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Journal of Medical and Life Science
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Impact of Essential Oils (Orange Peels) on Ehrlich Ascites Carcinoma Against Cardiac Damage in Female Mice

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DOI: 10.21608/jmals.2024.266129.1018

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.

Received: January 28, 2024. Accepted: March 2, 2024. Published: March 29, 2024

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 4º 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,

2.5×10^6 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% ± 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 (≥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 −20C 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 ± 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).

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)	$101.1^{b} \pm 6.48$	$105.4^{b} \pm 2.52$	$212.7^{a} \pm 4.42$	$115.3^{b} \pm 3.82$
Triglyceride (mg/dL)	$139.1^{c} \pm 2.46$	$133.4^{c} \pm 5.92$	$337.2^{a} \pm 4.22$	$242.6^{b} \pm 3.87$
Ck-mb (ug/mL)	$45.62^{b} \pm 1.43$	$22.67^{c} \pm 1.89$	$79.40^{a} \pm 3.36$	$38.97^{b} \pm 3.28$
Na (mmol/l)	$137.3^{a} \pm 7.54$	$146.3^{a} \pm 2.17$	$156.5^{a} \pm 1.29$	$141.9^{a} \pm 1.61$
K (mmol/l)	$10.93^{b} \pm 0.19$	$9.49^{c} \pm 0.04$	$16.23^{a} \pm 0.30$	$12.16^{a} \pm 0.46$

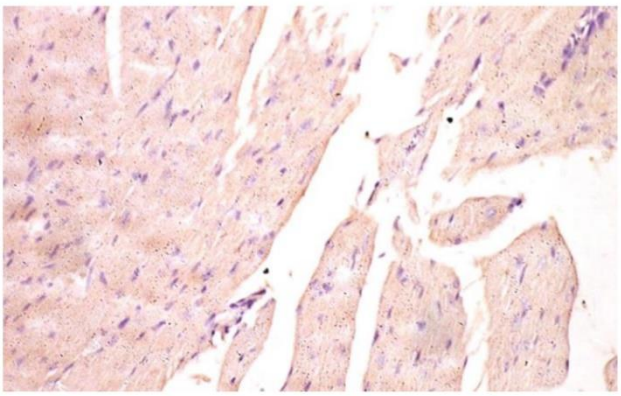
Values are expressed mean ± SE; n = 6 for each treatment group
Mean values within a raw not sharing common superscript letters were significantly different, $p<0.05$.
F: **F for One-way ANOVA test**, Pairwise comparison bet. each 2 groups was done using a **Post Hoc Test (Tukey)**
p: p-value for comparing between the studied groups.

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

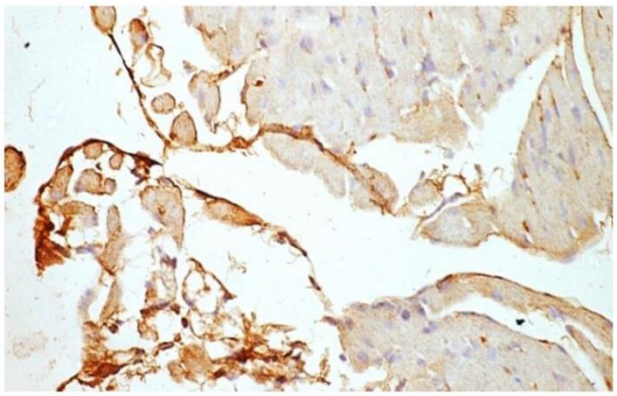
Parameters	Control	Essential oils	EAC	EAC +Essential oils
AFP (ng/mL)	$11.65^{c} \pm 0.20$	$11.75^{c} \pm 0.20$	$210.2^{a} \pm 0.45$	$61.25^{b} \pm 1.77$
CEA(ng/mL)	$1.10^{c} \pm 0.04$	$1.35^{c} \pm 0.02$	$42.75^{a} \pm 2.44$	$27.65^{b} \pm 0.20$

Values are expressed mean ± SE; n = 6 for each treatment group
Mean values within a raw 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.

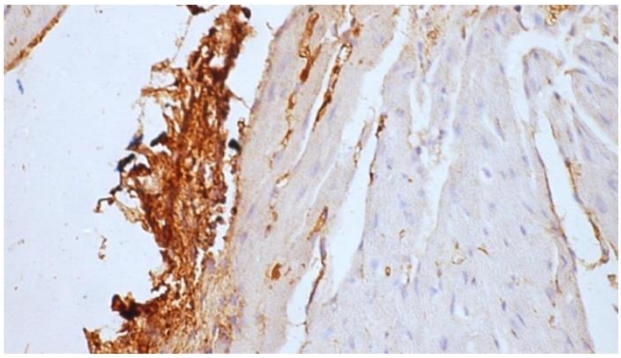
3.4. Desmine expressions in the heart:



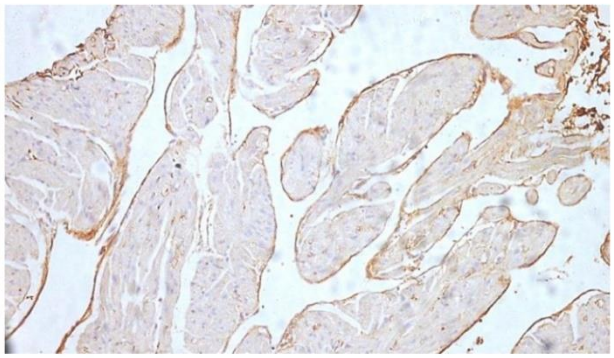
G1:.Heart control showing negative desmine immunopositive stain.



G2:. Heart Essential Oils showing intense desmine immunopostive stain.



G3:.Heart EAC showing destructed 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.

Funding: This research received no external funding.

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