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Antitumor efficacy of *Urtica sp.* leaves extract: *in vitro* and *in vivo* studies

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Abstract

Background: Natural products demonstrated potential for use in medicine and pharmaceuticals. **Objective:** This study evaluated the phytochemical composition of *Urtica sp.* leaves extract (USLE), and it's *in vitro* and *in vivo* antitumor effects. **Methods:** HepG-2 and MCF-7 cell lines were used to evaluate the IC_{50}, apoptosis, and cell cycle analysis of USLE. Forty female CD-1 mice were equally divided as follows; Gp1 was control; Gp2, Gp3, and Gp4 were inoculated with 1×10^6 of Ehrlich ascitic carcinoma (EAC)-cells/mouse. Then, Gp3 had injected with Cisplatin (2 mg/kg) intraperitoneally (i.p). Gp4 had injected with USLE (100 mg/kg) i.p. as in Gp3. Total tumor volume, total tumor cell count, and live and dead EAC-cells were determined, also biochemical and histopathological investigations were evaluated. **Results:** Data showed that the USLE had cytotoxic and antitumor effects against the HepG-2, MCF-7, and EAC-bearing mice. Biochemical and histopathological investigations showed an improvement in the liver and kidney tissues upon treatment of EAC-bearing mice with USLE. **Conclusion:** The results showed that the USLE can stop tumor growth and cause tumor cells to die.

Keywords: *Urtica sp.*, GC-MS, Antioxidants, Antitumor, EAC-bearing mice

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Introduction

Chemotherapeutic drugs are the most valuable choices by physicians for cancer treatment. Despite their efficacy, adverse effects on the vital organs are associated [1]. Therefore, in preclinical and clinical studies, finding new avenues to decrease the therapeutic doses of the chemotherapeutic agents through co-administration with other natural agents is necessitating [2]. Other studies were focused to find new settings to decrease the adverse effects of chemotherapy [3]. In addition, another set is focused to increase the effect of the chemotherapeutic agents by having natural products as potentiators for chemotherapies [4].

Most medicinal were investigated to exert their antitumor effect or screen their effect to ameliorate

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the toxic effects of the chemotherapy [5] For instance, the antitumor efficacy of *Rosmarinus officinalis*, *Spirulina platensis*, *Nigella sativa*, and scorpion venom were evaluated as anticancer agents [6].

Urtica sp. is an annual herb that belongs to the family *Urticaceae* and is widely distributed around the world. Its height is up to 0.6 m. Its flowers are monoecious. It prefers moist soil and cannot grow in shade. It exists in many nations, including Egypt. Although there is disagreement among experts over the number of species that make up the genus *Urtica*, 30 species have been documented [7]. Numerous phytochemical components found in *Urtica species*, including flavonoids, and phenolic compounds like diocanol, alcohols, and terpenes, have been linked to a variety of pharmacological effects [8]. The extracts of *Urtica sp.* have been known in traditional medicine. Extracts of different *Urtica sp.* have been used against various diseases in experimental animals [9]. It has been reported several pharmacological activities of *Urtica dioica* leaves. Furthermore, *Urtica sp.* has historically been used as a blood purifier, hemostatic, and stimulating tonic, as well as to increase hemoglobin levels [10]. In addition, *Urtica sp.* root could inhibit some of the consequences of prostatic hyperplasia and markedly improve the paclitaxel sensitivity of breast cancer cells [11].

Materials and Methods

Chemicals

Dimethyl sulfoxide (DMSO), propodium iodide (PI), and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). All biochemical kits were purchased from Bio-diagnostic Company (Egypt).

Preparation of *Urtica sp.* leaves extract

Urtica sp. leaves (USL) were obtained from the Crop Institute Agricultural Research Center, Giza, Egypt. Then transferred into the laboratory in the Zoology Department, Faculty of Science, Tanta University. 50 g of powdered leaves were added to 500 ml of 70% ethanol. The supernatants were filtered and left to dry to get the hydro-alcohol *Urtica sp.* leaves extract (USLE) for further use.

Human cell lines and determination of the cytotoxic effect of USLE

Human MCF-7 and HepG-2 cancer cell lines were obtained from VACSERA Tissue Culture Unit (El-Doki, Cairo, Egypt). Cells were propagated in their specific media. The USLE cytotoxic effects against HepG-2 and MCF-7 cells were assessed by MTT assay. Briefly, HepG-2 or MCF-7cells ($5x10^5$ cells/ ml) were seeded in DMEM in a 96-well plate. The plates were incubated at 37°C, 5% CO_2 for 48 h. Then, 10 µl MTT (5 mg/ml) was added into each well to generate formazan.

Determination of the USLE effects on apoptosis and cell cycle of MCF-7

Apoptotic cells were further analyzed by Annexin V-FITC assay. Briefly, MCF-7 cells were cultured and then treated with 1/10 of USLE IC_{50} as described earlier. After treatment for 24 h, the cells were harvested and re-suspended in 100 µL of kit binding buffer with the addition of 1 µL of FITC-Annexin V followed by 40 min. incubation at 4 °C. Then, the cells were analyzed using the flow cytometer BD FACS Calibur. To determine the effect of USLE on the cell cycle distribution of MCF-7 cells, cell cycle analysis was performed using the Cycle TEST^{TM} PLUS DNA Reagent Kit (Becton Dickinson Immuno-cytometry Systems, San Jose, CA). The MCF-7 cells were stained with PI stain following the procedure provided by the kit and then run on the cytometer.

Mice, tumor inoculation, and experimental design

Forty female CD-1 mice (20 ± 2 g) were purchased from National Research Center (NRC, Cairo, Egypt). Animals were housed (5/cage), in a 12 h/12 h dark/light cycle under laboratory conditions of temperature and humidity. The experimentations were performed in compliance with the ethical guidelines

approved by the animal care and use committee in the Faculty of Science, Tanta University, and numbered (IACUC-SCI-TU-0241). Mice were kept for a week before starting the experiment for adaptation. The EAC cells were collected from the tumor-bearing mice purchased from the National Cancer Institute (NCI, Cairo, Egypt). Then, tumor cells were adjusted at 1×10^6 cells/mouse for i.p inoculation. Mice were then divided into four groups (n=10). The 1st group (Gp1) was used as a negative control. Gp2, Gp3, and Gp4 were inoculated with 1×10^6 of EAC-cells/mouse. Then, Gp3 had injected with Cisplatin (2 mg/kg) i.p after 24 hours of EAC-cells inoculation for 6 consecutive days. Gp4 had injected with USLE (100 mg/kg) i.p under the same protocol treatment as in Gp3. All mice were bled to collect blood for biochemical assessments. Total tumor volume, total tumor cell count and live, and dead cells were determined in the ascetic tumor fluid. Liver and kidney tissues were harvested and fixed for oxidative stress parameters and histopathological investigations.

Determination of biochemical parameters

Serum ALT, AST activities, urea, and creatinine levels were determined. Furthermore, hepatic SOD, CAT activities, and MDA levels were determined by using Bio-diagnostic kits.

Histopathological investigation

The liver and kidney tissue samples were taken and preserved in 10% formalin. Different alcohol and xylene grades were used to process the tissues. Using a microtome (mark, company) to cut sections (5 mm) from paraffin blocks, the histopathological changes were examined under a light microscope (Optika light microscope, B-350) to look for obvious cellular damage [12].

Statistical analysis

ANOVA with a one-way analysis of variance was utilized to evaluate the significant variations between treatment groups. To demonstrate the significance of the treatment's impact, the Dunnet test was performed to compare each group to the control group. $P \leq 0.05$ was chosen as the threshold for statistical significance.

Results

The *in vitro* cytotoxic effect of USLE against HepG-2 cells and MCF-7 cells

The results showed that USLE had significant cytotoxic effects on HepG-2 and MCF-7 cells after 48 h of treatment. The IC_{50} of the USLE against HepG-2 and MCF-7 cells were 22.36 ± 2.45 and 40.52 ± 3.95 (Figure 1).

Effect of USLE on apoptosis and cell cycle of tumor cells

As shown in Figure 2, the percentages of the necrotic, early apoptotic, and late apoptotic HepG-2 cells in the untreated and USLE-treated conditions were 1.5, 0.27, 0.49%, 2.5, 7.7, and 9.2%, respectively. While the percentages of the necrotic, early apoptotic, and late apoptotic MCF-7 cells in the untreated and USLE-treated conditions were 0.95, 0.14, 0.35%, and 15.3, 5.8, and 2.3%, respectively.

The antitumor effect of USLE in EAC-bearing mice

The results showed that USLE treatment results in a significant decrease in the total tumor volume, total EAC-cells, and total live cells count, however, total dead cells count significantly increase as compared to tumor-bearing mice alone (Table 1).

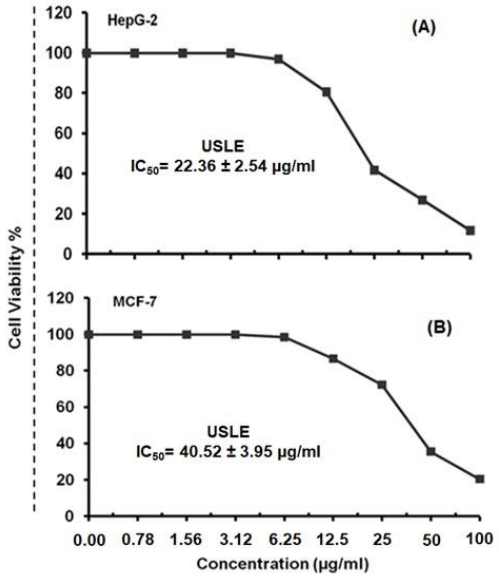

Figure (1): The IC_{50} of USLE against HepG-2 cells (A) and MCF-7 cells (B).

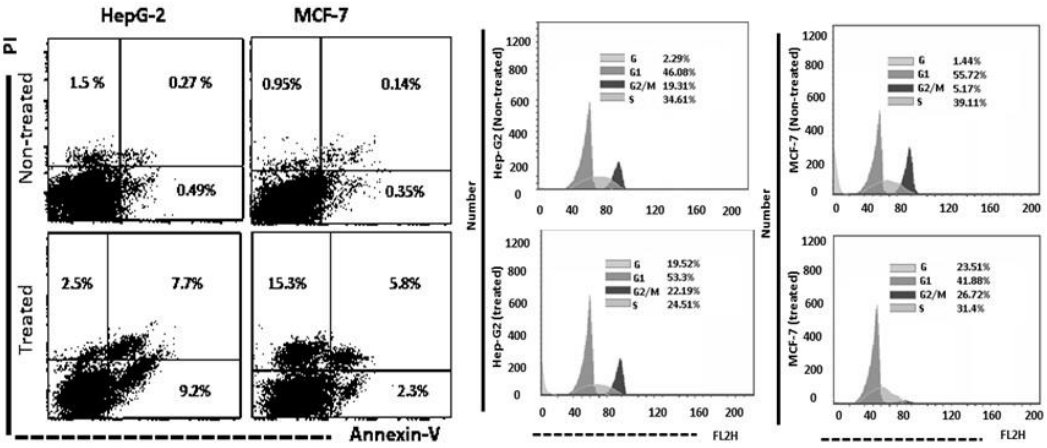

Figure (2): Apoptotic percentage **(A)** and Cell cycle **(B)**.

Table 1. The tumor volume, total cells count, live and dead cancer cells in different groups of mice under the study

Groups	Total tumor volume	Total cells count ×(10^6) /mouse	Total live cells count × (10^6) /mouse	Total dead cells count × (10^6)/mouse
EAC alone	10.5 ± 1.5	655 ± 19	625 ± 34	30 ±1.13
EAC/Cis	1.0 ± 0.8	120 ± 15	20 ± 2.9	100 ± 9.56
EAC/USLE	3.8 ± 1.2	260 ± 16	195 ± 8.5	65 ± 7.45

The values represented mean ± SD; USLE: *Urtica sp.* leaves extract.

Treatment with USLE after EAC-cells inoculation ameliorated hepato-renal dysfunctions

The result showed that EAC-cells inoculation led to a significant increase in the serum ALT and AST activities when compared to their values in the control group. However, treatment with USLE after EAC-cells inoculation decreased ALT and AST activities when compared to the EAC-cells inoculation group (Figure 3). Serum urea and creatinine levels were significantly increased in the EAC-cells inoculation group compared to the control group. However, USLE treatment results in a significant improvement in the serum levels of creatinine and urea.

Treatment with USLE improved the antioxidants/oxidants hemostasis

As shown in Figure 4, SOD and CAT activities were significantly decreased in EAC-bearing mice with a significant increase in MDA levels when compared to normal control. Treating EAC-bearing mice with USLE led to significant improvement in the antioxidant status evidenced by a significant increase in SOD and CAT activities and a significant decrease in MDA level.

Treatment with USLE restored histopathological alterations in the liver and kidney tissues

The control group (Gp1) showed normal architecture of hepatocytes radiation from the central vein. Narrow blood sinusoids surrounded by endothelial cells and unique phagocytic Kupffer cells alternated with the liver strands (Figure 5A). The liver sections of EAC-bearing mice (G2) exhibited marked disorganization of hepatic architecture, congested central veins, and cellular infiltration, some hepatocytes showed eosinophilia, and others have degenerated with darkly stained nuclei (pyknotic). Also, irregular, and dilated blood sinusoids were noticed (Figure 5B). Liver sections of EAC/Cis-treated mice showed an increase in hepatic disorganization with a centrilobular pattern of degeneration, necrosis, vacuolated hepatocytes, branched and congested central vein, and enlargement of blood sinusoids, pyknotic, vesicular nuclei, and megakaryocytic nuclei in their liver (Figure 5C). While treatment the mice with EAC/ USLE (G4) showed improvement with normal hepatic architecture with central vein, normal hepatic lobulation, binucleated hepatocytes, and regular blood sinusoids with distinct phagocytic Kupffer cells were distinct (Figure 5D).

The control group's (G1) kidney tissue had normal glomeruli with normal bowman's corpuscles, normal renal tubules, and normal renal parenchyma, all of which were visible as the cortical region (Figure 6A). Kidney sections of EAC- the treated group of mice (G2) showed disorganized and destructed glomeruli with distorted Bowman’s capsule with widening space, most of the renal tubules were disorganized, dilated, destructed, and degeneration of the epithelial cells lining was exhibited with hyaline casts (Figure 6B). Kidney sections of the EAC/Cis treated group (G3) showed an increase in renal damage and intertubular hemorrhage. Glomeruli appeared congested, shrunken, and destructed with irregular bowman's space; most of the renal tubules were injured with degenerated epithelial lining cells making them lose their characteristic appearance (Figure 6C). While kidney sections of the EAC/ USLE treated group (G4) exhibited improvement with a normal-like structure of the glomeruli with regular Bowman’s space and normal renal tubules (Figure 6D).

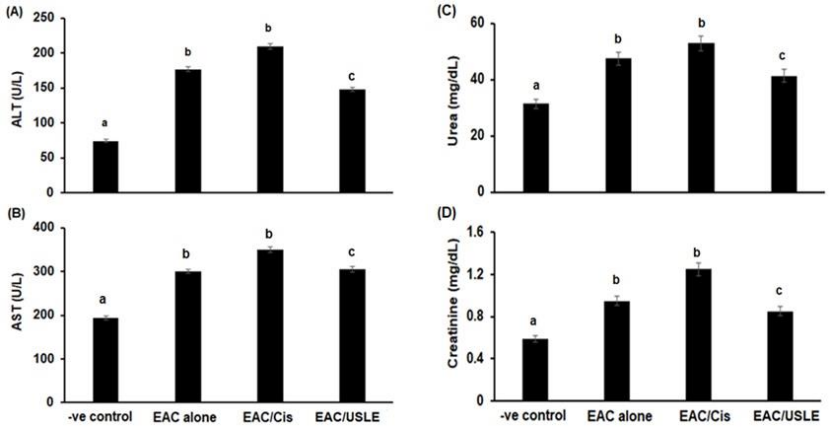

Figure 3. Treatment of EAC-bearing mice with USLE improves liver function enzymes. **(A)** Alanine transaminase (ALT), and **(B)** Aspartate transaminase (AST), **(C)** Urea, (D) Creatinine in different groups under study.

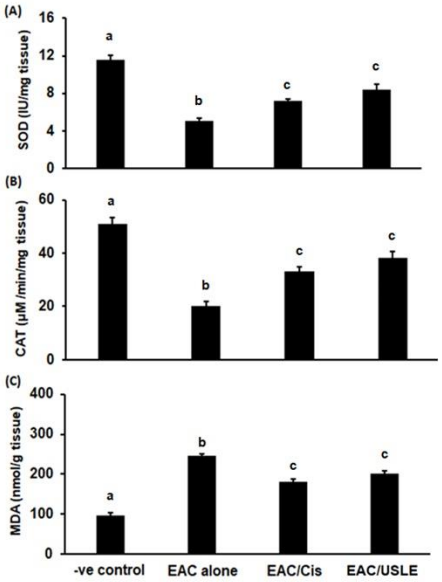

Figure 4. Treatment of EAC-bearing mice with USLE enhances antioxidant/oxidant status. (A) Superoxide dismutase (SOD), (B) Catalase (CAT), and (C) Malondialdehyde (MDA) in different experimental groups.

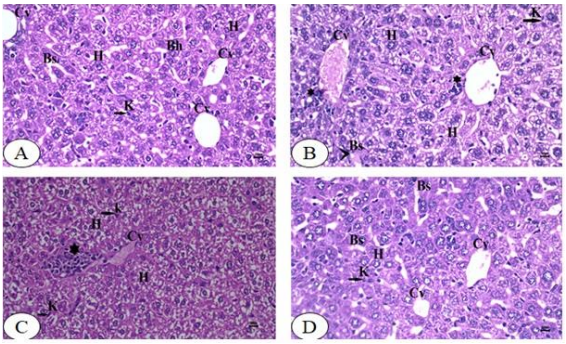

Figure 5. Photomicrographs of the liver sections stained with Haematoxylin & Eosin. (A) The liver section of the control group of mice (G1) exhibits normal hepatic structure, central vein (Cv), radial hepatic strands (H), and regular blood sinusoids (bs) lined with normal kupffer cells (K). (B) The liver section of EAC-treated mice (G2) exhibits marked disorganization of hepatic architecture, congested central veins (Cvs), cellular infiltration (*), some hepatocytes show eosinophilia (thick arrows), others have degenerated with pyknotic nuclei (thin arrows). Also, irregular, and dilated blood sinusoids (bs). (C) The liver section of the EAC/CIS treated group showing vacuolated hepatocytes (H), branched and congested central vein (Cv), large and irregular blood sinusoids, pyknotic, vesicular nuclei, and megakaryocytic nuclei, distinct kupffer cells (arrows) as well as cellular infiltration (*) were seen. (D) The liver section of the EAC/USLE -treated group (G4) shows a normal-like structure of hepatic architecture, normal strands of hepatocytes (H), some hepatocytes with megakaryocytic nuclei (arrows), others with karyolitic ones (thick arrows), slightly irregular and dilated blood sinusoids (bs) with kupffer cells (x 400).

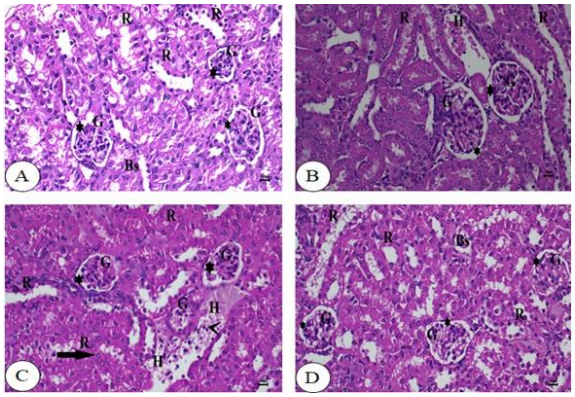

Figure 6. Photomicrographs of kidney sections stained with H&E. (A) Kidney section of the control group of mice (G1) reveals normal glomeruli (G) with Bowman's space (*) and regular renal tubules (R). (B) The kidney section of EAC treated group (G2) exhibits disorganized and destructed glomeruli (G), widening of Bowman's space (*), dilation of the renal tubules (R), mostly tubules lost their characteristic appearances, degeneration of the lining epithelial cells and existence of hyaline casts. (C) Kidney section of the EAC/CIS-treated group (G3) showing congested, shrunken, and destructed glomeruli (G), irregular bowman's space (*), intertubular hemorrhage (H, head arrow), the majority of the renal tubules were injured with degenerated epithelial lining cells make it losing their characteristic appearance were intermixed with each other (arrows). (D) Kidney section of EAC/USLE treated group (G4) showing a normal-like structure of the glomeruli (G), a slight widening of the Bowman's space (*), and more or less normal renal tubules (R), (x 400).

Discussion

Urtica dioica L. can be used as a functional food product that has significant contents of nutritional and biological compounds with a corresponding antioxidant capacity [13]. Kataki *et al.* (2012) indicated significant antioxidant activity of the *Urtica dioica* extract comparable to standard antioxidant compounds [14]. The present study evaluated the *in vitro* and *in vivo* antitumor effects of *Urtica sp.* leaves extract (USLE). This study reported the effect of USLE on the cytotoxic activity of cancer cell lines, where USLE showed a significant apoptotic effect on the HepG-2 cells and MCF-7 cancer cell line. In agreement with the previous study performed by Ahmed *et al.* (2020) who stated that *Urtica dioica* extract showed a significant apoptotic effect on the bladder cancer cell line [15].

According to this study, USLE has the highest levels of phenolic and flavonoids and the highest antioxidant activities, both of which are correlated with its cytotoxic effects on tumor cells. These results are following Hodroj *et al.* (2020) who indicated the presence of multiple chemical agents in *Urtica dioica* aqueous extract, such as flavonoids and phenolics, which may be attributed to the pro-apoptotic and anti-tumor effects [16]. Gaafar *et al.* (2020) attributed the cytotoxic effect of the *U. dioica* extract against breast cancer and colon cancer cells to its richness of oxygenated and nitrogen-containing compounds [17]. It has been shown that aqueous extracts of *Urtica dioica* leaves can reduce the proliferation of the MCF-7 cell line. [18]. A previous study determined the potential anticancer activity of *Urtica dioica* L. on human acute myeloid leukemia cells and apoptosis induction through the intrinsic pathway [19]. *U. dioica* leaves inhibited the growth and proliferation of human prostate cancer cells, showing an IC_{50} concentration of 15.54 μg/mL in 48 h exposure and a cell cycle arrest in the G2/M phase [20]. Fattahi *et al*. (2018) demonstrated that *Urtica dioica* affects the expression of the genes for ornithine decarboxylase and adenosine deaminase to cause apoptosis in breast cancer cells. [21].

Our data makes USLE treatment might represent an ideal therapeutic tool as an anticancer agent in agreement with Mohammadi *et al.* (2017) who stated that the *Urtica dioica* extract significantly reduced the tumor masses in the induced breast cancer mice model, diminished the size and weight of the tumors removed from the treated mice, and induced apoptosis [20]. Treatment with USLE significantly reversed the oxidative stress-associated changes, which could be due to the improvement of the antioxidant defense system in agreement with Kataki *et al.* (2012) who found that pretreatment of animals with *Urtica dioica* extract at the dose levels of 100, 200, and 400 mg/kg resulted in a significant decrease in MDA level as well as a significant increase in SOD level [14]. The same results have been shown by Pérez Gutiérrez *et al.* (2021) they demonstrated that the *Urtica dioica* extract increased the activity of enzymatic antioxidant enzymes (SOD, CAT) which heightened the levels of GSH, while decreasing the MDA in hepatic tissues in diabetic mice [23]. Moreover, Keleş *et al.* (2020) showed that *Urtica dioica* treatment produced a significant decrease in MDA and an increase in GSH as markers of oxidative stress in urinary calculi in rats [24]. Goorani *et al.* (2019) indicated that *U. dioica* significantly increased the levels of SOD and CAT when compared to the untreated group in diabetic mice [25]. The *U. dioica* leaves can lower the high degree of oxidative stress, especially flavonoids, that are present in malignant cells as a result of their high concentration of antioxidant and free radical scavenger components. [26].

The elevation levels of ALT and AST in EAC-tumor-bearing mice is an index of deterioration of hepatic functions due to cancer proliferation as observed in the EAC group and it suggested a functional impairment of hepatic cell membranes and a cellular leakage which demonstrated that EAC-induced

liver injury [27]. USLE induced a moderate degree of improvement in reversing these alterations in the hepatic enzymes as it can inhibit lipid peroxidation and prevent oxidative stress. *Urtica dioica* extract can protect the liver, particularly on membrane permeability due to its function as a radical scavenger [23]. Also, Kataki *et al.* (2012) observed a significant hepatoprotective effect of *Urtica dioica* extract as suggested by the decrease in serum ALT, AST, ALP, and total bilirubin levels towards normalization in hepatic injured rats [14]. In addition, Eldamaty (2018) stated that the diabetic rat fed on *Urtica dioica* leaves powder decreased the levels of AST, ALT, and ALP in comparison with the control positive [28]. Kisaka (2015) suggested that *U. dioica* had a hepatoprotective effect as it restored the ALT, AST, and albumin levels like control in acetaminophen-induced hepatotoxicity in mice [22].

Sayhan *et al.* (2012) suggested that *Urtica dioica* L treatment has a protective effect against renal damage, possibly due to its ability to inhibit renal damage, apoptosis, and cell proliferation [29]. Another study showed that *Urtica dioica* ethanol extracts significantly reduced serum levels of creatinine and urea which increased by ethylene glycol and was effective in the prevention and treatment of kidney stones, maybe due to its antioxidant effects and concentration of stone-forming components in the urine [24]. *U. dioica* significantly reduces the raised levels of blood urea and creatinine in diabetic mice [25]. Another study showed that administration of *Urtica dioica* extracts had potential nephroprotective effects as it significantly decreased the levels of plasma creatinine, blood urea nitrogen, and MDA on gentamicin-induced acute kidney injury rats, probably mediating this effect via its antioxidant compounds and anti-inflammatory activity [30]. Significant alterations in histological appearance characterized by necrosis and vacuolation of the liver and kidney were observed in Cis-injected mice. In the current work, the liver tissue of Cis-injected mice showed loss of cellular organization, degeneration, necrosis of many hepatocytes, and cytoplasmic vacuolation. Pronounced nuclear changes include pyknotic nuclei and congestion of blood vessels. Similar results were reported by El-Naggar et al. (2017). Treatment of EAC- bearing mice with natural products resulted in an improvement of the hepatic architecture, and branched central vein, and a slight widening of blood sinusoids, pyknotic nuclei, and vesicular ones was seen. These results were in line with our obtained results that USLE was effective for the prevention of Cis-induced hepatic damage in mice. Cis injection generated histopathological changes in the kidney tissues revealing intertubular bleeding, most renal tubules were damaged and lost their typical morphology, and the glomeruli were destroyed, shrank, and crowded with uneven bowman's space. These findings were supported by a previous study by [3].

Conclusion

The present study indicated that USLE can inhibit the growth of tumors *in vivo* and *in vitro* and induce apoptosis in EAC cells due to its promising phytochemical content.

No Funding

No conflict of interest

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