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Artículos originales


Naringin: antitumor potential in silico and in vitro on bladder cancer cells

Naringina: potencial antitumoral in silico e in vitro en células cancerosas de vejiga

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Conflict of interest

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Resumen

Introducción: el carcinoma urotelial es un problema de salud pública importante. El carcinoma de células de transición es el subtipo más común y representa aproximadamente el 90 % de todos los cánceres de vejiga. Se han estudiado protocolos quimioterapéuticos, pero algunos presentan alta toxicidad y baja tolerabilidad. La naringina es un compuesto polifenólico que se encuentra principalmente en los cítricos, cuya actividad antitumoral se ha estudiado en varios tipos de cáncer. Sin embargo, hay poca información sobre los efectos de la naringina en el cáncer de vejiga. Este estudio tuvo como objetivo evaluar el potencial antitumoral de la naringina *in silico* e *in vitro* utilizando dos líneas celulares de cáncer de vejiga.

Método: el análisis *in silico* se llevó a cabo mediante el software PASS Online. *In vitro*, se evaluaron los efectos del tratamiento con naringina (12,5 - 400 μ M) en cuanto a su citotoxicidad, supervivencia clonogénica, alteraciones morfológicas, progresión del ciclo celular, migración y mutagenicidad.

Resultados: los análisis *in silico* predijeron la actividad antitumoral a través de varios mecanismos de acción. Los resultados *in vitro* mostraron que la naringina presentó efectos citotóxicos, redujo el número de colonias, inhibió la migración celular y cambió la morfología y la progresión del ciclo celular de las dos líneas celulares evaluadas. Sin embargo, la naringina no presentó efectos mutagénicos.

Conclusiones: la naringina tiene actividad antiproliferativa y es un candidato prometedor para el tratamiento del cáncer de vejiga.

Palabras clave: cáncer de vejiga; flavonoide; *in silico*; naringina; progresión del ciclo celular

Abstract

Introduction: Urothelial carcinoma is a significant public health problem. Transitional cell carcinoma (TCC) is the most common subtype, accounting for approximately 90 % of all bladder cancers. Chemotherapeutic protocols have been studied, but some present high toxicity and low tolerability. Naringin is a polyphenolic compound found mainly in citrus fruits, which antitumor activity has been studied in several types of cancer. However, there is little information about naringin effects on bladder cancer. This study aimed to evaluate the antitumor potential of naringin *in silico* and *in vitro* using two bladder cancer cell lines

Method: *In silico* analysis was carried out by PASS Online software. *In vitro*, the effects of naringin treatment (12.5 - 400 μ M) were evaluated regarding its cytotoxicity, clonogenic survival, morphological alterations, cell cycle progression, migration, and mutagenicity

Results: *In silico* analyses predicted antitumor activity through several mechanisms of action. *In vitro* results showed naringin presented cytotoxic effects, reduced the number of colonies, inhibited cell migration, and changed the morphology and cell cycle progression of the two cell lines evaluated. However, naringin did not present mutagenic effects.

Conclusions: Naringin has antiproliferative activity and is a promising candidate for bladder cancer treatment.

Keywords: bladder cancer; cell cycle progression; flavonoid; *in silico*; naringin

Highlights

Several studies have demonstrated the therapeutic potential of naringin against diabetes, allergies, asthma, osteoporosis, hypertension and cancer, as well as protection of gastrointestinal tract. However, the antineoplastic effects of naringin in bladder cancer have been poorly studied. Our results showed naringin presented cytotoxic effects, reduced the number of colonies and inhibited cell migration, demonstrating naringin could be a promising candidate for bladder cancer treatment.

Introduction

Worldwide, 0.573 million new cases and almost 0.212 million deaths due to bladder cancer were estimated in 2020⁽¹⁾. Urothelial carcinoma and transitional cell carcinoma (TCC) are the most common type and subtype of bladder cancer, respectively. About 75 % of urothelial carcinoma patients have tumors confined to the bladder mucosa or submucosa, which are usually treated with transurethral resection of the tumor and intravesical therapy⁽²⁾. On the other hand, for that 25 % of patients with muscle-invasive disease, more aggressive therapy is necessary, including partial or total cystectomy and systemic chemotherapy with methotrexate, vinblastine, doxorubicin, and cisplatin, protocol known as MVAC⁽³⁾.

The main difficulty found for cancer therapy is the toxicity accompanied by high failure rates, which justifies the search for potential natural products as a therapeutic alternative⁽⁴⁾. Because of its diverse chemical structure, bioactive natural products are capable of modulating the cancer microenvironment and diverse cell signaling pathways⁽⁵⁾.

Among natural compounds with pharmacological potential, naringin, a flavonoid found mainly in citrus fruits, has been gaining attention because of its anti-inflammatory, antioxidant, and antitumor properties⁽⁶⁾. There is strong evidence for the association between treatment with naringin and antitumor activity, through modulation of several signaling cascades involved in cell proliferation, autophagy, apoptosis, angiogenesis, metastasis, and invasion⁽⁷⁾. Despite data showing that naringin can induce antiproliferative effects in many types of cancer cells, there is only one study demonstrating the effects of naringin on bladder cancer cells⁽⁸⁾.

In this context, this study aimed to investigate the antineoplastic potential of the naringin *in silico* and *in vitro* on bladder cancer cells by exploring its effect on cell proliferation, morphology, migration, cell cycle progression, and its ability to induce mutagenic effects.

Methods

Quantitative structure–activity relationship (QSAR) analysis

The naringin structure (Figure 1) was subjected to quantitative structure–activity relationship (QSAR) analysis using the platform Prediction of Activity Spectra for Substances (PASS online) to predict its antitumor potential and possible mechanisms of action. The naringin is a disaccharide derivative that is (S)-naringenin substituted by a 2-O-(α -L-rhamnopyranosyl)- β -D-glucopyranosyl moiety at position 7 via a glycosidic linkage (PubChem CID 442428). The results are provided as a probability that the compound is active (Pa) and inactive (Pi). Fourteen antitumor effects and mechanisms of action related to the tumorigenesis process were analysed. The results were expressed by difference Pa-Pi and were classified as Pa-Pi < 0.2: low potential, Pa-Pi \geq 0.5: moderate potential and Pa-Pi \geq 0.5: high potential⁽⁹⁾.

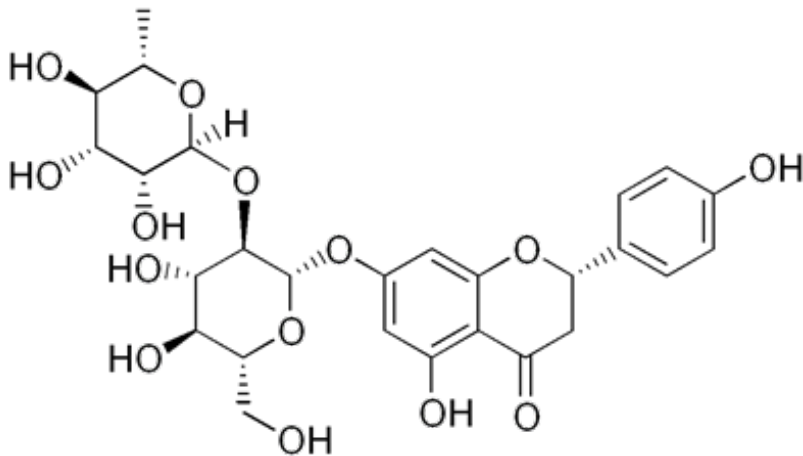


Figure 1. Naringin structure constructed using software ChemDraw.

Cell lines and naringin

The cell lines 5637 and T24 from urinary bladder tumors were kindly donated by Dr. Daisy Maria Fávero Salvadori from Universidade do Estado de São Paulo (UNESP), Brazil. The cell lines were maintained as described by Da Silva et al⁽¹⁰⁾. Naringin ($\geq 95\%$) was purchased from Sigma-Aldrich (Saint Louis, EUA) and was solubilized at $400 \mu\text{M}$ in culture medium with 2 % dimethylsulfoxide (DMSO) immediately before its use. Previous study of our research group has demonstrated that 2% DMSO does not alter the viability of 5637 and T24 cells⁽¹¹⁾.

Cytotoxicity

Cytotoxicity was assessed using the Cell Proliferation Kit II (XTT), from Roche Diagnostics (Manheim, Germany). Briefly, 1.0×10^4 cells/well were seeded in 96-well culture plates. After incubating for 24 hours, cells were treated with different concentrations of naringin (12.5, 25, 50, 100, 150, 200 and $400 \mu\text{M}$) for 24 hours. Untreated cells were cultured in parallel as a negative control. The culture medium was removed and cells were washed with Hank's solution (0.4 g KCl, 0.06 g KH_2PO_4 , 0.04 g Na_2HPO_4 , 0.35 g NaHCO_3 , 1 g glucose and 8 g NaCl in 1000 mL H_2O). XTT test solution was diluted in culture medium without phenol red and was added to each well. After 1 hour of incubation, the absorbance was measured using a microplate reader at the wavelength of 490 nm⁽¹²⁾. The absorbance results are proportional to the percentage of viable cells. Experiments were performed in technical triplicate.

Morphological analysis

To evaluate the effects of the treatment with naringin on morphology, 5637 and T24 cells were seeded at the density of 2.0×10^5 cells/well in 12-well culture plates and incubated for 24 hours. Afterwards, cells were treated with naringin (150 and $200 \mu\text{M}$) for 24 hours. Cells were observed and photographed using an inverted phase-contrast microscope at 200 x magnification⁽¹³⁾. Experiments were performed in technical triplicate.

Clonogenic assay

For evaluating the long-term effects of naringin, the cells were plated at a density of $1.0 \times 10^6/25 \text{ cm}^3$ culture flasks. After 24 hours, cells were treated with naringin (100, 150, 200, and $400 \mu\text{M}$) and were incubated for 24 hours. Subsequently, cultures were rinsed with Hanks' solution and the cells were removed using trypsin. Cells were plated into 12-well culture plate at 1.0×10^3 cells/well and allowed to grow for 10 days to form colonies. Afterwards, the cells were fixed with 10 % formaldehyde and stained

with 0.5 % crystal violet. Absorbance was measured using a microplate reader at 560 nm wavelength⁽¹¹⁾. Experiments were performed in technical triplicate.

Cell cycle analysis

To analyse the cell cycle progression after the treatment with naringin, cells were seeded at the density of 2.0×10^5 cells/well in 12-well culture plates and incubated for 24 hours. Cells were treated with naringin (100, 150, 200, and 400 μ M) for 24 hours. After the treatment, cells were washed with Hank's solution, trypsinized and centrifuged at 1,000 rpm for 10 minutes. The cell pellet formed was fixed with 70 % ethanol and maintained at -20 °C for 12 h. Subsequently, cells were washed with Hank's solution, centrifuged and resuspended in 200 μ L of labelling solution (0.0914 g of magnesium chloride; 0.0774 g of sodium citrate; 0.04766 g of Hepes; 10 μ L of Triton-X, 500 μ L of propidium iodide, and 9490 μ L of water). Cells were placed on ice and incubated protected from light for 30 minutes. The percentages of cells in the different cell cycle phases were measured using flow cytometry (BD FACSCalibur) and analysed using FlowJo® software⁽¹⁴⁾. Data from 30,000 cells were collected in each file. Experiments were performed in technical triplicate.

Wound healing assay

To assess the influence of the treatment with naringin on cell migration, the wound healing assay was performed. Cells were seeded at the density of 4.0×10^5 cells/well in 12-well culture plates. After 24 hours, the confluent cell monolayer was scraped with 200 μ L sterile pipette tip to create a wound. The wound was photographed using an inverted phase-contrast microscope at 40x magnification. Afterwards, cells were treated with naringin (100, 150, 200, and 400 μ M) for 24 hours. Finally, cells were again observed and photographed⁽¹⁵⁾. Cell migration was quantified through Image J® software by measuring the wound width in three random selected microscopic fields.

Cytokinesis-block micronucleus assay and nuclear division index (NDI)

The micronucleus assay with cytokinesis blockage was performed to assess the mutagenic potential of the treatment with naringin. Cells were plated at the density of 1.0×10^6 cell/plate in Petri dishes and incubated for 24 hours. Cells were treated with naringin (100, 150, 200, and 400 μ M) for 4 hours and with doxorubicin (0.4 g/ml) for 2 hours as a positive control. After the treatments, cells were washed with Hank's solution, cultured with 15 μ L of cytochalasin B (2 mg/mL) and incubated for more 24 hours. Afterwards, cells were trypsinized, hydrated with KCl 0.075 M and fixed on microscope slides with methanol and acetic acid. The microscope slides were then stained with 5 % Giemsa. One thousand binucleated cells were analyzed in each slide to determine the frequencies of micronucleated cells. Five hundred cells were analyzed in each slide to determine the NDI. The NDI was calculated as previously described by Fenech⁽¹⁶⁾: $NDI = M1 + 2 (M2) + 3 (M3) + 4 (M4) / N$, whereas M1-M4 is the number of cells with 1-4 nuclei and N is the total number of viable cells.

Statistical analysis

Statistical analyses were performed by GraphPad Prism® 6. The data were analysed by one-way ANOVA and *post hoc* analysis by Tukey's test. The results were considered statistically significant at $p < 0.05$.

Results

Quantitative structure–activity relationship (QSAR) analysis

The QSAR analysis showed that naringin presents high potential to act as anticarcinogenic, antineoplastic, and antimetastatic. The mechanisms of action with high potential are related to apoptosis (caspase 3 stimulant, caspase 8 stimulant, and apoptosis agonist), cytostatic effects and Myc inhibition. In addition, inhibition of DNA synthesis and expression inhibition of *RELA* and *MMP9* genes were predicted as moderate potential (Table 1).

Table 1. Quantitative Structure-Activity Relationship (QSAR) analysis for potential antitumor activities of naringin using the PASS online tool

Activities	Pa - Pi value
Anticarcinogenic	0.984
Caspase 3 stimulant	0.892
Antineoplastic	0.852
Cytostatic	0.805
Caspase 8 stimulant	0.727
Apoptosis agonist	0.713
Myc inhibitor	0.586
Antimetastatic	0.507
DNA synthesis inhibitor	0.479
<i>RELA</i> expression inhibitor	0.303
<i>MMP9</i> expression inhibitor	0.229
DNA repair enzyme inhibitor	0.114
P-glycoprotein inhibitor	0.102
DNA intercalator	0.039

Pa: potential to be active; Pi: potential to be inactive; Pa-Pi < 0,2: Low potential; Pa-Pi ≥ 0.5: Moderate potential; Pa-Pi ≥ 0.5: High potential

Cytotoxicity and cell morphology

The cytotoxicity results are shown in Figure 2-a. Significant reduction in cell viability was observed in 5637 cells after treatment with 100, 150, 200, and 400 μM. In T24 cells, significant reduction in cell viability was observed at 150, 200, and 400 μM of naringin.

The evaluation of cell morphological alterations caused by the treatment with naringin shows cellular debris and digitiform projections on both cell lines tested, as can be observed in Figure 2-b.

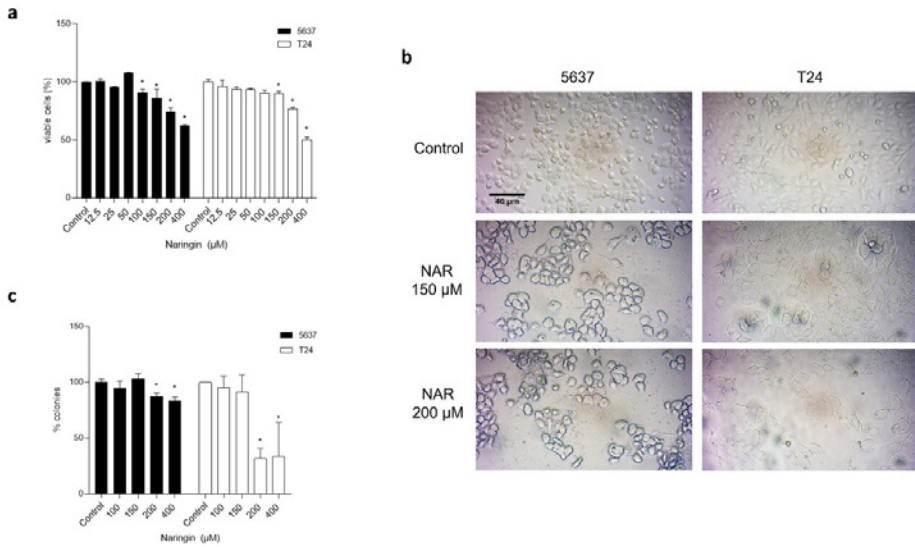


Figure 2. a) Percentages of viable 5637 and T24 cells after 24-h treatment with naringin. Control: untreated cells. * $p < 0.05$ compared with negative control. Each point represents the mean value obtained from three independent experiments. b) Photomicrographs of 5637 and T24 cells after 24-h treatment with naringin. Inverted phase-contrast optical microscope, 200x magnification. Control: untreated cells. NAR: naringin. c) Percentages of 5637 and T24 cell colonies after 24-h treatment with naringin. Control: untreated cells. * $p < 0.05$ compared with negative control. Each point represents the mean value obtained from three independent experiments.

Clonogenic survival

The results of clonogenic survival assay are presented on Figure 2-c. As can be observed, the long-term proliferation was changed after treatment with 200 µM and 400 µM for both cells.

Cell cycle progression

The results of the cell cycle analysis are shown in Figure 3. In 5637 cells, there was a significant increase in the number of cells in G0/G1 phase, followed by a significant reduction of cells in S and G2/M phases. In T24 cells, there was a significant increase in the number of cells in G2/M phase after treatment with the highest concentration of naringin.

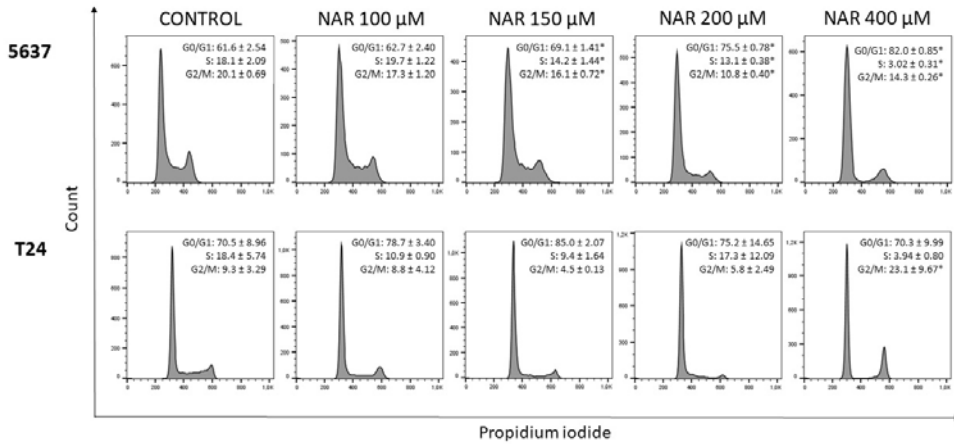


Figure 3. Quantification and representative histograms of cell cycle progression for 5637 and T24 cells after 24-h treatment with naringin. Control: untreated cells. * $p < 0.05$ compared with negative control. Each point represents the mean value obtained from three independent experiments.

Cell migration

The results of cell migration assay are presented in Figure 4. Naringin inhibited cell migration in both cell lines.

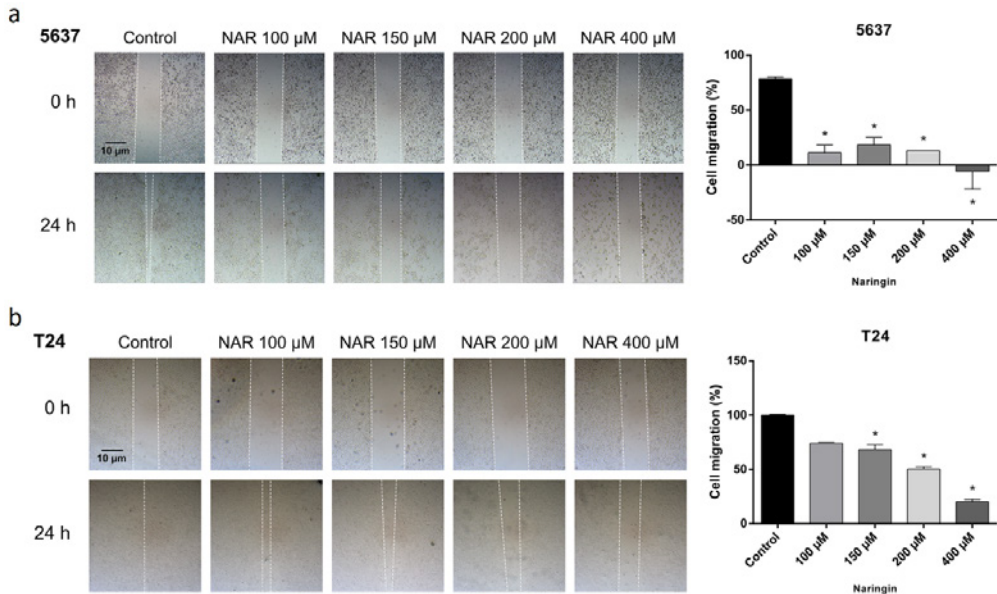


Figure 4. Photomicrographs and quantification of cellular migration of (A) 5637 and (B) T24 cells after 24-h treatment with naringin. Control: untreated cells. NAR: naringin. * $p < 0.05$ compared with negative control. Each point represents the mean value obtained from three independent experiments. Inverted phase-contrast optical microscope, 40x magnification.

Micronucleus assay and nuclear division index (NDI)

Naringin treatment did not cause an increase in the frequency of micronuclei (Table 2). In T24 cells, there was a significant decrease in NDI after treatment with naringin at the highest concentration (Table 3).

Table 2. Frequencies of micronucleated (%o) cells after treatment with naringin

	Control	Positive Control	Naringin 100 μ M	Naringin 150 μ M	Naringin 200 μ M	Naringin 400 μ M
5637	0.004 \pm 0.001	0.014 \pm 0.002*	0.003 \pm 0.002	0.004 \pm 0.002	0.004 \pm 0.001	0.005 \pm 0.001
T24	0.008 \pm 0.001	0.015 \pm 0.002*	0.006 \pm 0.001	0.009 \pm 0.001	0.008 \pm 0.002	0.006 \pm 0.001

Control: untreated control. Positive control: doxorubicin (0.4 μ g/mL). $p < 0.05$ compared to control.

Table 3. Nuclear division index (NDI) for the 5637 and T24 cells after the treatment with naringin

	Control	Naringin 100 μ M	Naringin 150 μ M	Naringin 200 μ M	Naringin 400 μ M
5637	1.721 \pm 0.014	1.705 \pm 0.031	1.697 \pm 0.036	1.707 \pm 0.018	1.668 \pm 0.010
T24	1.830 \pm 0.022	1.854 \pm 0.024	1.809 \pm 0,037	1.793 \pm 0.014	1.751 \pm 0.028*

Control: untreated control. $p < 0.05$ compared to control.

Discussion

We conducted this study aiming to explore the antitumor effects of naringin and its mechanism of action on bladder cells. The use of *in silico* studies are interesting tools for the drug discovery process, especially to understand more about poorly studied compounds in a fast and low-cost process⁽¹⁷⁾.

According to *in silico* analysis, the cell death caused by naringin in bladder cancer cells observed in the cytotoxicity assay might occur through apoptosis. In addition to the high probability to act as an apoptosis agonist, naringin also presents a high probability to stimulate caspase-8 and caspase-3. Caspase-8 is characterized as an enzyme that initiates the extrinsic apoptotic cell death pathway induced by the activation of death receptors⁽¹⁸⁾. Caspase-3 acts as an effector caspase, cleaving several substrates that cause the morphological and biochemical changes seen in apoptotic cells⁽¹⁹⁾. Indeed, in hepatocellular carcinoma cells, naringin treatment caused an increase in caspase-3 and caspase-8 activities⁽²⁰⁾. In addition, the morphological changes observed in T24 and 5637 cells are also associated with the apoptosis process^(21,22).

The *in silico* predicted moderate potential to inhibiting DNA synthesis was confirmed by clonogenic survival assay that determines the ability of a cell proliferates indefinitely, retaining its reproductive capacity after being exposed to a substance⁽²³⁾. Clonogenic survival the most appropriate endpoint for measuring cell death in tumor cell lines⁽²⁴⁾.

The naringin effect on cell cycle arrest corroborated the prediction of a high probability of naringin acting as a cytostatic agent. Several other studies also demonstrated the ability of naringin to modulate cell cycle progression^(25,26,27). Naringin effects on cell cycle seem to be related to modulation of the

complexes of cyclins, cyclin-dependent kinases, and cyclin-dependent kinases inhibitors⁽⁸⁾, in addition to inhibiting c-MYC expression^(28,29).

Also confirming the *in silico* prediction of antimetastatic effect, naringin inhibited cell migration in bladder cancer cells. Similar effects of naringin have been observed in other types of cancer^(30,31). Tan et al.⁽³²⁾ suggested that the anti-metastatic properties of naringin seem to be associated with the down-regulation of matrix metalloproteinases. Indeed, the *in silico* results suggested naringin ability to inhibit MMP9 expression, a matrix metalloproteinase with crucial role in cancer progression⁽³³⁾. Additionally, there is a moderate probability to naringin inhibit the *RELA* gene expression, which encodes the protein p65 that is upregulated in bladder cancer and is associated to cell migration⁽³⁴⁾.

Regarding to micronucleus assay, treatment with naringin was not capable of inducing mutagenicity to neither of the cell lines tested. In human lymphocytes, naringin also did not induce micronucleus formation⁽³⁵⁾, showing that this compound has not clastogenic or aneugenic potential.

In conclusion, our study corroborates the antiproliferative potential of naringin on bladder tumor cells and shows cytotoxicity, damage in the reproductive capacity, interference in the cell cycle progression, and alteration in cell migration by mechanisms unrelated to aneugenic or clastogenic activity. The *in silico* analysis showed that the mechanisms of action could be related to apoptosis (caspase 3 stimulant, caspase 8 stimulant, and apoptosis agonist) and Myc protein and *RELA* and *MMP9* gene inhibition.

References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. *Int J Cancer*. 2021;144(8):1941-1953. doi: 10.1002/ijc.31937
2. Sanli O, Dobruch J, Knowles MA, Burger M, Alemozaffar M, Nielsen ME, et al. Bladder cancer. *Nat Rev Dis Primers*. 2017;3(17022):1-19. doi: 10.1038/nrdp.2017.22
3. Chang SS, Bochner BH, Chou R, Dreicer R, Kamat AM, Lerner SP, et al. Treatment of non-metastatic muscle-invasive bladder cancer: AUA/ASCO/ASTRO/SUO Guideline. *J Urol*. 2017;198(3):552-559. doi: 10.1016/j.juro.2017.04.086
4. Lin SR, Chang CH, Hsu CF, Tsai MJ, Cheng H, Leong MK, et al. Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. *Br J Pharmacol*. 2020;177(6):1409-1423. doi: 10.1111/bph.14816
5. Dutta S, Mahalanobish S, Saha S, Ghosh S, Sil PC. Natural products: An upcoming therapeutic approach to cancer. *Food Chem Toxicol*. 2019;128:240-255. doi: 10.1016/j.fct.2019.04.012
6. Chen R, Qi QL, Wang MT, Li QY. Therapeutic potential of naringin: an overview. *Pharm Biol*. 2016;54(12):3203-3210. doi: 10.1080/13880209.2016.1216131
7. Ghanbari-Movahed M, Jackson G, Farzaei MH, Bishayee A. A systematic review of the preventive and therapeutic effects of naringin against human malignancies. *Front Pharmacol*. 2021;12:639840. doi: 10.3389/fphar.2021.639840
8. Kim DI, Lee SJ, Lee SB, Park K, Kim WJ, Moon SK. Requirement for Ras/Raf/ERK pathway in naringin-induced G1-cell-cycle arrest via p21WAF1 expression. *Carcinogenesis*. 2008;29(9):1701-1709. doi: 10.1016/j.pnrl.2017.11.001
9. Pereira GLDC, Almeida TC, Seibert JB, Amparo TR, Soares RDOA, Rodrigues IV, et al. Antitumor effect of *Cymbopogon densiflorus* (Linneus) essential oil in bladder cancer cells. *Nat Prod Res*. 2020; 2:1-5. doi: 10.1080/14786419.2020.1747453
10. Da Silva GN, de Castro Marcondes JP, de Camargo EA, Junior GASP, Sakamoto-Hojo, Salvadori DMF. Cell cycle arrest and apoptosis in TP53 subtypes of bladder carcinoma cell lines treated with cisplatin and gemcitabine. *Exp Biol Med* (Maywood). 2010;235(7):814-824. doi: 10.1258/ebm.2010.009322

- 11.** Lima APB, Almeida TC, Barros TMB, Rocha LCM, Garcia CCM, Da Silva GN. Toxicogenetic and antiproliferative effects of chrysin in urinary bladder cancer cells. *Mutagenesis*. 2020;1(5):1-11. doi: 10.1093/mutage/geaa021
- 12.** Sávio ALV, Da Silva GN, Salvadori DMF. Inhibition of bladder cancer cell proliferation by allyl isothiocyanate (mustard essential oil). *Mutat Res*. 2015;771:29-35. doi: 10.1016/j.mrfmmm.2014.11.004
- 13.** Da Silva GN, De Camargo EA, Salvadori DMF. Toxicogenomic activity of gemcitabine in two TP53-mutated bladder cancer cell lines: special focus on cell cycle-related genes. *Mol Biol Rep*. 2012;39(12):10373-10382. doi: 10.1007/s11033-012-1916-1
- 14.** Almeida TC, Guerra CCC, De Assis BLG, Soares RDOA, Garcia CCM, Lima AAL, et al. Antiproliferative and toxicogenomic effects of resveratrol in bladder cancer cells with different TP53 status. *Environ Mol Mutagen*. 2019;60(8):740-751. doi: 10.1002/em.22297
- 15.** Barros TMB, Lima APB, Almeida TC, Da Silva GN. Inhibition of urinary bladder cancer cell proliferation by silibinin. *Environ Mol Mutagen*. 2020;61(4):445-455. doi: 10.1002/em.22363
- 16.** Fenech M. The in vitro micronucleus technique. *Mutat Res*. 2000;455(1-2):81-95. doi: 10.1016/s0027-5107(00)00065-8
- 17.** Achary PGR. Applications of quantitative structure-activity relationships (QSAR) based virtual screening in drug design: A review. *Mini Rev Med Chem*. 2020;20(14):1375-1388. doi: 10.2174/1389557520666200429102334
- 18.** Elmore S. Apoptosis: A review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495-516. doi: 10.1080/01926230701320337
- 19.** Slee EA, Adrain C, Martin SJ. Executioner caspase-3, -6, and -7 perform distinct, non-redundant roles during the demolition phase of apoptosis. *J Biol Chem*. 2001;276:7320-7326. doi: 10.1074/jbc.M008363200
- 20.** Banjerdpongchai R, Wudtiwai B, Khawon P. Induction of human hepatocellular carcinoma HepG2 cell apoptosis by naringin. *Asian Pac J Cancer Prev*. 2016;17(7):3289-3294.
- 21.** Tsuboi A, Ohsawa S, Umetsu D, Sando Y, Kuranaga E, Igaki T, et al. Competition for space is controlled by apoptosis-induced change of local epithelial topology. *Curr Biol*. 2018;28(13):2115-2128. doi: 10.1016/j.cub.2018.05.029
- 22.** Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. *Biosci Rep*. 2019;39(1):1-17. doi: 10.1042/BSR20180992
- 23.** Munshi A, Hobbs M, Meyn RE. Clonogenic cell survival assay. *Methods Mol Med*. 2005;110:21-28. doi: 10.1385/1-59259-869-2:021
- 24.** Tannock IF, Lee C. Evidence against apoptosis as a major mechanism for reproductive cell death following treatment of cell lines with anti-cancer drugs. *Br J Cancer*. 2001;84:100-105. doi: 10.1054/bjoc.2000.1538
- 25.** Xu C, Huang X, Huang Y, Liu X, Wu M, Wang J, et al. Naringin induces apoptosis of gastric carcinoma cells via blocking the PI3K/AKT pathway and activating pro-death autophagy. *Mol Med Rep*. 2021;24(5):772. doi: 10.3892/mmr.2021.12412
- 26.** Lin R, Hu X, Chen S, Shi Q, Chen H. Naringin induces endoplasmic reticulum stress-mediated apoptosis, inhibits β -catenin pathway and arrests cell cycle in cervical cancer cells. *Acta Biochim Pol*. 2020;67(2):181-188. doi: 10.18388/abp.2020_5182
- 27.** Li H, Yang B, Huang J, Xiang T, Yin X, Wan J, et al. Naringin inhibits growth potential of human triple-negative breast cancer cells by targeting β -catenin signaling pathway. *Toxicol Lett*. 2013;220(3):219-228. doi: 10.1016/j.toxlet.2013.05.006
- 28.** Walz S, Lorenzin F, Morton J, Wiese KE, Eyss BV, Herold S, et al. Activation and repression by oncogenic MYC shape tumour-specific gene expression profiles. *Nature*. 2014;511:483-487. doi: 10.1038/nature13473

- 29.** Zhou J, Xia L, Zhang Y. Naringin inhibits thyroid cancer cell proliferation and induces cell apoptosis through repressing PI3K/AKT pathway. *Pathol Res Pract.* 2019;215(12):152707. doi: 10.1016/j.prp.2019.152707
- 30.** Aroui S, Aouey B, Chtourou Y, Meunier AC, Fetoui H, Kenani A. Naringin suppresses cell metastasis and the expression of matrix metalloproteinases (MMP-2 and MMP-9) via the inhibition of ERK-P38-JNK signaling pathway in human glioblastoma. *Chem Biol Interact.* 2016;244:195-203. doi: 10.1016/j.cbi.2015.12.011
- 31.** Ming H, Chuang Q, Jiashi W, Bin L, Guangbin W, Xianglu J. Naringin targets Zeb1 to suppress osteosarcoma cell proliferation and metastasis. *Aging.* 2018;10(12):4141-4151. doi: 10.18632/aging.101710
- 32.** Tan TW, Chou YE, Yang WH, Hsu CJ, Fong YC, Tang CH. Naringin suppress chondrosarcoma migration through inhibition vascular adhesion molecule-1 expression by modulating miR-126. *Int Immunopharmacol.* 2014;22(1):107-114. doi: 10.1016/j.intimp.2014.06.029
- 33.** Saleh M, Khalil M, Abdellateif MS, Ebeid E, Madney Y, Kandeel EZ. Role of matrix metalloproteinase MMP-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP-1) in the clinical progression of pediatric acute lymphoblastic leukemia. *Hematology.* 2021;26(1):758-768. doi: 10.1080/16078454.2021.1978763
- 34.** Zhu J, Li Y, Chen C, Ma J, Sun W, Tian Z, et al. NF- κ B p65 overexpression promotes bladder cancer cell migration via FBW7-mediated degradation of RhoGDI α protein. *Neoplasia.* 2017;19(9):672-683. doi: 10.1016/j.neo.2017.06.002
- 35.** Bacanlı M, Başaran AA, Başaran N. The antioxidant and antigenotoxic properties of citrus phenolics limonene and naringin. *Food Chem Toxicol.* 2015;81:160-170. doi: 10.1016/j.fct.2015.04.01