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Therapeutic Effect of Essential Oils (*Citrus sinensis*) against Ehrlich Ascites Model Induced Renal Toxicity in Female Mice

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ABSTRACT

Background: Cancer is one of the most dangerous diseases of the twentieth century and the speed of its spread is horrific. It is characterized by cellular growth and invades all tissues of the body. In recent years, all countries of the world have turned to cancer research in order to identify new drugs to treat cancer from various sources, Cancer is the most common disease that causes death in the world and this is attributed to many pathological factors, such as reactive oxygen species, which contribute to the initiation, spread and development of cancer.

Methods: Our current research aims to investigate the therapeutic role of essential oils (C.S.) against Ehrlich Tumor renal damage in mice. The mice were divided into four group (Gp1, control group, Gp2, essential oils (C.S) group, Gp3, EAC group, Gp4, EAC with essential oils (C.S).

Result: Results showed the Essential oils (C.S) in pretreated (G4) lead to an improvement in urea, creatinine levels when compared to the EAC group. Through our results, it was shown that treatment with essential oils (C.S.) improved the levels of urea and creatinine comparing to the Gp3. It was noted that treatment with essential oils (C.S.) improved kidney tissue and VEGF protein expression the Gp4 compared to the Gp3 Groups. It could be concluded that essential oils (C.S) has a possible benefit to the kidneys against EAC induce kidney Toxicity.

Key words: Ehrlich ascites carcinoma, Eosin, Essential oils (*Citrus sinensis*), Hematoxylin, Renal function, VEGF protein.

INTRODUCTION

Cancer is one of the most dangerous diseases of the twentieth century and the speed of its spread is horrific. It is characterized by cellular growth and invades all tissues of the body (Kaufman *et al*., 1996). In recent years, all countries of the world have turned to cancer research in order to identify new drugs to treat cancer from various sources (Sannigrahi *et al*., 2012; Hasan *et al*., 2024). Cancer is the most common disease that causes death in the world and this is attributed to many pathological factors, such as reactive - oxygen species (ROS), which contribute to the initiation, spread and development of cancer, Oxidative stress can cause genetic instability and be a cause of the development of cancer, Also, (ROS) is considered responsible for Transcription factors and disease induction (Adriana *et al*., 2006; Al-Dulimia *et al*., 2022; Hasan *et al*., 2024). The spread of the tumor inside the cells causes immunosuppression and inhibition of all the body's anti-tumor functions due to the weakness of the immune system (Singh *et al*., 2005). There are many methods of preventing or treating cancer, the most common of which is chemotherapy, which is considered more effective, but it is very important to conduct many researches in the field of cancer for the purpose of treating those affected without harming the patient's normal cells (Dash *et al*., 2007; Sharma *et al*., 2022; Naik *et al*., 2021). Ehrlich's ascites carcinoma (EAC) is a malignant tumor, but it is undifferentiated, has a short lifespan, has the ability to metastasize, does not have a tumor-specific culture antigen and is very dangerous (Hasan *et al*., 2024). Essential oils are plant extracts used as aromatherapy and are considered alternative medicine and have the ability to relieve pain (Manzur *et al*., 2023). Essential oils are originally a group of compounds extracted from fruits and seeds for the purpose of obtaining oil in several different concentrations. They directly target the olfactory receptors, affecting the central nervous system. These oils contain anti-bacterial, anti-cancer and anti-fungal activity (de Aquino *et al*., 2023; Rajkumar *et al*., 2023). Essential oils (orange peels) are concentrated and effective oils that have many

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benefits, including improving sleep quality, reducing depression and reducing anxiety. These oils contain a substance called (Limonene) as a natural substance that prevents the growth of cancer cells and kills them in many cases of colon, prostate and lung cancer (Amala Dev *et al*., 2023; Hamdi *et al*., 2023; Hamdan *et al*., 2024). Orange oils are rich in vitamin C, thymine and many antioxidants and have health benefits. Orange oil contains many chemical agents that stimulate blood circulation and preserve the skin, such as myrcene, limonene, camphor and eugenol (Brito *et al*., 2021; Li *et al*., 2021).

MATERIALS AND METHODS

Essential oils (*Citrus sinensis*)

These oils were extracted from (*Citrus sinensis*) by evaporation method (Cleavenger) as per the method described by (Khalaf *et al*., 2021).

Animals

40 mice (female albino Swiss) were purchased. Their weight ranged between (20-25 grams) and their age ranged from 11 to 12 weeks. They were warmed at a temperature of 25°C and placed in special cages according to method of Mutar *et al*. (2020). The present study was carried out In the Department of Plant Biotechnology, Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq.

Induction of ehrlich ascites model celle

EAC cells were purchased from the Egyptian National Cancer Institute and these cells were placed in an equal saline solution, after which each mouse was injected with these cells intraperitoneally (ip) at a dose of (2.5×10^6 cells/20 g body weight). The use of the animal for the experiment was approved by the Institutional Animal Care and Use Committee approval number - IACUC-SCI-TU-0233. All the mice were monitored on a daily basis until the tumor appeared on the eighth, ninth, tenth and Fourteenth days.

Experimental design

The mice were divide into four groups of (10 mice each/ group).

1st group: Natural mice.

2nd group: Mice treated with essential oils (*Citrus sinensis*) at an oral dose (25 mg/Kg. body weight/day) according to (Parmar *et al*., 2008).

3rd group: Mice treated with EAC cell line intraperitoneally according to (Hasan *et al*., 2023).

4th group: Mice treated with EAC cells and treated with essential oils (*Citrus sinensis*) for fourteen days in a row. This study began in July 2020 and the practical part was completed in April 2022.

Blood and tissue sample collection

Following the conclusion of the trial, the mice were put in a cage containing the sedative sodium pentobarbital (≥100 mg/kg) for the purpose of anesthetizing the mice and dissecting them. Blood was drawn from the inferior vena cava into tubes containing heparin and the plasma was separated by a centrifugation at 3000 g for 20 minutes and stored in tubes. at -20°C. Kidney tissue was also collected and placed in a saline solution containing 10% diluted formalin, to perform histological examination and immunohistochemistry.

Biochemical assays

Serum urea and creatinine concentration tests were conducted by Diamond Company in Egypt according to (Ezz *et al*., 2023; Hasan *et al*., 2022). Rewrite this sentence as "Serum urea and creatinine were estimated using diagnostic kits obtained from (Kandeler *et al*., 2024), following the methods of Eisenhofer *et al*. (2023).

Immunohistochemical investigation

Kidney 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 and H) for histological examination according to method of Alankooshi *et al*. (2023); Hasan *et al*. (2024).

Histopathological examination

The kidney tissues were fixed with 10% formalin for 48 hours. Kidney sections were fixed and dried in a layer of paraffin and stained with Hematoxylin and Eosin according to method of Hameed *et al*. (2023) and Hasan *et al*. (2021).

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 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.

RESULTS AND DISCUSSION

Recently, scientists have become widely interested in the field of discovering new treatments for cancer and essential oils (*Citrus sinensis*) have been of interest due to their broad effectiveness as an antioxidant. The main benefit of our research is knowing the therapeutic role of essential oils (*Citrus sinensis*) as an antidote to kidney toxicity caused by EAC. Our results indicate that EAC initiates changes in kidney function as detected by elevations in urea levels and depletion of creatinine levels. The results showed that EAC cells cause changes in kidney function in the EAC group (Fig 1 and 2), as they cause an increase in urea levels compared to the control group. These results were supported by the findings of Aldubayan *et al*. (2019) and a

decrease in creatinine levels in the EAC group compared to the control group. These results differ from the findings of Mutar *et al*. (2020). Our results indicate that EAC causes damage to kidney tissue compared to the essential oil group and these results were supported by the findings of Hasan *et al*. (2023). Our current results showed that treatment with essential oils (*Citrus sinensis*) treats kidney functions, as we notice a decrease in urea levels and an increase in creatine levels. Our results agreed with findings of Mohamed *et al*. (2010) and Alharbi *et al*. (2023). Also, our results showed an improvement in kidney tissue in the fourth group (*Citrus sinensis*) compared to the EAC group (Fig 3). These results were supported by (the findings of Soji-Omoniwa *et al.* (2014) and Bouzenna *et al.* (2016). Vascular endothelial growth factor (VEGF), was initially identified as a mitogen unique to endothelial cells, VEGF is generated by a variety of cell types, including as renal mesangial cells, keratinocytes, macrophages, platelets and tumor cells.

In the immunochemical examination of VEGF proteins, our results showed a positive reaction in renal tubules and glomeruli in kidney tissue for EAC cells in the third

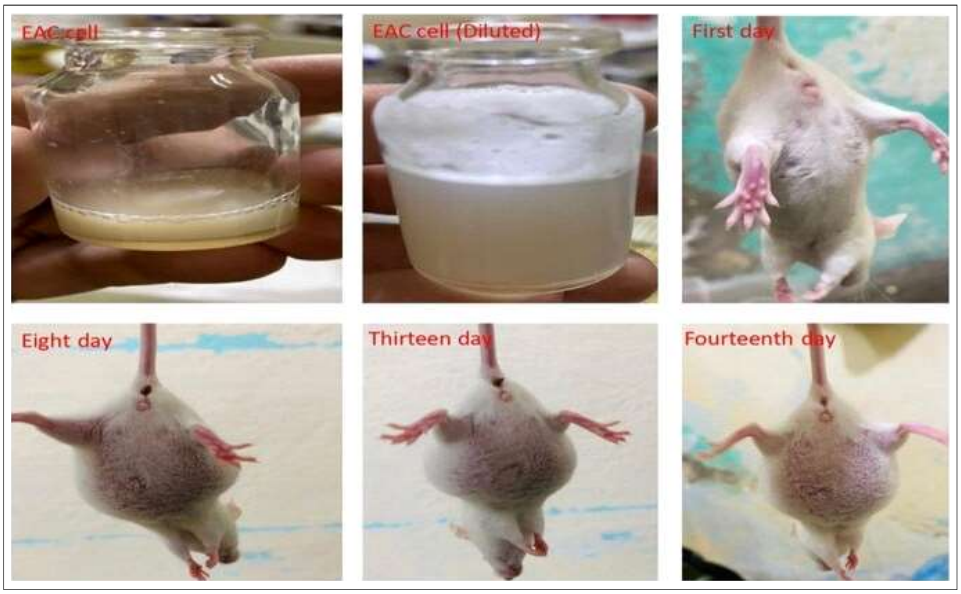
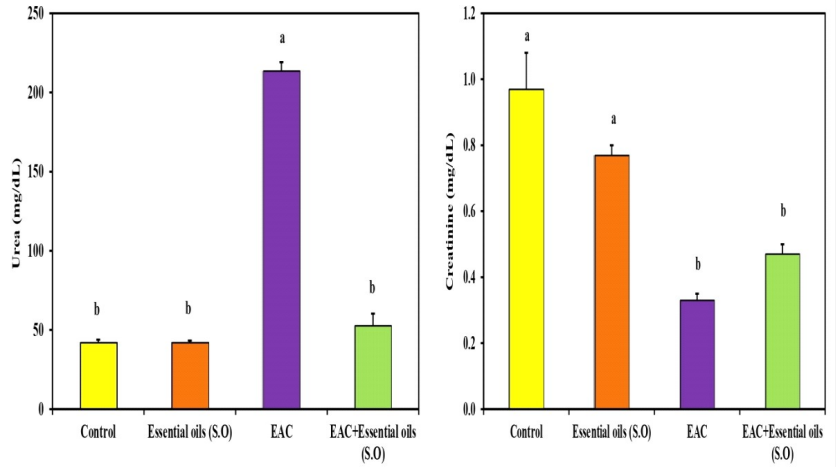
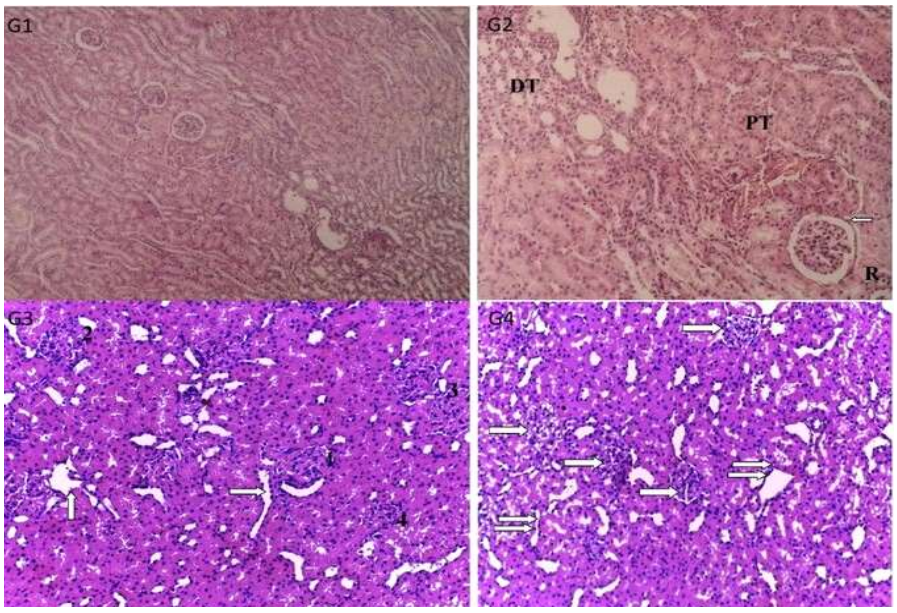


Fig 1: A set of figures showing the growth of EAC cells from the first day to the fourteenth day according to (Hameed *et al*., 2024).



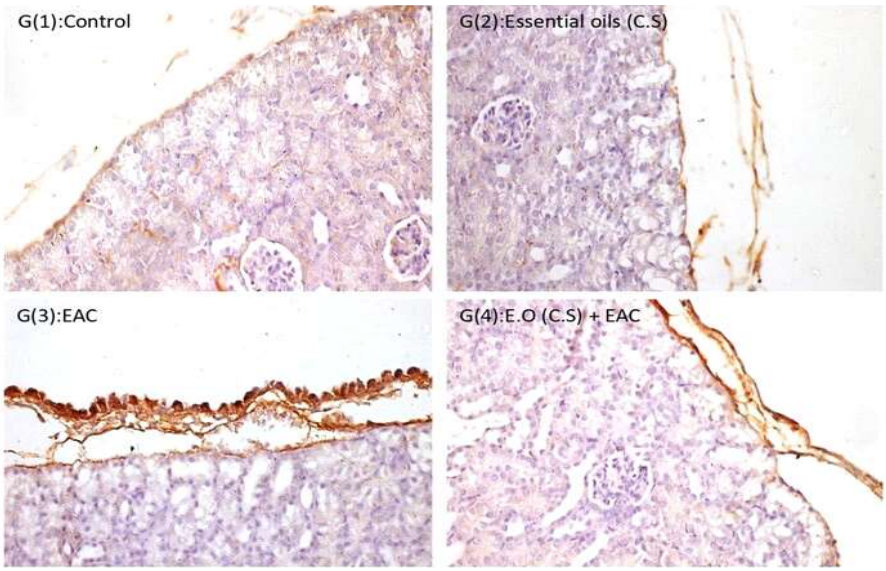
Serum urea levels were significantly (P<.05) increased in EAC Group as compared with control mice. However, were significantly (P<.05) decreased in Essential oils (C.S) + EAC Group as compared with Essential oils (C.S) mice. Serum creatinine levels were significantly (P<.05) decreased in EAC Group as compared with control mice. However, were significantly (P<.05) increased n essential oils (C.S) + EAC Group as compared with essential oils (C.S) mice.

Fig 2: Effect of essential oils (C.S) on renal function parameters in the serum of EAC bearing mice.



G1 and G2: Mice kidney sections in control and Essential oils (C.S) groups revealed normal structure of bowrmans capsules and renal tubules. G3: Mice kidney sections in EAC groups showed marked degenerated (white arrows) and dilatation of kidney tubules, coagulative necrosis (black arrows) and congestion of blood vessels with RBCs. G4: Kidney sections in mice treated with EAC and essential oils (C.S) showed mild intertubular haemorrhage, moderate leucocytic infiltrations and degenerated (white arrows) of kidney tubules.

Fig 3: Essential oils (C.S) improved renal tissue against EAC induced histopathological changes photomicrographs of kidney sections in the different experimental groups stained with haematoxylin and eosin.



G1: Immunopositive staining in newly formed blood capillaries in between renal tubuli. G2: Kidney showing mild iimunopositive staining. G3: EAC Kidney showing brown iimmmunopostive VEGF in kidney capsule. G4: Kidney showing -ve immunostaining in the kidney capsule.

Fig 4: Effect of Essential oils (C.S) on Renal VEGF protein expression photomicrographs of kidney sections in the different experimental groups stained with Vascular endothelial growth factor (VEGF).

group compared to the control group according to Deluque *et al.* (2022) and Elhady *et al.* (2022).

The method of treatment with essential oils (*Citrus sinensis*) for tumor-bearing mice in the fourth group showed an improvement and a decrease in VEGF protein in kidney tissue compared to the EAC group (Fig 4) due to its effects on the cells. Our results agreed with (Rojas-Armas *et al*., 2022; Miastkowska *et al*., 2021).

CONCLUSION

These results indicate that Essential oils (*Citrus sinensis*) may be an innovative and dependable therapy For EAC, confirm the malignant activity even more of essential oils (*Citrus sinensis*) as a possibility Based on what we've found, it appears that Essential oils (*Citrus sinensis*) can serve as a successful course of therapy against EAC, We advise more testing and investigation into its efficacious application in the management of various ailments.

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Data availability

Data supporting our study results are accessible from the relevant author whenever needed.

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Conflict of interest

The authors declare no conflict of interest.

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