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## Impact of Essential Oils (Orange Peels) on Ehrlich Ascites Carcinoma Against Cardiac Damage in Female Mice

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### Abstract

Cancer is defined as the accumulation of abnormal cells that are formed by cell proliferation and cause damage to all organs. According to reports from the World Health Organization, the number of people infected with this disease exceeds ten million, Essential oils are a blend of several non-terpenoid chemicals that are beneficial in food flavoring, medications, and cosmetics. This study focused on studying the preventive role of essential oils as a treatment for cancer. This study's goal was to ascertain the effects of Essential oils (orange peels) against Ehrlich Ascites Carcinoma-induced cardiac toxicity. 40 mice were divided into four groups at random (G1, control; G2, Essential oils (orange peels) (25 mg/kg bw/day) orally for 2 weeks; G3, EAC and G4, EAC + Essential oils (orange peels)). Current findings showed, that EAC induced a significant elevation in creatine kinase myoglobin (CK-MB), cholesterol, triglycerides, potassium, and sodium, a significant increase in serum AFP, CEA, and Desmine expressions when compared to Essential oils (orange peels), and control groups. EAC + Essential oils (orange peels) adjust these modifications. EAC-induced cardiac dysfunction and Essential oils (orange peels) can improve cardiac functions, and post-treated EAC with Essential oils (orange peels) improved, AFP and CEA Levels and Desmine expressions. It is possible to utilize essential oils (orange peels) as a supportive treatment for cardiac ascites caused by hepatocellular carcinoma, Schistosoma, and hepatitis.

**Keywords:** EAC, Essential Oils (Orange Peels). Cardiac Functions, AFP, CEA, electrolytes.

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## 1. Introduction

Cancer is abnormal cells that form due to cell proliferation and cause damage to other organs within the body (1). Annually, based on the World Health Organization reports, above ten million people are infected with this disease, and it is considered the most important cause of death (2). Breast Cancer is among the most dangerous varieties of cancer that affects women. Regarding 1.41 million cases and 46,000 deaths have been discovered every year. Breast cancer causes an increase in deaths (3,4,5).

EAC causes the majority of organs in the body to fuse due to oxidative stress (6). This study was conducted and EAC was induced in mice, and the tumor was cultured to study the effect of this cancer (7).

EAC is recognized to be lacking distinction, grows rapidly inside the mouse, has a short lifespan, and is considered malignant (8). The majority common type of medical care used to treat Ehrlich's cancer is chemotherapy, which kills malignant cells through programmed cell death (9). This tumor has been used in many in vivo experiments and is a rapidly spreading type of malignant breast cancer that has the potential to develop within the body (10). Over time, the effects of EAC begin to appear and their rate of reproduction increases gradually, and swelling begins to appear due to the presence of ascitic fluid (11). Essential oils have health benefits such as eliminating complex diseases and improving digestion (12). It can reduce lymphocyte proliferation and increase the serum level in the blood (13). Recent studies have proven that essential oils have many properties, including anti-

diabetic, anti-inflammatory, anti-hypertension, and getting rid of many chronic diseases (14,15). Essential oils are manufactured by plants. These oils contain active biological substances and have been used for medicinal and cosmetic purposes for centuries and also in the field of nutrition (16). Sweet orange is considered the most important type of citrus fruit and is considered a significant supply of phenolic compounds (mostly hesperidin and ascorbic acid) and antioxidants and nicotinic acid (17,18). Orange peels contain high concentrations of phenols and high amounts of beta-carotene (19,20). The component of orange peel essential oil is d-limonene, which is the only hydrocarbon present (21,22).

## 2. Material and methods

### 2.1. Orange Peels Essential oil extraction

The essential oils of these plants were isolated from orange peels. (250g) of dried parts by steam distillation method (Cleavenger).

The plant material with D.W (1.2L) was boiled for 3h; the essential oil was kept at 4° C until used according to (23).

### 2.2. Induction of Ehrlich Ascites Carcinoma

Ehrlich Ascites Carcinoma (EAC) cells were obtained, which were acquired from the Egyptian National Cancer Institute - Cairo University, Egypt, 7-day-old EAC cells were collected from infected mice, and then mixed with sterile saline solution,  $2.5 \times 10^6$  cells/20 g body weight were transplanted into all mice, The growth of EAC cells was observed in all mice that were treated with these cells on the seventh and fourteenth days after imaging the mice, as Fig (1) according to (24).



**Figure.1:** Mice Bearing EAC on the fourteenth day before anatomy.

### 2.3. Animals

Forty adult Mice (male Swiss albino) weighing 20-25 g and Their ages were 9 to 10 weeks, Mice were obtained from the animal house at the Egyptian National Cancer Institute - Cairo University, Egypt. They were placed in cages randomly and kept at a temperature between 22 and 25C, humidity of 55%  $\pm$  5%, and water daily for fourteen days.

### 2.3. Experimental design and mice groups

Four groups of mice were assigned to the mice equally:

**Gp1:** Control Gp wherein mice received no therapy.

**Gp2:** Essential oils from orange peels mice injected in with received Essential oils (O.P) orally (25 mg/Kg body weight/day) by stomach tube around fourteen days according to (25).

**Gp3:** EAC group, mice injected once intraperitoneal I.P with Ehrlich cells with about 2,500,000 EAC/mouse according to (26).

**Gp4:** (EAC + Essential oils (O.P)) EAC cells were intraperitoneally administered once on the first day and treatment was carried out directly on the second day using essential oils from orange peels orally for 14 days.

### 2.4. Blood and Tissue Sampling

After the trial, anesthesia was administered to every mouse using a sedative sodium pentobarbital ( $\geq$ 100 mg/kg), They were then dissected, and the EAC cells were isolated by the protein cavity of all the mice that were injected with EAC cells.

Blood samples were isolated through the inferior vena cava into tubes containing heparin and mixed well to prevent clots in the samples. After that, the samples were separated by a centrifuge at 3000 g for 20 min and kept in a clean stopper vial at  $-20^{\circ}\text{C}$  until biochemical analysis.

The heart tissue was then collected, cleaned with saline solution, and preserved in plastic tubes containing formalin-fixed in 10%. This is to

conduct a histological examination.

### 2.5. Measurement of cardiac biomarkers, Lipid profiles, and electrolytes

The activity of CK-MB in serum, cholesterol, and triglyceride levels was tested by a kinetic method according to (27).

### 2.6. Measurement of Alpha-fetoprotein marker (AFP) and Carcinoembryonic antigen (CEA)

Serum Alpha-Fetoprotein (AFP) and Carcinoembryonic antigen (CEA) were measured by using the quantitative sandwich immunoassay, MyBioSource Mouse Carcinoembryonic Antigen Elisa Kit (MyBioSource, San Diego, USA) according to (28).

### 2.7. Immunohistochemical detection

Heart tissue was extracted after cutting from all groups and fixed with 10% formalin for a period between (24-48 hours), and the tissue sections were stained with eosin and hematoxylin (E& H) for histological examination according to (29,30,31).

### 2.8. Statistical analysis

The Statistical Package for the Social Sciences (SPSS software version 16) was used to analyze the findings. The data were displayed as mean  $\pm$  standard error of the mean (SEM) and subjected to one-way analysis of variance (ANOVA) and Dunnett test statistical analysis. Comparisons using the Dunnett test were used to determine how significant the differences between the groups were. To compare the significant difference between groups, an unpaired T-test was used.  $P < 0.05$  was established as the threshold for statistical significance.

## 3. Results

### 3.1. Toxicity

The results showed that the essential oils extracted from orange peels did not have negative effects on the mice that were treated with oils only. It had an essential role in reducing the size of the tumor for the mice that were treated with EAC +Essential oils, as in Figure (2).



After treatment with Essential oils (orange peels).



Before treatment with Essential oils (orange peels).

Figure 2: Mice Bearing EAC were treated with essential oils extracted from orange peels on the fourteenth day before anatomy (before and after treatment).

### 3.2. Effect of Essential oils from orange peels on cardiac marker and lipid profiles and electrolytes

When compared to the control and essential oil groups (Gp1 & Gp2), Table (1) showed a substantial spike in the levels of cholesterol, triglycerides, Ck-mb, and sodium and potassium ions in the sera on the EAC group (Gp3).

Following the co-treatment of EAC with essential oils, there was a substantial ( $p < 0.05$ ) decrease in the elevations of cholesterol, triglycerides, and

potassium ions, and an increase in the depletion of sodium ions (Gp4).

### 3.3. Effect of Essential oils from orange peels on AFP and CEA in serum

Table (2) demonstrated that the levels of AFP and CEA in the sera on the EAC group (Gp3) were significantly higher than those in the control and essential oil groups (Gp1 & Gp2). The elevations of AFP, CEA group (Gp4) were significantly ( $p < 0.05$ ) reduced after co-treating EAC with essential oils.

**Table (1): Comparison between the different groups under study to lipid profile and cardiac marker and electrolytes**

Parameters	Control	Essential oils (O.P)	EAC	EAC + Essential oils (O.P)
Cholesterol (mg/dL)	101.1 <sup>b</sup> ± 6.48	105.4 <sup>b</sup> ± 2.52	212.7 <sup>a</sup> ± 4.42	115.3 <sup>b</sup> ± 3.82
Triglyceride (mg/dL)	139.1 <sup>c</sup> ± 2.46	133.4 <sup>c</sup> ± 5.92	337.2 <sup>a</sup> ± 4.22	242.6 <sup>b</sup> ± 3.87
Ck-mb (ug/mL)	45.62 <sup>b</sup> ± 1.43	22.67 <sup>c</sup> ± 1.89	79.40 <sup>a</sup> ± 3.36	38.97 <sup>b</sup> ± 3.28
Na (mmol/l)	137.3 <sup>a</sup> ± 7.54	146.3 <sup>a</sup> ± 2.17	156.5 <sup>a</sup> ± 1.29	141.9 <sup>a</sup> ± 1.61
K (mmol/l)	10.93 <sup>b</sup> ± 0.19	9.49 <sup>c</sup> ± 0.04	16.23 <sup>a</sup> ± 0.30	12.16 <sup>a</sup> ± 0.46

Values are expressed mean ± SE; n = 6 for each treatment group

Mean values within a row not sharing common superscript letters were significantly different,  $p < 0.05$ .

F: F for One-way ANOVA test, Pairwise comparison bet. each 2 groups was done using a Post Hoc Test (Tukey)

p: p-value for comparing between the studied groups.

**Table (2): Comparison between the different studied groups according to tumor marker**

Parameters	Control	Essential oils	EAC	EAC +Essential oils
AFP (ng/mL)	11.65 <sup>c</sup> ± 0.20	11.75 <sup>c</sup> ± 0.20	210.2 <sup>a</sup> ± 0.45	61.25 <sup>b</sup> ± 1.77
CEA(ng/mL)	1.10 <sup>c</sup> ± 0.04	1.35 <sup>c</sup> ± 0.02	42.75 <sup>a</sup> ± 2.44	27.65 <sup>b</sup> ± 0.20

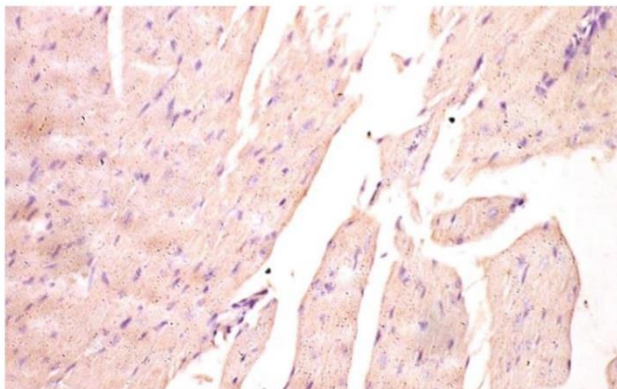
Values are expressed mean ± SE; n = 6 for each treatment group

Mean values within a row not sharing common superscript letters were significantly different,  $p < 0.05$ .

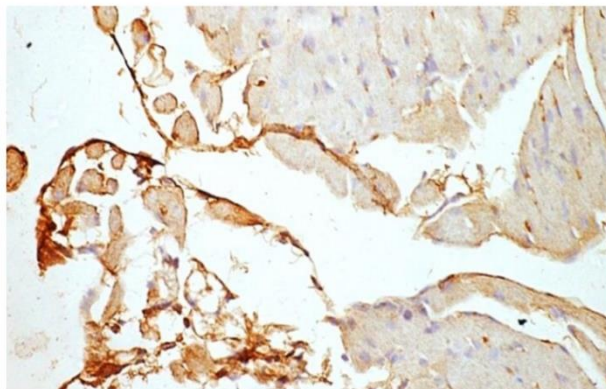
F: F for One-way ANOVA test, pairwise comparison bet. each 2 groups were done using a Post Hoc Test (Tukey)

p: p-value for comparing between the studied groups.

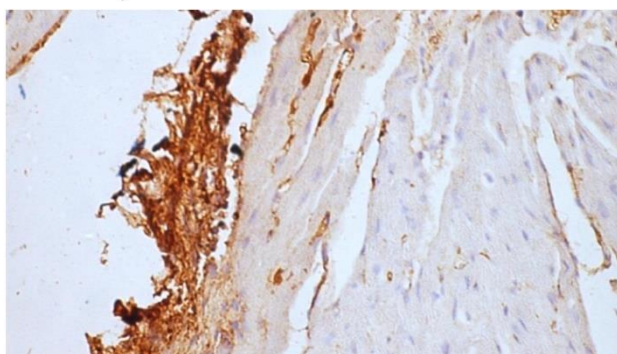
### 3.4. Desmine expressions in the heart:



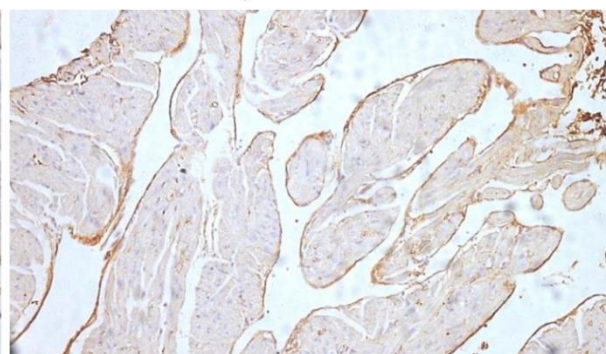
G1: Heart control showing negative desmine immunopositive stain.



G2: Heart Essential Oils showing intense desmine immunopositive stain.



G3: Heart EAC showing destroyed muscle fibers with desmine immunopositive.



G4: Heart treated with oranges oils showing mild +ve desmine stain.

## 4. Discussion

EAC tumor is a type of cancer that grows rapidly and turns into a liquid, this tumor is originally considered a malignant breast tumor that can implant and grow quickly, Ehrlich ascites fluid is considered the primary source on which the tumor depends during growth. This fluid causes major changes that destroy the heart muscle. On this basis, a study was conducted on the effect of essential oils extracted from orange peels as an agent in a treatment against EAC Tumors (32, 33). The results showed that the EAC in the third group resulted from an increase in the size of the mouse and changes in the external appearance of the mice that were treated with the tumor, as shown in Figure (1), while in the fourth group, due to the

treatment of the tumor-bearing mice with essential oils extracted from orange peels, the size of the tumor decreased and decreased. Tumor growth in mice, EAC tumor destroys the heart muscle and causes an increase in CK-Mb activity due to an increase in lipid peroxides, which causes enzyme breakdown and damage to cell membranes (34). These results were agreed with according to (23). EAC tumors in mice caused an increase in triglyceride levels, cholesterol, sodium, and potassium ions) 35). When treating mice with EAC tumors with essential oils extracted from orange peels, the levels of cholesterol, triglycerides, sodium, and potassium ions were improved compared to the group of mice that were treated with EAC. This means that these oils can improve

the heart's ability and restore blood circulation. These results are (36, 37). EAC caused an increase in the tumor factor AFP in the third group of mice that were treated with tumors only. As for the mice treated with essential oils (O.P.), the level of tumor factor AFP was reduced. These results were similar to (38). From the results obtained, it is clear that the EAC tumor causes an increase in CEA for the mice that were injected with EAC in contrast to the group under authority. Therapy using essential oils (O.P.) reduced CEA compared to mice that were treated with EAC. These results were consistent with (39).

Desmine is an intermediate filament-type protein found in cardiac striated muscle and, more broadly, in all muscle cells (40). Cancer is a tumor that results from defects and changes in the mechanism of the cell cycle as a result of the occurrence of abnormal cells that cause programmed cell death (23). Desmine immunopositive heart EAC, destructed muscle fibers with desmine immunopositive heart EAC Heart with minor +ve desmine stain after treatment with orange oils. These results agreed with both (41).

## 5. Conclusions

EAC causes a high rise in the levels of cholesterol, triglycerides, Ck-mb, sodium, and potassium ions, and an increase in the levels of AFP and CEA, in addition to damage to the heart muscle. Treatment with essential oils (O.P) led to an improvement in lipid levels and activity and can be used in the future as a treatment for liver and kidney cancer.

## Conflict of interest

There are no conflicts of interest for all authors.

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## 6. Reference

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers

in 185 Countries. *CA Cancer J Clin.* 2021 May;71(3):209-249.

2. Giaquinto AN, Miller KD, Tossas KY, Winn RA, Jemal A, Siegel RL. Cancer statistics for African American/Black People 2022. *CA Cancer J Clin.* 2022 May;72(3):202-229.

3. Oeffinger KC, Fontham ET, Etzioni R, Herzig A, Michaelson JS, Shih YC, Walter LC, Church TR, Flowers CR, LaMonte SJ, Wolf AM, DeSantis C, Lortet-Tieulent J, Andrews K, Manassaram-Baptiste D, Saslow D, Smith RA, Brawley OW, Wender R; American Cancer Society. Breast Cancer Screening for Women at Average Risk: 2015 Guideline Update from the American Cancer Society. *JAMA.* 2015 Oct 20;314(15):1599-614.

4. La Rocca E, Meneghini E, Lozza L, Fiorentino A, Vitullo A, Giandini C, Bonfantini F, Di Cosimo S, Gennaro M, Sant M, Pignoli E, Valdagni R, De Santis MC. Older age and comorbidity in breast cancer: is RT alone the new therapeutic frontier? *J Cancer Res Clin Oncol.* 2020 Jul;146(7):1791-1800.

5. Pagani O, Senkus E, Wood W, Colleoni M, Cufer T, Kyriakides S, Costa A, Winer EP, Cardoso F; ESO-MBC Task Force. International guidelines for management of metastatic breast cancer: can metastatic breast cancer be cured? *J Natl Cancer Inst.* 2010 Apr 7;102(7):456-63.

6. Elgharabawy RM, El Tantawy El Sayed I, Abd-Allah Rezk N, Tousson E. Therapeutic Impact of *Costus (Saussurea lappa)* Against Ehrlich Solid Tumor-Induced Cardiac Toxicity and DNA Damage in Female Mice. *Front Pharmacol.* 2021 Jun 28; 12:708785.

7. Oshiba RT, Touson E, Elsherbini YM, Abdraboh ME. Melatonin: A regulator of the interplay between FoxO1, miR96, and miR215 signaling to diminish the growth, survival, and metastasis of

- murine adenocarcinoma. *Biofactors*. 2021 Sep;47(5):740-753.
8. Tousson E, El Sayed I. E, El-Aleim H. A, Elabd M, Karhib M, Gebreel D. T. Impact of Poria Cocos Nanoparticles Extract Against Ehrlich Solid Tumour Induced Toxicity, Oxidative Stress and Apoptosis in Female Mice Kidney. *Biomed Pharmacol J* 2022;15(4).
9. Tousson E, Hafez E, Zaki S, Gad A. P53, Bcl-2 and CD68 expression in response to amethopterin-induced lung injury and ameliorating role of L-carnitine. *Biomed Pharmacother*. 2014 Jun;68(5):631-9.
10. Abd Eldaim MA, Tousson E, El Sayed IET, Abd El-Aleim AEH, Elsharkawy HN. Grape seeds proanthocyanidin extract ameliorates Ehrlich solid tumor induced renal tissue and DNA damage in mice. *Biomed Pharmacother*. 2019 Jul; 115:108908.
11. OZASLAN, Mehmet, et al. Ehrlich ascites carcinoma. *African Journal of Biotechnology*, 2011, 10.13: 2375-2378.
12. Erkin, Z., Franz, M., Guajardo, J., Katzenbeisser, S., Lagendijk, I., & Toft, T. (2009). Privacy-preserving face recognition. In *Privacy Enhancing Technologies: 9th International Symposium, PETS 2009, Seattle, WA, USA, August 5-7, 2009. Proceedings 9* (pp. 235-253). Springer Berlin Heidelberg.
13. Bureau C, Thabut D, Jezequel C, Archambeaud I, D'Alteroche L, Dharancy S, Borentain P, Oberti F, Plessier A, De Ledinghen V, Ganne-Carrié N, Carbonell N, Rousseau V, Sommet A, Péron JM, Vinel JP. The Use of Rifaximin in the Prevention of Overt Hepatic Encephalopathy After Transjugular Intrahepatic Portosystemic Shunt: A Randomized Controlled Trial. *Ann Intern Med*. 2021 May;174(5):633-640.
14. Cheli F, Baldi A. Nutrition-based health: cell-based bioassays for food antioxidant activity evaluation. *J Food Sci*. 2011 Nov-Dec;76(9): R197-205.
15. Miguel MG. Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules*. 2010 Dec 15;15(12):9252-87.
16. Burt S. Essential oils: their antibacterial properties and potential applications in foods--a review. *Int J Food Microbiol*. 2004 Aug 1;94(3):223-53.
17. Fromer, M., Roussos, P., Sieberts, S. et al. Gene expression elucidates functional impact of polygenic risk for schizophrenia. *Nat Neurosci* 19, 1442–1453 (2016).
18. Manthey JA. Fractionation of orange peel phenols in ultrafiltered molasses and mass balance studies of their antioxidant levels. *J Agric Food Chem*. 2004 Dec 15;52(25):7586-92.
19. ELVIÑA, Rolando O.; MOJICA, Elmer-Rico E. Orange peel essential oil as component of a metal sensor for Lead (II) ion determination in aqueous solutions. 2005.
20. ABBOOD, Alaa Saadi; LAZM, Anwar M.; HASAN, Ahmed F. Study of histopathological changes and the levels of TNF- $\alpha$  in Preterm Preeclamptic women. *Journal of AL-Farabi for Medical Sciences*, 2023, 1.1: 10-10.
21. AL-DULIMIA, Ali G.; HASAN, Ahmed F.; AL-MOGADAMY, Osama A. Anti-tumor Activity of Gold nanoparticles by Use High Content Screening Technique (HCS). *Journal of Medical and Life Science*, 2022, 4.3: 27-40.
22. Ezz, A. M., Zaki, S., & Tabl, G. A. (2023). Anti-diabetic effects of pomegranate peel extract and L-carnitine on streptozotocin induced diabetes in rats. *Biomedical and Pharmacology Journal*, 16(3), 1827-1835



23. Khalaf, A. N., & Abed, I. J. (2021). Evaluating the in vitro Cytotoxicity of Thymus vulgaris Essential Oil on MCF-7 and HeLa Cancer Cell Lines. Iraqi Journal of Science, 62(9), 2862–2871.
24. Mutar TF, Tousson E, Hafez E, Abo Gazia M, Salem SB. Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice. Environ Toxicol. 2020 Apr;35(4):528-537.
25. Parmar HS, Kar A. Antiperoxidative, antithyroidal, antihyperglycemic and cardioprotective role of Citrus sinensis peel extract in male mice. Phytother Res. 2008 Jun;22(6):791-5.
26. Hasan, A. F., Alankooshi, A. A., Abbood, A. S., Dulimi, A. G., Mohammed Al-Khuzaay, H., Elsaedy, E. A. & Tousson, E. (2023). Impact of B-Glucan Against Ehrlich Ascites Carcinoma Induced Renal Toxicity in Mice. OnLine Journal of Biological Sciences, 23(1), 103-108.
27. Alotaibi B, Tousson E, El-Masry TA, Altwaijry N, Saleh A. Ehrlich ascites carcinoma as model for studying the cardiac protective effects of curcumin nanoparticles against cardiac damage in female mice. Environ Toxicol. 2021 Jan;36(1):105-113.
28. Aldubayan MA, Elgharabawy RM, Ahmed AS, Tousson E. Antineoplastic Activity and Curative Role of Avenanthramides against the Growth of Ehrlich Solid Tumors in Mice. Oxid Med Cell Longev. 2019 Jan 13; 2019:5162687.
29. Hasan, A. F., Mutar, T. F., Tousson, E. M. & Felemban, S. G. (2021). Therapeutic Effects of Turnera diffusa Extract Against Amitriptyline-Induced Toxic Hepatic Inflammation. OnLine Journal of Biological Sciences, 21(2), 395-408.
30. Hasan A. F, Hameed H. M, Tousson E, Massoud A, Atta F, Youssef H, Hussein Y. Role of Oral Supplementation of Damiana (Turnera diffusa) Reduces the Renal Toxicity, Apoptosis and DNA Damage Associated with Amitriptyline Administration in Rats. Biomed Pharmacol J 2022;15(3).
31. Hameed, H. M., Hasan, A. F., Razoooki, Z. H., Tousson, E. & Fatoh, S. A. (2023). Orlistat induces renal Toxicity, DNA Damage, and Apoptosis in Normal and Obese Female Rats. OnLine Journal of Biological Sciences, 23(1), 25-32.
32. Ahmed, H., Chatterjee, B.P. & Debnath, A.K. Interaction and in vivo growth inhibition of Ehrlich ascites tumor cells by jacalin. J. Biosci. 13, 419–424 (1988).
33. Segura JA, Barbero LG, Márquez J. Ehrlich ascites tumour unbalances splenic cell populations and reduces responsiveness of T cells to Staphylococcus aureus enterotoxin B stimulation. Immunol Lett. 2000 Oct 3;74(2):111-5.
34. Maghamiour N, Safaie N. High Creatine Kinase (CK)-MB and Lactate Dehydrogenase in the Absence of Myocardial Injury or Infarction: A Case Report. J Cardiovasc Thorac Res. 2014;6(1):69-70.
35. Alankooshi, A. A., Alankooshi, A. A., Hasan, A. F., Tousson, E., El-Atrsh, A. & Mohamed, T. M. (2023). Impact of Coriander Seeds Extract Against Thyroidectomy Induced Testicular Damage and DNA Replication in Male Rats. OnLine Journal of Biological Sciences, 23(2), 193-201.
36. Wang L, Zhang Y, Fan G, Ren JN, Zhang LL, Pan SY. Effects of orange essential oil on intestinal microflora in mice. J Sci Food Agric. 2019 Jun;99(8):4019-4028.
37. Park MK, Cha JY, Kang MC, Jang HW, Choi YS. The effects of different extraction methods on essential oils from orange and tangor: From the peel to the essential oil. Food Sci Nutr. 2023 Oct 25;12(2):804-814.
38. Tousson E, Hafez E, Abo Gazia MM, Salem SB, Mutar TF. Hepatic ameliorative role of vitamin

B17 against Ehrlich ascites carcinoma-induced liver toxicity. *Environ Sci Pollut Res Int.* 2020 Mar;27(9):9236-9246.

39. Elsamie, G. H. A., El-Banna, S. G., Tousson, E., Felemban, S. G. & Hussein, M. S. (2021). Impact of Vitamin B17 Against Growth of Colitis Bearing Mice Induced Variations in Colon Structure, AFP, CEA and PCNA Immunoreactivity. *OnLine Journal of Biological Sciences*, 21(3), 228-234. <https://doi.org/10.3844/ojbsci.2021.228.234>.

40. Protonotarios A, Brodehl A, Asimaki A, Jager J, Quinn E, Stanasiuk C, Ratnavadivel S, Futema

M, Akhtar MM, Gossios TD, Ashworth M, Savvatis K, Walhorn V, Anselmetti D, Elliott PM, Syrris P, Milting H, Lopes LR. The Novel Desmin Variant p. Leu115Ile Is Associated with a Unique Form of Biventricular Arrhythmogenic Cardiomyopathy. *Can J Cardiol.* 2021 Jun;37(6):857-866.

41. Abed, I. J., Hussein, A. R., Abdulhasan, G. A., & Dubaish, A. N. (2022). Microbiological Effect of Lemongrass *Cymbopogon Citratus* and Spearmint *Mentha Spicata* Essential Oils as Preservatives and Flavor Additives in Yogurt. *Iraqi Journal of Science*, 63(7), 2839–2849.