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Mitochondrial targeting cancer therapy by naringin in Ehrlich ascites carcinoma-bearing mice

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ABSTRACT

Naringin (NRG), a natural flavanone that has recently gained attention for its pharmacological properties. The present study investigated the mitochondrial-mediated anticancer effects of NRG in EAC-bearing mice. Mice were treated with NRG, and its effects were compared with untreated tumor-bearing controls and cisplatin (Cis)-treated groups. Tumor profile, oxidative stress markers, and mitochondrial functional integrity were assessed. Furthermore, the gene expression analysis was performed using real time PCR to assess apoptotic signaling. The results demonstrated that the treatment with NRG led to significant inhibition of tumor growth, markedly reduced lipid peroxidation, enhanced antioxidant defense systems, and stabilized mitochondrial membrane potential. Additionally, the treatment with NRG triggered the intrinsic apoptotic pathway enhancing tumor cell programmed death. The NRG exerts potent anticancer activity and decreased hepatotoxicity in EAC-bearing mice through mitochondrial targeting mechanisms that induce oxidative stress modulation and intrinsic apoptosis. These findings highlight NRG's potential as a promising natural compound for mitochondrial-based cancer therapy. NRG could serve as an adjuvant to chemotherapy to reduce toxicity and enhance efficacy.

Introduction

Cancer remains one of the most severe diseases impacting lives worldwide. Although cancer typically originates in a specific area, it can spread to various organs and tissues throughout the body through a process known as metastasis, which includes invasion and migration **(Sung *et al.*, 2021)**. Metastasis is the primary reason for deaths associated with cancer, mainly due to resistance to various cytotoxic agents and to the process of apoptosis. This leads to high rates of mortality and morbidity in individuals, as current chemotherapy treatments fail to successfully eliminate cancer cells without harming healthy ones. Anticancer treatments are frequently complicated by the cancer cells' ability to withstand drugs and the severe side effects of the therapy **(Ghanbari-Movahed *et al.*, 2021)**. Modern treatment options now encompass therapies based on hormones, and immunotherapy. The adverse effects linked to conventional cancer therapies underscore the potential of innovative cancer treatment approaches. Various novel treatment systems targeting malignancies include strategies against the angiogenic capabilities of tumors. These therapies are applied to various cancers **(Anand *et al.*, 2022)**.

Mitochondria are crucial double-membraned organelles that possess complex structures and a wide range of functions within cells. Irregular mitochondrial metabolism is strongly linked to a variety of diseases, including cancers **(Wang et al., 2025)**. Mitochondrial dysfunction is linked to various diseases, contributes to the malignant transformation of cells **(Escrig-Larena *et al.*, 2023)**.

Apoptosis is a complicated process with numerous paths. Anywhere along these pathways, there might be flaws that result in tumor spread, resistance to anticancer medications, and the malignant transformation of the impacted cells **(Chaudhry *et al.*, 2022)**. Natural products have been thoroughly explored for their potential against cancer, bringing natural products into clinical application and unveiling new therapeutic possibilities **(Chunarkar-Patil *et al.*, 2024)**. The history of anticancer drug discovery has been significantly influenced by natural products. A variety of commonly used anticancer medications have their origins in natural sources **(Huang *et al.*, 2018)**. Citrus flavonoids, naringin (NRG) being particularly prominent in grapefruit flowers that showed various pharmacological and biological properties **(Zeng *et al.*, 2020; Miles and Calder, 2021; Yang *et al.*, 2022)**. This study investigated the role of NRG in EAC-bearing mice by targeting mitochondria.

Materials and methods

Chemicals

Naringin (≥95% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2) (30% w/v; purity ≥99%), and phosphate buffer salts (0.1 M, pH 7.4) were purchased from Merck (Darmstadt, Germany).

Experimental animals

The experimental design was approved with the code (IACUC-SCI-TU-443). Fifty male albino mice (20 ± 2 g) were divided into five groups (n=10 per group): Group 1 received normal diet *ad*

libitum. Group 2, inoculated intraperitoneally with 1 × 10^6 EAC-cells/mouse **(El-Naggar and El-Said, 2020)**. Group 3, EAC/Cis group: injected with Cis (2 mg/kg) **(El-Naggar and El-Said, 2020)**. Group 4, EAC/NAR group: injected with NAR (100 mg/kg) **(Knežević et al., 2011)**. Group 5, EAC/Cis/NAR group: Co-treated with Cis (2 mg/kg) and NAR (100 mg/kg). All treatments were interperitoneally (i.p) injections after 24 hours. Body weight changes, the volume of ascitic tumor fluids were measured, count of the live and dead tumor cells were determined. Ascitic tumor fluid was collected and diluted with saline for molecular analysis. Sera samples were collected for determination of biochemical parameters.

Hematological parameters assessment

The electronic blood counter was used to measure the hemoglobin content (Hb g/dl), red blood cells (RBCs), total platelets, total white blood cells (WBCs), and their differential counts. The automated Dirui BCC-3600, MA, USA automated hematological analyzer was used to count the CBC.

Biochemical analysis

Serum levels of rat's ALT (catalogue number: MBS269614), AST (catalogue number: MBS264975), ALP (catalogue number: MBS165203), hepatic levels of SOD (catalogue number: MBS036924), CAT (catalogue number: MBS2600683), GSH (catalogue number: MBS265966), and MDA (catalogue number: MBS738685) were determined using their rat's specific ELISA kits from My BioSource (Inc., San Diego, CA, USA).

Assessment of mitochondrial parameters

Mitochondrial membrane potential was assessed using the fluorescent dye Rhodamine 123 (Rh123). A decrease in fluorescence intensity indicates mitochondrial depolarization. The ROS generation was determined using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). The ATP levels were determined using a luciferin–luciferase bioluminescence assay kit (catalogue number: FLAA) (Sigma-Aldrich, USA).

Gene expression analysis

The expression of the apoptotic related genes, P53, Bcl-2, BAX, and caspase-9 genes was assessed in EAC cells of the different EAC-bearing groups of mice under the study after different treatments using GAPDH as a housekeeping gene. The primers were prepared using the Primer-Blast program from NCBI **Table (1) (Livak and Schmittgen, 2001)**.

Statistical analysis

One-way ANOVA was employed to compare the groups, and Tukey post hoc comparisons were conducted among the various groups. *P* values < 0.05 were statistically significant.

Table (1): Forward and reverse primer sequences for RT PCR

Gene	Accession No.	Forward sequence (5′-3′)	Reverse sequence (5′-3′)
P_{53}	NM_001429993.1	GTTCCGAGAGCTGAATGAGG	TTTTATGGCGGGACGTAGAC
Bcl-2	NM_016993.2	CATGCCAAGAGGGAAACACCAGAA	GTGCTTTGCATTCTTGGATGAGGG
BAX	NM_017059.2	GGCTGGACACTG GACTTCCT	GGTGAGGACTCCAGCCACAA
Caspase-9	NM_001277932.2	TTCATTATTCAGGCCTGCCGAGG	TTCTGACAGGCCATGTCATCCTCA
GAPDH	NM_017008.4	GAGAAACCTGCCAAGTATG	GGAGTTGCTGTTGAAGTC

Results

Administration of NRG improves body weight changes

The % b.wt changes of the EAC-bearing mice were significantly increased ($p < 0.05$) up to 37.68% ± 4.36. Treatment with Cis (2 mg/kg b.wt) or NRG (100 mg/kg) for 6 consecutive days led to significant decrease in % b.wt changes to 8.35% ± 2.97, or 14.79% ± 2.61, respectively. Combinatorial treatment with Cis/NRG resulted in much more decrease in the % b.wt changes up to 7.15% ± 2.36 when compared to single treatment. The combination of the therapeutic dose of Cis and NRG led to synergistic effect on the decrease in the % b.wt changes due to the decrease in the ascitic tumor fluid **Table (2)**.

Table (2): Initial body weights, final body weights, % change of body weight of groups under the study

Groups	I. B. wt. (g)	F. B. wt. (g)	% change of B.wt.
Group 1	22.95 ± 2.65 [a]	28.12 ± 3.44 [a]	22.53 ± 3.02[a]
Group 2	23.14 ± 3.32 [a]	31.86 ± 4.15 [a]	37.68± 4.36[e]
Group 3	22.29 ± 2.89 [a]	24.15 ± 3.52 [a, b]	8.35 ± 2.97 [b]
Group 4	23.70 ± 2.34 [a]	27.13 ± 4.84 [a]	14.79 ± 2.61 [c]
Group 5	22.37 ± 2.45 [a]	23.97 ± 3.14 [a, b]	7.15 ± 2.36 [b]

The values represented as means ± S.D. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Total tumor volume, viable and dead EAC-cells after different treatments

There were significant decreases in the total ascitic tumor volume of EAC-bearing mice that had been treated with Cis or/and NRG (9.4 mL ± 0.85). The co-treatment with Cis and NRG led to synergistic effect on the reduction of the tumor volume when compared to the EAC-bearing mice that were treated with the Cis alone. The combinatorial treatment with Cis and NRG resulted in significant reduction in the T.T.C to 42×10^6 ± 5.13 when compared to single treatment. Additionally, the number of viable tumor cells decreased post treatment with Cis or/and NRG **Table (3)**.

Table (3): Total volume, viable and dead EAC-cells in the different groups.

Groups	T.T.V (ml)	T.T.C ($\times10^6$ /mouse)	T.L.C ($\times10^6$ /mouse)	T.D.C ($\times10^6$ /mouse)
Group 2	9.4 ± 0.85 [a]	621 ± 28.35 [a]	580 ± 27.45 [a]	41 ± 3.5 [a]
Group 3	2.1 ± 0.31 [b]	76 ± 7.51 [b]	15 ± 1.7 [b]	61 ± 3.9 [a, b]
Group 4	4.3 ± 0.39 [c]	289 ± 13.69 [c]	115 ± 6.5 [c]	174 ± 7.5 [c]
Group 5	1.4 ± 0.25 [b]	42 ± 5.13 [f]	8 ± 0.6 [e]	34 ± 0.95 [a]

The values represented as means ± S.D. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Effect of the treatment with Cis or/and NRG on the hematological parameters

The total RBCs count, Hb level, Hct (%), and platelets count were significantly decreased ($p < 0.001$) in EAC-bearing mice. However, the treatment with the Cis alone or in combination with NRG for 6 consecutive days improved these hematological parameters **Table (4)**. The total count of WBCs was significantly increased ($p < 0.001$) in the EAC-bearing mice ($29.44 \times 10^3/\mu L \pm 3.25$) when compared to naïve mice ($9.37 \times 10^3/\mu L \pm 1.78$). In EAC-bearing mice treated with Cis or NRG, the total WBCs counts increased to $15.91 \times 10^3/\mu L \pm 3.63$ or $11.75 \times 10^3/\mu L \pm 3.29$, respectively. Moreover, the co-treatment with Cis/NRG days did alter the total WBCs count closed to the normal control ($10.15 \times 10^3/\mu L \pm 2.99$) **Table (5)**.

Table (4): The hematological parameters in different groups under the study

Groups	RBCs ($x10^6$ /µL)	Hb (g/dL)	Hct. (%)	Platelets ($x10^3$ /µL)
Group 1	9.5 ± 0.89 [a]	13.6 ± 1.15 [a]	46.7 ± 3.65 [a, b]	769.6 ± 114.5 [b]
Group 2	6.7 ± 0.79 [b]	8.9 ± 0.88 [b]	30.5 ± 2.85 [c]	652.5 ± 97.9 [c]
Group 3	8.4 ± 1.05 [b, c]	9.4 ±1.13 [b, c]	36.7 ± 2.69 [c]	707 ± 89.9 [b, c]
Group 4	8.7 ± 0.97 [b, c]	10.4 ± 0.88 [b, c]	38.5± 3.15 [b, c]	719.5 ± 94.5 [b, c]
Group 5	9.2 ± 0.96 [b, c]	11.8 ± 0.94 [b, c]	41.6 ± 4.32 [a,b,c]	732 ± 128.4 [b, c]

The values represented as means ± S.D. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Table (5): The percentages of the leucocytes and their differential in the different groups under the study

Groups	WBCs ($x10^3$ /µL)	Lymphocytes (%)	Neutrophiles (%)	Monocytes (%)
Group 1	9.37± 1.78 [a]	67.28 ± 5.79 [b]	23.12 ± 1.47 [b]	9.15 ± 0.95 [b]
Group 2	29.44 ± 3.25 [b]	75.09 ± 8.33 [a, b]	21.25 ± 3.65 [b]	4.17 ± 2.89 [a, b]
Group 3	15.91 ± 3.63 [a,b]	64.15 ± 7.21 [b]	20.55 ± 2.18 [b]	8.87 ± 0.97 [b]
Group 4	11.75 ± 3.29 [a]	66.89 ± 9.22 [b]	19.46 ± 3.16 [b]	9.56 ± 4.39 [b]
Group 5	10.15 ± 2.99 [a]	69.37 ± 7.15 [b]	18.99 ± 2.86 [b]	8.46 ± 3.78 [b]

The values represented as means ± S.D. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Co-treatment of Cis with NRG mitigated the hepatic dysfunctions

The activities of liver transaminases enzymes and ALP were significantly increased ($p < 0.05$) in the EAC-bearing mice due to liver injury caused by tumor, which represented 185 ± 10.5 U/L, 209.5 ± 12.9 U/L, and 275.6 ± 11.6 U/L, respectively when compared to the normal control group (41.8 ± 3.9 U/L, 87.6 ± 7.5 U/L, and 90.8 ± 5.3 U/L, respectively) **Table (6)**. However, the co-treatment with NRG and Cis resulted in significant mitigation of liver damage that was induced by tumors much more than single treatments as significant decrease in the sera activities of ALT, AST, and ALP. The combinatorial

treatment with NRG and Cis led to significant improvement of liver injury much more than single treatments by significant increase in the total protein levels **Table (6)**.

Table (6): Serum activities of ALT, AST, ALP, and the total protein levels in the different groups under the study

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	T. protein (g/dL)
Group 1	41.8 ± 3.9 [a]	87.6 ± 7.5 [a]	90.8 ± 5.3 [a]	6.85 ± 0.39 [a]
Group 2	185 ± 10.5 [c]	209.5 ± 12.9 [b]	275.6 ± 11.6 [b]	3.15 ± 0.22 [b]
Group 3	131.5 ± 8.6 [b]	153.5 ± 9.8 [c]	204.7 ± 9.5 [c]	4.75 ± 0.29 [c]
Group 4	122.8 ± 6.9 [b,d]	130.3 ± 7.4 [c,d]	171.3 ± 8.6 [c,d]	5.63 ± 0.18 [c]
Group 5	88.7 ± 8.9 [e]	111.5 ± 8.3 [d]	127.6 ± 6.8 [a,e]	6.14 ± 0.22 [a,c]

The values represented as means ± S.D. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Co-treatment of Cis with NRG ameliorated oxidative stress

The result demonstrated that the EAC-bearing mice showed significant decreased ($p < 0.05$) in the hepatic SOD, CAT, and GSH levels. The MDA levels in liver tissues homogenates of the EAC-bearing mice were significantly increased ($p < 0.05$). The treatment with the therapeutic dose of Cis led to significant improvement in the antioxidants/oxidants status that was evidenced by increase in SOD, CAT, and GSH levels accompanied by significant decrease in the levels of MDA in the liver tissues homogenates. Moreover, the treatment with Cis and NRG showed much more improvement in their antioxidant properties **Fig. (1)**.

Co-treatment of Cis with NRG targeted mitochondrial dysfunctions

The EAC-bearing mice showed a marked decrease in mitochondrial membrane potential (ΔΨm), indicating mitochondrial depolarization. Notably, combined Cis and NRG treatment significantly restored ΔΨm close to that of the control group, suggesting synergistic protection against mitochondrial dysfunction **Table (7)**.

A significant alteration in ROS production was observed among the different experimental groups. The EAC inoculation led to a marked elevation of mitochondrial ROS, indicating oxidative stress and mitochondrial dysfunction. The combined treatment with both Cis and NRG exhibited the lowest ROS levels among the treated groups, approaching near-normal values. Additionally, A significant alteration in mitochondrial ATP levels was observed across the experimental groups. EAC inoculation caused a marked reduction in ATP content, indicating impaired mitochondrial oxidative phosphorylation. The combination of Cis and NRG showed the highest restoration of ATP content, approaching normal control values **Table (7)**.

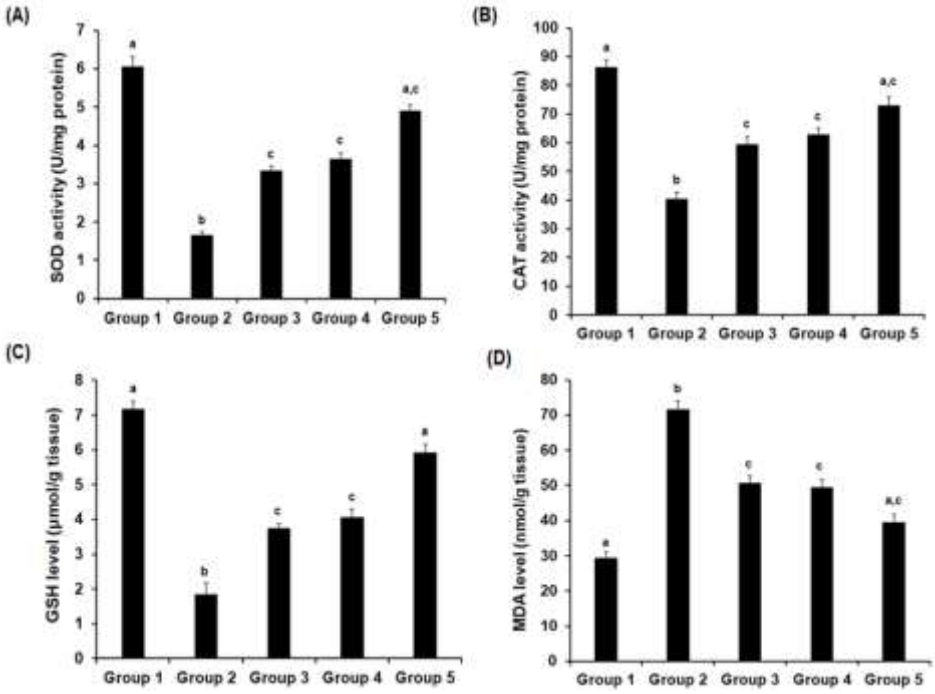

Fig. (1): Hepatic levels of SOD **(A)**, CAT **(B)**, GSH **(C)**, and MDA **(D)** in the different groups under the study. Means that do not share a letter are significantly different ($p < 0.05$).

Table (7): Effect of NRG and Cis on mitochondrial dysfunctions in liver mitochondria.

Groups	ΔΨm (% of control)	ROS Production (RFU/mg protein)	ATP Content (µmol/mg protein)
Group 1	100.00 ± 3.12 [a]	100.00 ± 4.25 [a]	4.82 ± 0.21 [a]
Group 2	54.37 ± 2.98 [c]	215.67 ± 6.83 [b]	2.15 ± 0.14 [b]
Group 3	70.21 ± 3.45 [b]	165.22 ± 5.76 [c]	3.01 ± 0.19 [b, c]
Group 4	82.46 ± 2.71 [b, e]	132.48 ± 4.92 [e]	3.85 ± 0.17 [a, c]
Group 5	93.58 ± 2.84 [e]	110.73 ± 4.28 [a]	4.41 ± 0.20 [a]

The values represented as means ± S.D. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Effect of the treatment with Cis or/and NRG on apoptosis-related gene expression in EAC-cells

The results of qPCR showed that the treatment with the Cis induced apoptosis *in vivo* in EAC-cells via the mitochondrial-dependent pathways indicated by a significant ($p < 0.05$) increase in the mRNA relative expression level of the P_{53} gene by around 10 folds. Concomitant treatment with the Cis/NRG led to enhancement of apoptosis that indicated significant increase in the P_{53} gene expression to 12.54 ± 0.28, when compared to the single treatments **Fig. (2)**. The results showed that mice injection with Cis resulted in significant decrease ($p < 0.05$) in the mRNA relative expression level of Bcl-2 gene to 0.44 ± 0.05. Co-treatment with Cis/NRG led to synergistic effect on the reduction of

Bcl-2 gene expression in EAC-cells when compared with single injections. Co-treatment with Cis/NRG led to enhancement of carcinoma cells' apoptosis and synergistic effect on the raising of BAX gene expression in EAC-cells when compared with single injections. Moreover, combinatorial treatment of Cis with NRG in EAC-bearing mice showed the highest level of caspase-9 gene expression **Fig. (2)**.

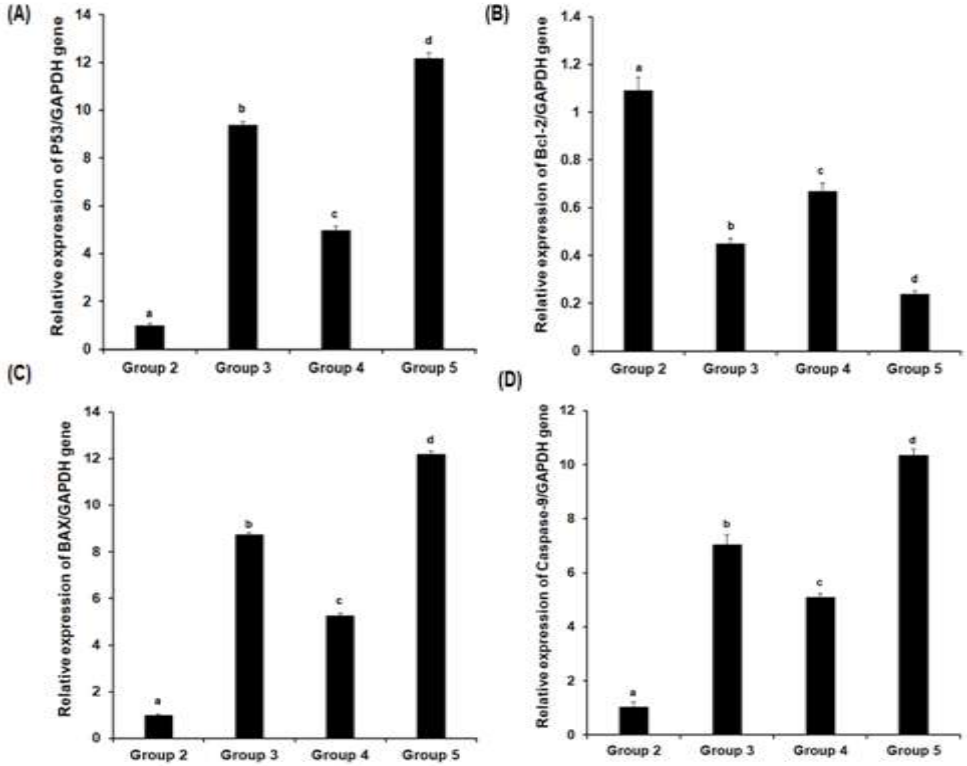

Fig. (2): Gene expression analysis by RT-PCR in the different groups under the study shows the relative expression of P53 **(A)**, Bcl-2 **(B)**, BAX **(C)**, and Caspase-9 **(D)** using GAPDH gene as housekeeping gene. The values represented as means ± S.D. Means that do not share a letter are significantly different ($p < 0.05$).

Discussion

Conventional chemotherapy is an effective approach for cancer treatment. However, chemotherapy treatment led to wide side effects on some vital organs and induces resistance of the tumor cells to the treatment. Cis is an effective chemotherapeutic agent, however, it has toxic effects on vital organs **(Dasari et al., 2022; El-Naggar and El-Said, 2020)**. Resistance to chemotherapy and its adverse effects remain the major problems in its cancer treatment regimen Therefore, decreasing its resistance and adverse effects without any limitation to its anticancer efficacy is necessary **(Bukowski *et al*., 2025)**. Novel biologically active compounds derived from natural products will undoubtedly continue to provide a plethora of opportunities as sources of novel anticancer therapeutic leads **(Santaniello *et al*., 2023)**. This study investigated the role of NRG for cancer therapy in EAC-bearing mice by targeting mitochondria.

This study showed that the inoculation of EAC-cells led to an increase in the percentage of body weight change, and this could be due to the proliferation of EAC-cells. This finding agreed with previous study by **El-Naggar *et al*. (2019**). Treatment with Cis or NRG led to significant decrease in the percentage of body weight change. This finding was in accordance with previous studies **(El-Naggar and El-Said, 2020; Ibrahim *et al*., 2022)**. In this study, the co-treatment with the Cis and NRG led to significant reduction in the tumor profile. These results were in line with previous study demonstrated the antitumor efficacy of combinatorial treatment of Cis and natural compounds that could be enhanced in EAC-bearing mice by increasing the percentages of dead tumor cells **(El-Naggar *et al*., 2019; Hashem *et al*., 2021; Morsi *et al*., 2022)**. Furthermore, the total RBCs count, Hb level, Hct % were decreased in EAC-bearing mice. The total WBCs count was increased in EAC-bearing mice, while the treatment with Cis or/and NRG resulted in improvement in hematological parameters and decreased the WBCs count. This effect could be due to the protection of the hematopoietic system **(Nafie et al., 2020; Fawzy et al., 2023)**.

The progression of EAC-tumor led to elevation in liver transaminase enzymes (ALT and AST). These enzymes were elevated in serum of EAC–bearing mice **(Radwan et al., 2024)**. Elevation of ALT and AST enzymes may be due to the cytotoxic effect of EAC tumors which led to damage of liver cells and canaliculi. The results showed that NRG had antitumor effects *in vivo* studies. In addition, the co-treatment with NRG could protect liver tissues against Cis toxicity. These results agreed with the previous studies reported the efficacy of the co-treatment with Cis and natural products **(Nafie *et al*., 2020; Hashem *et al*., 2021; Abd Rashid *et al*., 2022)**. The present study showed that the levels of ALT, AST, ALP, and total proteins in the group that treated Cis were significantly decreased. This finding was in line with previous study of **El-Naggar *et al*. (2019)**. Combinatorial treatment with Cis/NRG led to a significant decrease in the levels of these transaminases in EAC-bearing mice compared to single injections that indicate the ameliorative effects of NRG on hepatotoxicity. The ALT, AST, and ALP enzymes were decreased in the group treated with a combination of Cis/NRG.

Keleş *et al*. (2020) demonstrated that treatment with natural constituents led to a significant decrease in MDA and an increase in GSH as markers of oxidative stress in urinary calculi in rats. **Abd-Elghany et al. (2022)** assess the potential anticancer activity of the natural products in EAC-bearing mice, and reported that liver functions were improved, and oxidative stress improved in tumor, liver tissues.

The EAC inoculation resulted in a pronounced loss of ΔΨm, indicating severe mitochondrial dysfunction. This reduction may result from excessive ROS generation leading to loss of membrane polarization and subsequent apoptotic signaling. These findings are in line with earlier studies reporting depolarized mitochondrial membranes in tumor-bearing models **(El-Naggar and El-Said, 2020)**. Treatment with Cis alone partially restored ΔΨm, which can be attributed to its cytotoxic action on tumor cells and the subsequent reduction

of oxidative stress load; however, Cis itself is known to cause mitochondrial toxicity, limiting its protective effect. Conversely, naringin treatment markedly enhanced ΔΨm compared with EAC-bearing mice, suggesting potent mitochondrial stabilization. The observed improvement can be explained by naringin's antioxidant and free radical scavenging properties, which preserve mitochondrial structure and prevent mPTP opening **(Knežević et al., 2011)**. Importantly, the combination of Cis and naringin exhibited a synergistic effect, with ΔΨm values approaching those of the control group. This indicates that naringin effectively mitigates Cis-induced mitochondrial injury, possibly by modulating oxidative stress, restoring mitochondrial biogenesis.

In this study, EAC inoculation induced a pronounced increase in mitochondrial ROS, indicating a disturbed redox equilibrium and compromised mitochondrial integrity. This elevation likely results from high metabolic activity and increased oxygen consumption by tumor cells, consistent with earlier reports of mitochondrial oxidative stress in tumor-bearing models **(El-Naggar and El-Said, 2020)**. Treatment with Cis moderately reduced ROS levels compared with EAC mice. While Cis exerts antineoplastic effects through DNA crosslinking and induction of oxidative stress in cancer cells, it may also cause collateral mitochondrial damage in normal cells, limiting its protective potential. Administration with NRG significantly suppressed ROS production. This attenuation can be attributed to naringin's antioxidant and radical-scavenging capacity, as well as its ability to enhance endogenous antioxidant defenses. Naringin's structural hydroxyl groups enable direct scavenging of superoxide anions and hydroxyl radicals, thus stabilizing the mitochondrial redox environment **(Knežević et al., 2011)**.

Notably, the combination therapy demonstrated the most profound reduction in ROS, bringing levels close to those of the control group. This suggests that naringin mitigates Cis-induced oxidative damage and enhances its therapeutic selectivity by protecting non-tumor mitochondria. The synergistic effect may arise from naringin's ability to modulate mitochondrial enzymes, inhibit ROS-producing pathways, and restore the mitochondrial membrane potential, collectively maintaining mitochondrial bioenergetics and reducing oxidative stress burden. These results highlight the pivotal role of naringin in attenuating mitochondrial oxidative stress and its potential use as a mitochondria-targeted adjuvant to improve chemotherapeutic safety and efficacy in EAC-bearing mice.

The ATP generation by mitochondria is the hallmark of cellular energy metabolism and reflects the efficiency of oxidative phosphorylation. In cancer-bearing animals, altered mitochondrial function disrupts ATP synthesis due to oxidative stress, mitochondrial DNA damage, and impaired respiratory chain activity. In this study, Group 2 showed a significant decline in mitochondrial ATP content, suggesting severe mitochondrial dysfunction and energy failure. The reduction in ATP likely results from increased ROS generation and structural damage to the electron transport chain complexes events characteristic of tumor-induced mitochondrial impairment. These findings are consistent with previous studies

reporting reduced ATP synthesis in tumor models due to mitochondrial oxidative stress **(El-Naggar and El-Said, 2020)**.

Treatment with Cis moderately improved ATP levels, reflecting its cytotoxic effect on tumor cells and partial restoration of normal mitochondrial activity. However, Cis's known mitochondrial toxicity limits full recovery of ATP synthesis, as it can induce mitochondrial DNA crosslinking and inhibit respiratory chain enzymes. Treatment with NRG significantly enhanced ATP content compared to the EAC group, demonstrating its potential to stabilize mitochondrial bioenergetics. Naringin's antioxidant and mitochondrial-protective effects likely contribute to restoring electron transport chain function, preserving membrane potential, and maintaining ADP/ATP translocation efficiency. Previous reports have shown that flavonoids like naringin enhance mitochondrial biogenesis and promote ATP synthesis by upregulating PGC-1α and related pathways **(Motallebi et al., 2022)**. The combined treatment exhibited the highest ATP levels, approaching those of normal control mice. This synergistic improvement indicates that naringin mitigates Cis-induced mitochondrial damage, thereby enhancing overall mitochondrial function and ATP yield. The dual therapy appears to balance Cis's antitumor efficacy with naringin's mitochondrial protection, representing a promising strategy for energy preservation during cancer therapy. These results reinforce the concept that naringin effectively restores mitochondrial ATP production, maintaining energy homeostasis and counteracting tumor- and drug-induced mitochondrial dysfunctions.

The results indicated that there was a significant increase in the mRNA relative expression level of the P_{53} gene of the EAC-cells post treatment with Cis alone or in combination with NRG. These findings indicated the induction of apoptosis *in vivo* in EAC-cells via the mitochondrial-dependent pathways **(Elmorsy *et al*., 2024)**. The preventive and therapeutic effects of naringin against human malignancies have been reviewed **(Ghanbari-Movahed et al., 2021)**. Co-treatment with Cis and NRG enhanced the antitumor activity by inducing apoptosis in EAC-cells that evidenced by significant decrease in the relative expression level of the anti-apoptotic Bcl-2 gene with significant increase in the relative expression level of the apoptotic BAX and caspase-9 genes. These findings agreed with the previous studies reported the effects of the treatment with natural constituents on apoptosis of EAC-bearing mice **(Ghanbari-Movahed et al., 2021; Hashem et al., 2021)**.

Conclusion

The co-treatment with NRG with Cis lad to enhancement of the antitumor efficacy of Cis by inducing apoptosis and decrease its toxic effects on the liver tissues via improvement of hepatorenal functions and antioxidant status. Taken together, these findings suggest that naringin's mitochondrial-targeting potential lies in its ability to restore TCA cycle enzyme activities, maintain mitochondrial integrity, and alleviate oxidative damage associated with both tumor progression and Cis treatment.

Declaration statement

Authors confirm that the manuscript is the author's own work, has not been

published elsewhere, and that all sources are properly cited.

Conflict of interest

Authors declare that there was no conflict of interest.

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استهداف الميتوكوندريا لعلاج السرطان باستخدام النارينجين في فئران مصابة بورم الإستسقاء البطني إيرليش

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النارينجين، وهو فلافانون طبيعي اكتسب مؤخرًا اهتمامًا كبيرًا لخصائصه الدوائية. بحثت هذه الدراسة في التأثيرات المضادة للسرطان المتواسطة بالميتوكوندريا للنارينجين في فئران مصابة بسرطان استسقاء إيرليخ. عولجت الفئران بالنارينجين، وقورنت آثاره مع فئران غير مصابة بالسرطان، وفئران عولجت بالسيسبلاتين تم تقييم نمط الورم، وعلامات الإجهاد التأكسدي، والسلامة الوظيفية للميتوكوندريا. علاوة على ذلك، تم تحليل التعبير الجيني باستخدام تفاعل البوليميراز المتسلسل في الوقت الحقيقي (PCR) لتقييم إشارات موت الخلايا المبرمج. أظهرت النتائج أن العلاج بالنارينجين أدى إلى تثبيط كبير لنمو الورم، وانخفاض ملحوظ في بيروكسيد الدهون، وتعزيز أنظمة الدفاع المضادة للأكسدة، واستقرار جهد غشاء الميتوكوندريا. بالإضافة إلى ذلك، حفّز العلاج بـالنارينجين مسار موت الخلايا المبرمج الداخلي، مما عزز موت الخلايا المبرمج. اظهر النارينجين نشاطًا قويًا مضادًا للسرطان، ويُقلل من سمية الكبد لدى الفئران الحاملة لـورم الاستسقاء البطني من خلال آليات استهداف الميتوكوندريا التي تُحفز تعديل الإجهاد التأكسدي وموت الخلايا المبرمج الداخلي. تُبرز هذه النتائج إمكانات النارينجين كمركب طبيعي واعد لعلاج السرطان المعتمد على الميتوكوندريا. يُمكن استخدام النارينجين كعامل مساعد للعلاج الكيميائي لتقليل السمية وتعزيز الفعالية.