteostasis[15,25].

Synthetic agents are potent autophagy inducers, acting through more specific and direct routes. Metformin activates AMPK, inhibits mTOR, and improves mitochondrial function[5,7]. Rapamycin directly inhibits mTORC1, triggering a powerful autophagic response to eliminate dysfunctional organelles and damaged proteins[15,21].

In terms of efficacy, synthetic agents are more potent in inducing autophagy due to their direct action on mTOR inhibition[21]. However, natural agents offer the advantage of activating multiple pathways, potentially providing additional benefits such as reducing oxidative stress and improving metabolic health[15,25].

2. Safety and Bioavailability

One of the main challenges in using these compounds, both natural and synthetic, is bioavailability and safety profiles, particularly for long-term application in humans.

Resveratrol, while safe at moderate doses, has low bioavailability due to rapid metabolism and elimination[13,15], limiting its clinical efficacy unless formulations improve absorption. Spermidine has higher bioavailability when ingested through diet and has been shown to be safe even in long-term human studies[12].

Metformin is well-tolerated and has significant usage experience in type 2 diabetes, though it may cause gastrointestinal side effects[3,5]. Rapamycin, despite its effectiveness, has a more complex adverse effect profile, including immunosuppression, which increases the risk of infections[15,21].

Overall, natural agents are safer and better tolerated for prolonged use, although their efficacy is limited by bioavailability challenges[15,13]. Synthetic agents, while more potent, require careful monitoring due to their potential side effects[5,15].

3. Clinical Applications and Therapeutic Potential

The therapeutic potential of CRMs in anti-aging is the focus of extensive research. Their clinical applicability depends on their ability to induce autophagy, safety, impact on longevity, and prevention of aging-related diseases.

Resveratrol and spermidine could be used as safe dietary supplements to enhance metabolic health and delay aging[13,25], potentially serving as a preventive strategy for older adults seeking to improve quality of life without resorting to more aggressive drugs[5,15].

Metformin has demonstrated reduced incidence of chronic diseases such as cancer and cardiovascular conditions in diabetic patients[3,5]. Ongoing trials are evaluating its impact on longevity in non-diabetic individuals.

Rapamycin, still in experimental stages due to its immunosuppressive effects, is less applicable for healthy individuals[5,21] although it has been suggested that this could be improved through an intermittent dosing regimen[14].

Natural compounds could be more easily integrated as dietary supplements due to their safety profile[13,25], while synthetic agents may have more restricted applications, targeting specific populations at high risk of aging-related diseases[5,15]. In the future, one of these substances might be routinely used in anti-aging medicine following clinical research to establish its safety.

Advantages and Limitations of CRMs

CRMs mimic the benefits of CR without its challenges. However, both natural and synthetic agents present advantages and limitations.

Natural Agents:

These stand out for their safety and dietary availability[13,25]. They improve mitochondrial function, reduce inflammation, and activate multiple pathways such as SIRT1 and Nrf2, promoting cellular protection[6,15]. However, they face bioavailability issues[13,15], and further studies are needed to establish optimal doses and long-term effects in humans[25].

Synthetic Agents:

These are highly effective. Metformin is safe but can cause gastrointestinal issues and, in some cases, reduced vitamin B12 absorption[29]. Rapamycin, while effective in promoting autophagy and improving metabolic health, has immunosuppressive effects that increase infection risk[15,21], making it inadvisable for routine use.

Regarding the research conducted, it presents certain limitations that deserve discussion. Firstly, the review predominantly relies on preclinical studies conducted in animals, which poses a challenge for extrapolation to humans. While animal models provide valuable insights into molecular mechanisms, their physiological differences limit the generalizability of the findings. Furthermore, variability in the experimental protocols used in the studies complicates direct comparisons of the results.

Another significant limitation is the lack of longitudinal studies in humans evaluating the long-term impact of CRMs. Most of the reviewed studies are characterized by small sample sizes and limited follow-up periods, which restrict the ability to assess prolonged effects. The available data on potential adverse effects are insufficient to establish a solid safety profile.

Lastly, a lack of integration between experimental and clinical approaches was identified. While preclinical studies emphasize molecular mechanisms, clinical trials rarely measure the same biomarkers, creating a gap in translational interpretation

Challenges and Future Directions in Research

Despite advances in CRM research, significant challenges remain for their clinical implementation as anti-aging interventions. The lack of longitudinal studies in humans confirming their long-term effects on longevity and health is a critical obstacle.

Limited bioavailability of natural agents is another significant challenge[15,25]. Enhancing their absorption and stability through advanced formulations or pharmaceutical delivery technologies is crucial[13,15].

Dosage optimization to maximize benefits without adverse effects is essential, especially for compounds with complex safety profiles. Controlled clinical trials are indispensable for establishing safe and effective long-term parameters with larger population samples that evaluate both the benefits and risks, with a particular focus on key biomarkers, and adopting comparative designs that analyze combinations of natural and synthetic CRMs, assessing possible synergies between them.

CRMs represent an emerging paradigm in anti-aging medicine, allowing intervention in the underlying biological processes driving aging and associated diseases. If optimized, they could significantly impact the prevention of chronic pathologies such as cardiovascular diseases, cancer, and neurodegenerative conditions[6,9], which have a significant incidence in populations over 50, posing a substantial economic burden on healthcare systems.

Metformin could be repurposed as a preventative treatment to reduce the risk of age-related diseases in healthy people. In the case of rapamycin, further studies are required for its safe use, as well as trials to determine whether its intermittent administration could mitigate undesired effects[14], and it could be a valuable option to prevent mitochondrial dysfunction and chronic inflammation that are a driving force behind health problems associated with aging.

The therapeutic combination of natural and synthetic compounds could offer a synergistic approach, maximizing benefits and minimizing risks[13,25]. Personalized strategies based on genetic and metabolic profiles could transform anti-aging medicine into a more effective and individually tailored approach. With adequate research, CRMs have the potential to revolutionize chronic disease prevention, improve health, and extend longevity.

Conclusions

Research on CRMs has shown that both natural and synthetic agents are promising tools for inducing autophagy and promoting longevity. These compounds act by modulating key molecular pathways such as AMPK, mTOR, and SIRT1, improving metabolic parameters, reducing chronic inflammation, preventing aging-related diseases, and potentially extending lifespan.

Aging is one of the primary risk factors for developing chronic metabolic diseases, cardiovascular diseases, cancer, and neurodegenerative conditions. CRMs offer an innovative approach to address these pathologies by targeting the cellular mechanisms driving aging.

By inducing autophagy and reducing cumulative cellular damage, they can slow the aging process and extend healthy life. This implies not only living longer but also with better quality of life, reducing reliance on costly medical treatments and improving overall well-being.

Optimizing bioavailability by improving the absorption and stability of natural compounds and their dosing is essential to expand their clinical effectiveness. Developing new formulations and delivery systems could make these compounds more accessible and effective.

The effective combination of natural and synthetic agents to maximize benefits without increasing the risk of side effects represents a new approach, potentially including personalization based on individual genetic and metabolic profiles.

References

1. Anson RM, Jones B, de Cabo R. The diet-mimetic effect of intermittent fasting on slow-aging processes. Cell Metab. 2005;2(1):25-35. Doi: 10.1016/j.cmet.2005.06.001.

2. Speakman JR, Mitchell SE. Caloric restriction. Mol Aspects Med. 2011;32(3):159-221. doi: 10.1016/j.mam.2011.07.001.

3. López-Otín C, Galluzzi L, Freije JM, Madeo F, Kroemer G. Metabolic control of longevity. Cell. 2016;166(4):802-821. Doi: 10.1016/j.cell.2016.07.031.

4. Fontana L, Partridge L, Longo VD. Extending healthy lifespan—from yeast to humans. Science. 2010;328(5976):321-326. Doi: 10.1126/science.1172539.

5. Martel J, Chang SH, Wu CY, Peng HH, Hwang TL, Ko YF, Young JD, Ojcius DM. Recent advances in the field of caloric restriction mimetics and anti-aging molecules. Ageing Res Rev. 2021;66:101240. Doi: 10.1016/j.arr.2020.101240.

6. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol. 2018;14(10):576-590. Doi: 10.1038/s41574-018-0059-4.

7. Hardie DG. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev. 2011;25(18):1895-1908. Doi: 10.1101/gad.17420111.

8. Madeo F, Zimmermann A, Maiuri MC, Kroemer G. Essential role for autophagy in lifespan extension. J Clin Invest. 2015;125(1):85-93. Doi: 10.1172/JCI73946.

9. Madeo F, Carmona-Gutierrez D, Hofer SJ, Kroemer G. Caloric restriction mimetics against age-associated disease: targets, mechanisms, and therapeutic potential. Cell Metab. 2019;29(3):592-610. Doi: 10.1016/j.cmet.2019.01.001.

10. Speakman JR, Hambly C. Starving for life: what animal studies can and cannot tell us about the use of caloric restriction to prolong human lifespan. J Nutr. 2007;137(4):1078-1086. Doi: 10.1093/jn/137.4.1078.

11. Imai S, Guarente L. NAD+ and sirtuins in aging and disease. Trends Cell Biol. 2014;24(8):464-471. Doi: 10.1016/j.tcb.2014.04.002.

12. Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol. 2010;5:253-295. Doi: 10.1146/annurev.pathol.4.110807.092250.

13. Madeo F, Eisenberg T, Pietrocola F, Kroemer G. Spermidine in health and disease. Science. 2018;359(6374). Doi: 10.1126/science.aan2788.

14. Nassar K, El-Mekawey D, Elmasry AE, Refaey MS, El-Sayed Ghoneim M, Elshaier YAMM. The significance of caloric restriction mimetics as anti-aging drugs. Biochem Biophys Res Commun. 2024;692:149354. Doi: 10.1016/j.bbrc.2023.149354

15. Yessenkyzy A, Saliev T, Zhanaliyeva M, Masoud AR, Umbayev B, Sergazy S, Krivykh E, Gulyayev A, Nurgozhin T. Polyphenols as Caloric-Restriction Mimetics and Autophagy Inducers in Aging Research. Nutrients. 2020;12(5):1344. Doi: 10.3390/nu12051344.

16. Longo VD, Panda S. Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. Cell Metab. 2016;23(6):1048-1059. Doi: 10.1016/j.cmet.2016.06.001.

17. Madeo F, Zimmermann A, Maiuri MC, Kroemer G. Essential role for autophagy in lifespan extension. J Clin Invest. 2015;125(1):85-93. Doi: 10.1172/JCI73946.

18. Pang L, Jiang X, Lian X, Chen J, Song EF, Jin LG, Xia ZY, Ma HC, Cai Y. Caloric restriction-mimetics for the reduction of heart failure risk in aging heart: with consideration of gender-related differences. Mil Med Res. 2022;9(1):33. Doi: 10.1186/s40779-022-00389-w

19. Gabandé-Rodríguez E, Gómez de Las Heras MM, Mittelbrunn M. Control of Inflammation by Calorie Restriction Mimetics: On the Crossroad of Autophagy and Mitochondria. Cells. 2019;9(1):82. Doi: 10.3390/cells9010082.

20. Rubinsztein DC, Marino G, Kroemer G. Autophagy and aging. Cell. 2011;146(5):682-695. Doi: 10.1016/j.cell.2011.07.030.

21. Selman C, Tullet JM, Wieser D, et al. Ribosomal protein S6 kinase 1 signaling regulates mammalian lifespan. Science. 2009;326(5949):140-144. Doi: 10.1126/science.1177221.

22. Sharma, A., & Singh, A. K. Molecular mechanism of caloric restriction mimetics-mediated neuroprotection of age-related neurodegenerative diseases: an emerging therapeutic approach. Biogerontology 2023;24(5): 679–708. Doi:10.1007/S10522-023-10045-Y

23. Cuervo AM, Wong E. Chaperone-mediated autophagy: roles in disease and aging. Cell Res. 2014;24(1):92-104. Doi: 10.1038/cr.2013.153.

24. Sandri M. Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome pathways. Nat Rev Mol Cell Biol. 2013;14(6):345-350. Doi: 10.1038/nrm3606.

25. Martin-Montalvo A, Mercken EM, Mitchell SJ, et al. Metformin improves healthspan and lifespan in mice. Nat Commun. 2013;4:2192. doi: 10.1038/ncomms3192.

26. Fontana L, Partridge L, Longo VD. Extending healthy life span—from yeast to humans. Science. 2010;328(5976):321-326. Doi: 10.1126/science.1172539.

27. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell. 2008;132(1):27-42. Doi: 10.1016/j.cell.2007.12.018.

28. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol. 2007;8(9):741-752. Doi: 10.1038/nrm2239.

29. Sánchez H, Masferrer D, Lera L, Arancibia E, Ángel B, Albala C. Vitamin B12 deficiency associated with high doses of metformin in diabetic older adults. Nutr Hosp. 2014;29(6):1394-400. Doi: 10.3305/nh.2014.29.6.7405.