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Urtica pilulifera leaves exacerbate the cisplatin effect in Ehrlich ascites carcinoma-bearing mice

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Abstract

Conventional chemotherapy is an effective approach to cancer treatment. However, several side effects limited its uses. Natural products have been reported for their anticancer potential. This study evaluated the anticancer efficacy of *Urtica pilulifera* leaves extract (UPLE) alone or in combination with the Cis chemotherapeutic drug Cis in Ehrlich ascites carcinoma (EAC)-bearing mice. Phytochemical constituents were determined in UPLE by quantitative methods. Seventy mice were divided into seven groups (n = 10) as follows: Gp1 was used as a negative control, from Gp2 to Gp7 were inoculated with 1×10^6 EAC-cells/mouse, then Gp2 left as a positive control, Gp3 was injected with Cis (2 mg/kg), Gp4 was injected with UPLE (100 mg/kg), Gp5 was co-treated with Cis as Gp3 and UPLE as Gp4. Gp6 was injected with a low dose of Cis (0.5 mg/kg), and Gp7 was co-treated with a low dose of Cis as Gp6 and UPLE as Gp4. The body weight change percentages (b.wt%) were calculated. On day 14, all groups were sacrificed, the ascitic fluids were harvested, and the total tumor volume, count, and live and dead tumor cells were measured. Sera samples were collected for biochemical parameters assessment. Liver tissues were collected for the determination of oxidants/antioxidants biomarkers. The results showed that combinatorial treatment of the high or low doses of Cis with UPLE led to a synergistic effect on the reduction in the % b.wt changes due to the decrease in the ascitic tumor fluid. Cotreatment with Cis and UPLE exacerbates the antitumor efficacy of Cis with a significant decrease in liver dysfunctions induced by Cis and enhances the hepatic antioxidant status.

Keywords: *Urtica pilulifera*; Phytochemical; Antitumor; Cisplatin; Hepatotoxicity.

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Introduction

Cancer is an incurable global health problem that is characterized by uncontrolled cell growth that can metastasize to other parts of the body, it remains a significant challenge for societies and healthcare systems [1]. Cancer ranks as a leading cause of death, it is the second biggest cause of premature death, after cardiovascular diseases [2]. For cancer treatments, conventional treatment approaches including chemotherapy can be used, it is required to create a new revolution in neoplastic cancer or medications that target the various tumor entities' routes and traits [3, 4]. Chemotherapy is considered the most effective and widely used modality in treating several types of cancers, however, they cause numerous side effects due to increasing the oxidative stress agents and consequently led to toxicity of vital organs [5]. Constraints of traditional chemotherapeutic methods include lack of selectivity, quick metabolism, and mostly negative side effects [6].

One of the most used chemotherapeutic medications for treating various cancers is cisplatin (Cis), whose induction of oxidative stress has been identified as a key contributor to toxicity [7]. The ability to target certain pathways involved in the advancement of cancer therapies has greatly improved with combinatorial strategies, it hits important pathways in a manner that is typically additive or synergistic. Co-treatment with ibuprofen and Cis accelerates the apoptosis of cancer cells [8]. This strategy may lessen medication resistance while also having therapeutic anti-cancer advantages [9].

Natural antioxidants have shown promise in locating free radicals and counteracting their damaging effects, perhaps treating cancer [3]. Plant-derived bioactive compounds are natural constituents used as preventive and therapeutic agents due to their antioxidant properties [10, 11]. Recently, phytochemicals have gained much attention in cancer research due to their pleiotropic impacts and safe behavior. However, to comprehend the potential anticancer properties of these phytochemicals as well as the processes underlying their action, additional research is required [12].

Urtica pilulifera belongs to the family Urticaceae and is classified as a popular plant cultivated in the Mediterranean region, it is an annual herb with a straight, square-shaped leafy stem and a troublesome, branching, rhizome [13]. Different parts of *U. pilulifera* have been reported for their potential antioxidant properties and phytochemical content [14]. Various pharmacological effects have been connected to several phytochemical elements found in *Urtica*. *Urtica* extracts have been known in traditional medicine to protect against various diseases in experimental animals [15, 16]. Numerous species of *Urtica* have been used extensively to treat a variety of conditions, including rheumatism, asthma, coughs, dandruff, diabetes, eczema, gout, scurvy, and tuberculosis [17]. Additionally, Urtica species have primarily been used as diuretics and for the treatment of prostate hypertrophy [18]. It has been reported that *U. pilulifera* root could markedly improve the paclitaxel sensitivity of breast cancer cells [19]. Furthermore, Lower urinary tract infection symptoms can be relieved by *U. pilulifera* preparations [20]. Therefore, this study investigated the anticancer efficacy of *Urtica pilulifera* leaves extracts (UPLE) alone or in combination with the Cis chemotherapeutic drug Cis in EAC-bearing mice.

Materials and Methods

Chemicals

Chemicals for phytochemical assessments were purchased from Sigma (St. Louis, Mo., USA). Cisplatin (50 mg/50 mL vial) was purchased from Merk Ltd. (Cairo, Egypt). Biochemical kits were purchased from Bio-diagnostic Company, Egypt.

Collection of plant materials and extract preparation

Urtica pilulifera leaves were purchased from the local market in Tanta City, Gharbia, Egypt. The plant

materials were identified and authenticated by a taxonomist at the Botany Department, Faculty of Science, Tanta University. Leaves were dried, and crushed and a certain weight of powder was mixed with ethanol. The hydroalcoholic *Urtica pilulifera* leaves extract (UPLE) was weighed and stored at 4 ˚C for further processing.

Phytochemical analysis

The total phenolic contents of the 9 extracts were determined by using the Folin-Ciocalteau reagent according to Singelton *et al*. [21]. The flavonoid content was assessed by using the aluminum chloride colorimetric method according to Zhishen *et al*. [22]. The phosphomolybednum method was used to determine the total antioxidant capacities (TAC) of all extracts [23]. The free radicals scavenging capacity spectrophotometrically were assessed according to the method of Blois [24].

Mice and experimental design

Ehrlich ascitic carcinoma (EAC) cells were collected from the tumor-bearing mice purchased from the National Cancer Institute (NCI, Cairo, Egypt). The number of tumor cells was adjusted at 2×10^6 cells/mouse for intraperitoneal (i.p) inoculation. Seventy male Swiss albino mice (20 ± 2 g) were given drinking tap water and normal experimental pelleted animal food *ad libitium*. Mice were divided into seven groups as follows: Gp1 was used as a negative control, from Gp2 to Gp7 were inoculated with 1×10^6 EAC-cells/mouse, then Gp2 left as a positive control, Gp3 was injected with Cis (2 mg/kg), Gp4 was injected with UPLE (100 mg/kg) [25], Gp5 was co-treated with Cis (2 mg/kg) and UPLE (100 mg/kg). Gp6 was injected with the low dose of Cis (0.5 mg/kg), and Gp7 was co-treated with the low dose of Cis (0.5 mg/kg) and UPLE (100 mg/kg).

All treatments were intraperitoneally (i.p) injections after 24 hours of EAC-cells inoculation for 6 consecutive days. All groups were weighted at the beginning (initial b.wt) and at the end of the experiment (final b.wt). The percentage of the change in the total body weight was calculated. On day 14, mice from all groups were sacrificed. By using 10 ml syringes, the ascitic fluids were harvested from all groups under the study. The volume of ascitic tumor fluids was measured. To determine the number of live and dead tumor cells, the trypan blue exclusion method was used. Blood samples were collected, and the sera were separated and frozen at -20 ˚C until used for the determination of biochemical parameters. Liver homogenates were used for the determination of oxidants/antioxidants biomarkers.

Biochemical analyses

Sera alanine transaminase (ALT), and aspartate aminotransferase (AST) activities were assayed according to the method of Reitman and Frankel (1957) [26]. Alkaline phosphatase was estimated according to Belfield and Goldberg, (1971) [27]. Total proteins were assessed according to Gornall *et al*. (1949) [28]. Superoxide dismutase (SOD) and catalase (CAT) activities were measured [29, 30]. Reduced glutathione was assayed according to the method of Beutler *et al*. (1963) [31]. The malondialdehyde (MDA) levels were assayed according to the method of Esterbauer and Cheeseman (1990) [32].

Statistical analysis

All data are presented as mean ± SD. One-way analysis of variance (ANOVA) was used to assess the significant differences among treatment groups. The SPSS statistics program was used for data analysis. The criterion for statistical significance was set at $p < 0.05$.

Results

Phytochemical constitutes of *Urtica pilulifera* leaves extract (UPLE)

The results showed that the *U. pilulifera* yield an adequate extract amount (9 %). The results showed that the total phenolic amount of UPL was 19.65 ± 2.35 mg GAE/mg of dry samples (Table 1). The total flavonoid content was 10.31 ± 6.82 mg QE/g of dry samples. The total antioxidant capacity (TAC) of the leaves extract was 0.31 ± 0.08 mg AAE/g DW, and DPPH scavenging

activity (%) was 73 %. The amount of leaf extract which able to reduce 50% of DPPH was 6.95 ± 3.56 mg/mL (Table 1).

Administration of UPLE improves the decrease in the percentage of body weight changes

The results indicated that the % b.wt changes of the EAC-bearing mice were significantly increased ($p < 0.05$) up to 32.56% ± 4.21 when compared to the negative control group (18.78% ± 3.15). Treatment of EAC-bearing mice with the therapeutic dose of Cis (2 mg/kg b.wt) or UPLE (100 mg/kg) for 6 consecutive days led to a significant decrease in % b.wt changes to 7.10% ± 2.14, or 14.89% ± 2.33, respectively when compared to EAC-bearing mice alone (Figure 1). Combinatorial treatment of a high dose of Cis/UPLE resulted in a much more decrease in the % b.wt changes up to 4.69% ± 1.67 when compared to a single treatment. EAC-bearing mice that were treated with the low dose of Cis showed a significant decrease in % b.wt changes (11.67 ± 1.58) but not as the treatment with the therapeutic dose, however, the combination of a low dose of Cis and UPLE led to a synergistic effect on the decrease in the % b.wt changes due to the decrease in the ascitic tumor fluid (Figure 1).

Table (1): Quantitative phytochemical analysis of *Urtica pilulifera* leaves.

Phytochemical analysis	**UPLE**
Total phenolic content (mg GAE/g DW)	19.65 ± 2.35
Total flavonoids contents (mg QE/mg DW)	10.31 ± 6.82
Total antioxidant capacity (TAC) (mg AAE/g DW)	0.31 ± 0.08
DPPH scavenging activity (%)	73 % ± 5.44
IC_{50} of DPPH (mg/ml)	6.95 ± 3.56

GAE: Gallic acid equivalent; QE: Quercetin equivalents; DW: Dry weight; TAC: Total antioxidant capacity; AAE: Ascorbic acid equivalent; DPPH: Diphenyl-1-picrylhydrazyl; IC_{50}: Inhibitory concentration of 50%.

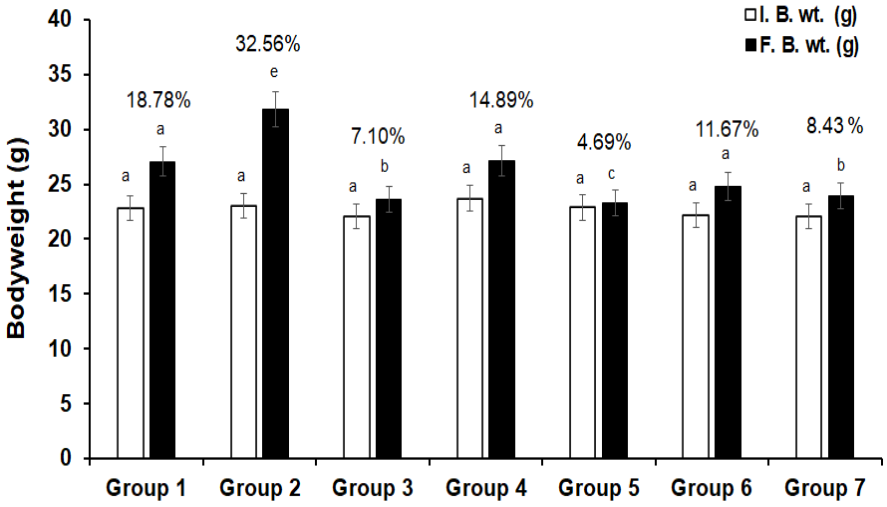

Figure (1): Initial, and final body weights, and the percentages of body weight changes in the different groups under the study. The values represented as means ± S.D.; Means that do not share a letter are significantly different ($p < 0.05$).

Total tumor volume, viable and dead EAC-cells after different treatments in the different groups of EAC-bearing mice

The results indicated that there were significant decreases in the total ascitic tumor of EAC-bearing mice that had been treated with Cis (2 mg/kg) or/and UPLE (1.9 mL ± 0.52, 4.1 mL ± 0.35, 1.1 mL ± 0.23) when compared to EAC-bearing mice alone (9.2 mL ± 0.79). Moreover, EAC-bearing mice that were treated with the low dose of Cis showed a significant reduction in the total tumor volume (3.8 mL ± 0.50) when compared to its value in EAC-bearing mice alone (9.2 mL ± 0.79). Co-treatment with a low dose of Cis and UPLE led to a synergistic effect on the reduction of the tumor volume that represented 2.2 mL ± 0.48 when compared to the EAC-bearing mice that were treated with the low dose of Cis alone (Table 2 and Figure 2). As compared to the EAC-bearing mice, the treatment with a high dose of Cis led to a significant decrease ($p < 0.001$) in the total tumor cell counts (T.T.C). Also, the treatment of EAC-bearing mice with UPLE led to a significant decrease in the T.T.C ($221 \times 10^6 \pm 12.2$) when compared to EAC-bearing mice alone ($593 \times 10^6 \pm 25.0$). Furthermore, the co-treatment with a high dose of Cis and UPLE resulted in a significant reduction in the T.T.C to $30 \times 10^6 \pm 6.2$ when compared to a single treatment. Treatment of EAC-bearing mice with a low dose of Cis or in combination with UPLE caused a marked decrease in the T.T.C ($64 \times 10^6 \pm 8.7$ and $40 \times 10^6 \pm 5.4$, respectively). The number of viable tumor cells decreased, and the number of dead EAC-cells was increased after different treatment protocols when compared to their numbers in the EAC-bearing mice (Table 2).

Table (2): Total volume, viable and dead EAC-cells in the different groups of EAC-bearing mice.

Groups	T.T.V (ml)	T.T.C ($\times 10^6$/mouse)	T.L.C ($\times 10^6$/mouse)	T.D.C ($\times 10^6$/mouse)
Group 2	9.2 ± 0.79 [a]	593 ± 25.0 [a]	571 ± 28.3 [a]	22 ± 3.1 [a]
Group 3	1.9 ± 0.52 [b]	43 ± 7.1 [b]	11 ± 2.6 [b]	32 ± 3.3 [a]
Group 4	4.1 ± 0.35 [c]	221 ± 12.2 [c]	106 ± 7.5 [c]	115 ± 8.4 [c]
Group 5	1.1 ± 0.23 [b]	30 ± 6.2 [b, f]	7 ± 0.6 [e]	23 ± 0.8 [a]
Group 6	3.8 ± 0.50 [c]	64 ± 8.7 [d]	44 ± 1.5 [d]	20 ± 1.2 [a]
Group 7	2.2 ± 0.48 [d]	40 ± 5.4 [b]	11 ± 0.7 [b]	29 ± 2.1 [a]

The values represented mean ± SD. T.T.V: Total tumor volume, T.T.C: Total tumor count, T.L.C: Total live cells, T.D.C: Total dead cells. This means that do not share a letter in each column is significantly different ($p < 0.05$).

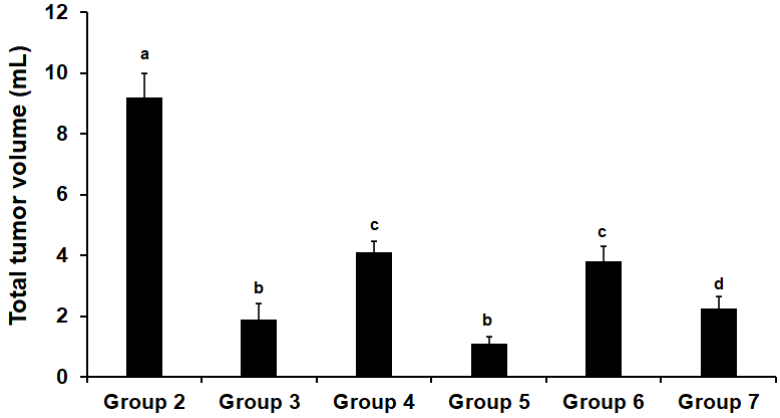

Figure (2): Total tumor volume (ml) mouse of EAC-bearing groups under the study. This means that do not share a letter are significantly different ($p < 0.05$).

Co-treatment of Cis with UPLE mitigated liver dysfunctions in EAC-bearing mice

The results showed that in EAC-bearing mice, there was significant increase ($p < 0.05$) in the serum ALT, AST, and ALP enzymes due to liver dysfunctions, which represented 194 ± 10.1 U/L, 202.3 ± 13.3 U/L, and 264.37 ± 9.7 U/L, respectively when compared to the normal control groups (43.5 ± 2.9 U/L, 83.2 ± 7.2 U/L, and 93.78 ± 4.2 U/L, respectively) **(Table 3)**. The data demonstrated that the treatment of EAC-bearing mice with high or low doses of Cis led to significant improvement in liver functions evidenced by a significant decrease in the serum ALT, AST, and ALP activities when compared to the group of EAC-bearing mice alone. However, the co-treatment of EAC-bearing mice with UPLE and Cis (high or low doses) resulted in significant alleviation of liver injury much more than single treatments and a significant decrease in the sera activities of ALT, AST, and ALP **(Table 3)**. Their EAC-bearing mice showed a significant decrease ($p < 0.05$) in their total protein levels when compared to the negative control groups. However, the treatment of EAC-bearing mice with high or low doses of Cis led to a significant increase in the sera protein levels due to improvement in liver functions. Moreover, the combinatorial treatment of EAC-bearing mice with UPLE and Cis (high or low doses) resulted in significant alleviation of liver injury much more than single treatments by a significant increase in the total protein levels **(Table 3)**.

Co-treatment of Cis with UPLE improved hepatic antioxidants/oxidants status in EAC-bearing mice

Antioxidants/oxidants biomarkers including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) levels were determined post-different treatments in the different groups under the study. The result indicated that EAC-bearing mice showed significant decreased ($p < 0.05$) in the SOD, CAT, and GSH levels to 1.65 ± 0.08 U/mg protein, 45.31 ± 2.3 U/mg protein, and 1.85 ± 0.33 µmol/g tissue, respectively when compared to the negative control groups that represented 6.05 ± 0.26 U/mg protein, 86.27 ± 2.6 U/mg protein, and 7.18 ± 0.52 µmol/g tissue, respectively. On the contrary, levels of MDA in liver tissues of EAC-bearing mice were significantly increased ($p < 0.05$) up to 71.59 ± 2.5 nmol/g tissue when compared to the normal control group that represented 32.44 ± 1.7 nmol/g tissue **(Figure 3)**. The data obtained from the current study demonstrated that treatment of EAC-bearing mice with high or low doses of Cis led to significant improvement in the antioxidants/oxidants status evidenced by an increase in SOD, CAT, and GSH levels accompanied by a significant decrease in the levels of MDA in the liver tissues homogenates. Moreover, the treatment of EAC-bearing mice with Cis and UPLE showed much more improvement in their antioxidant capacity **(Figure 3)**.

Table (3): 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	43.5 ± 2.9 [a]	83.2 ± 7.2 [a]	93.78 ± 4.2 [a]	6.72 ± 0.28 [a]
Group 2	194 ± 10.1 [c]	202.3 ± 13.3 [b]	264.37 ± 9.7 [b]	3.24 ± 0.19 [b]
Group 3	133.7 ± 7.9 [b]	155.7 ± 10.7 [c]	200.64 ± 8.5 [c]	4.81 ± 0.25 [c]
Group 4	120.5 ± 6.8 [b,d]	132.4 ± 7.5 [c,d]	181.2 ± 7.6 [c,d]	5.55 ± 0.17 [c]
Group 5	90.4 ± 8.7 [e]	112.5 ± 7.5 [d]	129.6 ± 5.5 [a,e]	6.05 ± 0.18 [a,c]
Group 6	153.7 ± 10.2 [b]	173.1 ± 8.4 [b,c]	224.56 ± 10.3 [b,c]	4.35 ± 0.05 [c]
Group 7	120.1 ± 8.9 [b]	140.1 ± 6.3 [c,d]	195.3 ± 11.7 [c,d]	5.75 ± 0.21 [a,c]

The values represented as means ± S.D.; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase. This means that do not share a letter in each column is significantly different ($p < 0.05$).

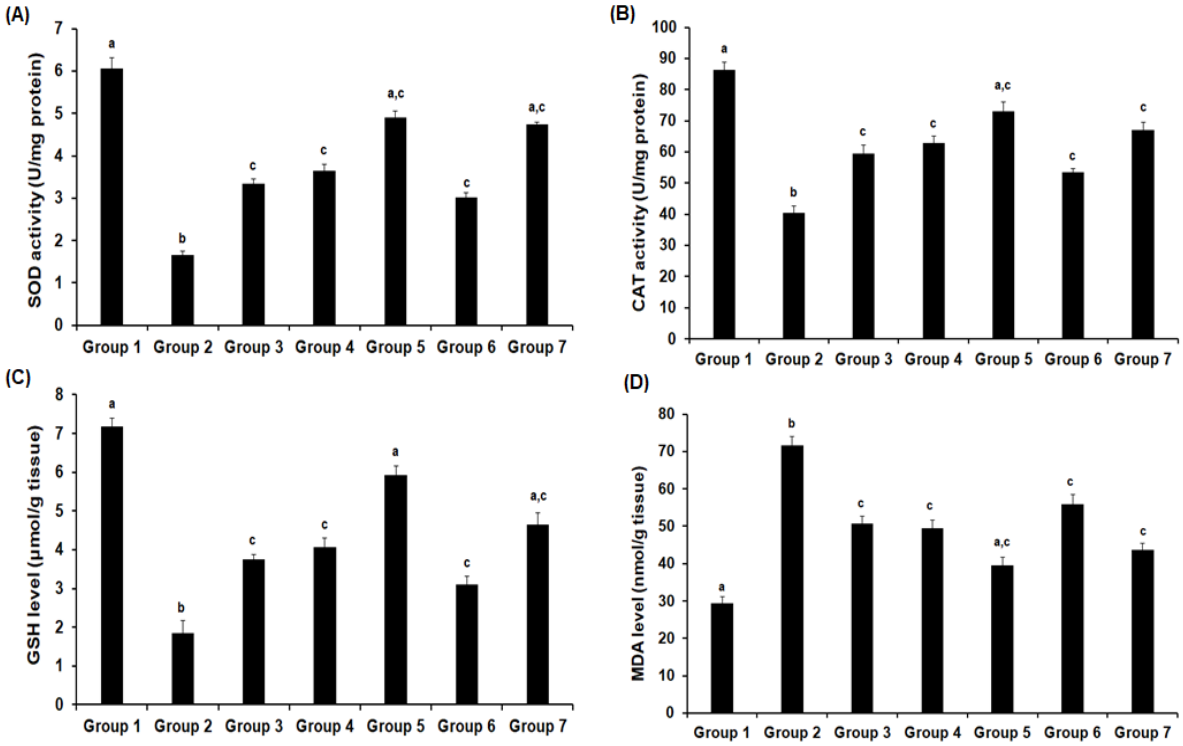

Figure (3): Hepatic superoxide dismutase (SOD) activity (A), catalase (CAT) activity (B), reduced glutathione (GSH) level (C), and malondialdehyde (MDA) level in the different groups under the study. The values represented as means ± S.D.; Means that do not share a letter are significantly different ($p < 0.05$).

Discussion

Conventional chemotherapy is an effective approach to cancer treatment. However, chemotherapy treatment led to wide side effects on some vital organs and induces resistance of the tumor cells to the treatment. Cisplatin (Cis) is an effective chemotherapeutic agent that is used to treat different types of cancer; however, it has toxic effects on the liver, and other organs [33]. 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 [34].

Natural constituents have been tested for their ability to fight cancer [35]. *Urtica* herbs have been found worldwide with multiple health benefits. Phytochemicals like polyphenols, including phenolic acids and flavonoids, as well as amino acids and vitamins, are all abundant in the perineal herbaceous plant known as *Urtica pilulifera* [36]. A broad spectrum of biological and pharmacological activities of *Urtica sp* have been reported including anticancer, antioxidant, antitumor agent, antibacterial, antimicrobial, antifungal, and antiviral effects [37]. This study investigated the anticancer efficacy of UPLE alone or in combination with high or low doses of Cis in EAC-bearing mice. The naturally occurring plant substances known as phytochemicals are important sources of new drugs and sources for cancer therapy by boosting antioxidant status, inactivating carcinogens, reducing proliferation, and inducing cell cycle arrest and apoptosis [38]. The current study showed that UPLE had an adequate amount of phytochemical constituents including phenolic and flavonoids. A previous study demonstrated that there were various phytochemical components found in *U. pilulifera* including flavonoids, and phenolic compounds like diocanol, alcohols, and terpenes, which have been linked to a variety of pharmacological effects [37].

This study showed that 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 inside the peritoneal cavity of mice. This finding agreed with a previous study by El-Naggar *et al.* (2019), who reported that there was a significant increase in the total body weight change in EAC-bearing mice compared to naïve mice [33]. Treatment of EAC-bearing mice with Cis or UPLE led to a significant decrease in the percentage of body weight change. This finding agreed with the previous study of Ibrahim et al. (2022) [39]. Furthermore, combinatorial treatment with high or low doses of Cis and UPLE increases the reduction in the percentage of body weight change this could be due to the inhibition of EAC-cells growth in the peritoneal cavity of mice and decrease in tumor growth compared to single treatments. Previous studies reported that co-treatment of Cis with natural products enhanced the antitumor activity and resulted in a reduction in the percentage of body weight change of EAC-bearing mice [33, 40, 41].

In this study, the treatment of EAC-bearing mice with high or low doses of Cis, UPLE led to a significant reduction in the total tumor volume, total tumor count, and total live tumor cells, however, the low dose of Cis or UPLE did not completely treat the EAC-bearing mice. This could be due to the low doses of Cis not being enough to eliminate or stop the tumor cells completely. Co-treatment with UPLE increased the efficacy of the low doses of Cis as anticancer agents in EAC-bearing mice. This finding was supported by the decrease in the total volume, total tumor count, total live tumor cells, and increase in the total dead tumor cells. These results were in line with a previous study that reported that the antitumor efficacy of low doses of Cis could be enhanced in EAC-bearing mice by increasing the percentages of dead tumor cells [33, 42, 43].

Elevated ALT and AST levels in EAC tumor-bearing mice are a sign of deteriorating hepatic

functions brought on by cancer proliferation due to functional impairment of hepatic cell membranes and cellular leakage, which showed that EAC-induced liver injury [44]. In the present study, the protective and antitumor effects of UPLE were addressed in EAC-bearing mice. Elevation of ALT and AST enzymes in EAC-bearing mice may be due to the cytotoxic effect of EAC tumors which led to damage of liver cells and canaliculi. The results showed that UPLE had antitumor effects *in vivo* studies. In addition, the co-treatment with UPLE could protect liver tissues against Cis toxicity. These results agreed with the previous studies that reported the efficacies of the co-treatment with Cis and natural products [42, 45]. Combinatorial treatment with Cis/UPLE led to a significant decrease in the levels of these transaminases in EAC-bearing mice compared to single injections which indicates the ameliorative effects of UPLE on hepatotoxicity. The ALT, AST, and ALP enzymes were decreased in the group of EAC-bearing mice treated with a combination of Cis/UPLE. Decreasing the hepatic toxicity upon treatment with this combination indicates that the UPLE has a protective effect against liver dysfunction and cellular injury of the liver. A previous reported a significant impact of *Urtica sp.* extract as a hepatoprotective agent by the decrease in serum ALT, AST, ALP, and total bilirubin levels towards normalization in hepatic injured rats. In addition, it has been reported that *Urtica sp.* leaves powder decreased the levels of AST, ALT, ALP, and albumin [46].

The present study stated that UPLE induced significant improvement in reversing the alterations in the hepatic antioxidant/oxidant hemostasis as it can inhibit lipid peroxidation and prevent oxidative stress. In agreement with Kataki et al. (2012), who discovered that pretreatment of animals with Urtica sp. extract led to a significant decrease in MDA level as well as a significant increase in SOD level, treatment with USLE significantly reversed the oxidative stress-associated changes. This could be because the antioxidant defense system was improved [47]. Similar findings were made by Pérez Gutiérrez et al. (2021), who showed that the Urtica dioica extract boosted the antioxidant enzymatic activity of SOD and CAT, which increased the levels of GSH while lowering the MDA in hepatic tissues in mice [48]. *U. dioica* leaves have been shown to have a high concentration of antioxidant and free radical scavenger components, which can reduce the high level of oxidative stress found in malignant cells [49].

Conclusion

The co-treatment with UPLE and Cis led to enhancement of the antitumor efficacy of low-dose Cis and decrease its hepatotoxic effects via improvement of liver functions and antioxidant status.

Conflict of interest

All authors declared that there were no conflicts of interest.

References

1. **M. S. Copur,** "State of Cancer Research Around the Globe", *Oncology (Williston Park)*, Vol. 33, pp. 181–185 (2019).
2. **H. Sung, J. Ferlay, R. L. Siegel, et al.,** "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries", *CA Cancer J Clin*, Vol. 71, pp. 209–249 (2021).
3. **D. T. Debela, S. G. Muzazu, K. D. Heraro, et al.,** "New approaches and procedures for cancer treatment: Current perspectives", *SAGE Open Med*, Vol. 9, pp. 49–57 (2021).
4. **K. S. El-Said, E. M. Ali, K. Kanehira, et al.,** "Effects of toll-like receptors 3 and 4 induced by titanium dioxide nanoparticles in DNA damage-detecting sensor cells", *J Biosens Bioelectron*, Vol. 4, pp. 144.
5. **A. El-Hussein, S. L. Manoto, and S. Ombinda-Lemboumba,** "A review of chemotherapy and photodynamic therapy for lung cancer treatment",

Anticancer Agents Med Chem, Vol. 21, pp. 149–161 (2021).

6. **J. Mondal, A. K. Panigrahi, and A. R. Khuda-Bukhsh,** "Conventional chemotherapy: problems and scope for combined therapies with certain herbal products and dietary supplements", *Austin J Mol Cell Biol*, Vol. 1, pp. 10 (2014).
7. **M. M. El-Sawalhi, and L. A. Ahmed,** "Exploring the protective role of apocynin a specific NADPH oxidase inhibitor in cisplatin-induced cardiotoxicity in rats", *Chemico-biol Interact*, Vol. 207, pp. 58–66 (2014).
8. **H. Endo, M. Yano, Y. Okumura, et al.,** "Ibuprofen enhances the anticancer activity of cisplatin in lung cancer cells by inhibiting the heat shock protein 70", *Cell Death and Dis,* Vol. 5, pp. 1027 (2014).
9. **R. Bayat Mokhtari, T. S. Homayouni, N. Baluch, et al.,** "Combination therapy in combating cancer", *Oncotarget*, Vol. 8, pp. 38022–38043 (2017).
10. **S. Bernardini, A. Tiezzi, V. Laghezza Masci, et al.,** "Natural products for human health: an historical overview of the drug discovery approaches", *Nat Prod Res*, Vol. 32, pp. 1926–1950 (2018).
11. **S. Chikara, L. D. Nagaprashantha, J. Singhal, et al.,** "Oxidative stress and dietary phytochemicals: role in cancer chemoprevention and treatment", *Cancer Lett,* Vol. 413, pp. 122–134 (2019).
12. **P. Marino, G. Pepe, M. G. Basilicata, et al.,** "Potential role of natural antioxidant products in oncological diseases", *Antioxidants,* Vol. 12, pp. 704 (2023).
13. **Z. Zamani, S. M. A. Razavi,** "Physicochemical, rheological, and functional properties of Nettle seed (*Urtica pilulifera*) gum", *Food Hydrocolloids*, Vol. 112, pp. 106304 (2021).
14. **T. Ozen, C. Zeynep, and H. Korkmaz,** "Antioxidant properties of *Urtica pilulifera* root, seed, flower, and leaf extract", *J Med Food*, Vol. 13, pp. 1224–1231(2010).
15. **M. Hussain,** "Medicinal plant genus Urtica Traditional uses phytochemical and pharmacological review", *Int J of Sci Engin Res,* Vol. 10, pp. 557–607 (2019).
16. **V. K. Alluri, C. Rao, and D. Sundararaju,** "Anti-inflammatory activity of Urtica dioica bark extracts against arthritis in Sprague Dawley rat", *Am J of Infec Disea*. Vol. 5, pp. 68–73 (2009).
17. **D. Kregiel, E. Pawlikowska, and H. Antolak,** "*Urtica sp*: ordinary plants with extraordinary properties", *Molecules,* Vol. 23, pp. 1664 (2018).
18. **A. Kumar, A. Singh Bisht, S. Joshi, et al.,** "Pharmacognostical and phytochemical study of a plant Urtica parviflora Roxb a review", Journal of Pharmacognosy and Phytochemistry (2017)., Vol. 6, pp. 42–45 (2017).
19. **M. Saponaro, I. Giacomini, G. Morandin, et al.,** "*Serenoa repens* and *Urtica dioica* fixed combination: In-Vitro validation of a therapy for benign prostatic hyperplasia (BPH)" , *Int J of Mole Sci,* Vol. 21, pp. 9178 (2020).
20. **N. Lopatkin, A. Sivkov, S. Schlafke, et al.,** "Efficacy and safety of a combination of Sabal and Urtica extract in lower urinary tract symptoms-long-term follow-up of a placebo-controlled, double-blind, multicenter trial", *Int Urol Nephrol*, Vol. 39, pp.1137–1146 (2007).
21. V. R. Singelton, R. Orthifer, and R. M. Lamuela-Raventos, "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent", *Methods Enzymol*, Vol. 299, pp. 152–178 (1999).
22. J. Zhishen, T. Mengcheng, and W. Jianming, "The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals", *Food Chem*, Vol. 64, pp. 555–559 (1999).
23. P. Prieto, and M. Pineda, "Spectrophotometric quantitation of antioxidant capacity through the

formation of a phosphomolybdenum complex: specific application to the determination of vitamin E", *Anal Biochem*, Vol. 269, pp. 337–341 (1999).

24. M. S. Blois, "Antioxidant determinations by the use of a stable free radical", *Nature*, Vol. 181, pp. 1199–1200, (1958).
25. **R. S. Fakher El Deen, S. A. El-Naggar, E. El-Nahass, et al.,** "Antitumor efficacy of Urtica sp. leaves extract: *in vitro* and *in vivo* studies", *J Biosci App Res*, Vol. 8, pp. 264–274 (2022).
26. **S. Reitman, and S. Frankel,** "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases", *American J Clin Pathol*, Vol. 28, pp. 56–63 (1957).
27. A. Belfield, and D. M. Goldberg, "Colorimetric determination of alkaline phosphatase activity", *Enzyme*, Vol. 12, pp. 561–568 (1971).
28. A. G. Gornall, C. J. Bardawill, and M. M. David, "Determination of serum proteins by means of the biuret reaction", *Biol Chem*, Vol. 177, pp. 751–766 (1949).
29. M. Nishikimi, N. A. Roa, and K. Yogi, "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen", *Biochem Biophys Res Comm*, Vol. 46, pp. 849–854 (1972).
30. **Aebi, H.** "Catalase *in vitro*", *Methods Enzymol*, Vol. 105, pp. 121–126 (1984).
31. **E. Beutler, O. Duron, and B. M. Kelly,** "Improved method for the determination of blood glutathione", *Lab Clin Med*, Vol. 61, pp. 882–888 (1963).
32. **H. Esterbauer, and K. H. Cheeseman,** "Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal", *Methods Enzymol*, Vol. 186, pp. 407–421 (1990).
33. **S. A. El-Naggar, K. S. El-Said,** "Antitumor efficacy of EDTA co-treatment with cisplatin in tumor-bearing mice", *Braz J Pharm Sci*, Vol. 56, pp. 18536 (2020).
34. **S. A. El-Naggar, K. S. El-Said, M. Mobasher, et al.,** "Enhancing antitumor efficacy of cisplatin low dose by EDTA in Ehrlich ascetic carcinoma bearing mice", *Braz Arch Biol Technol*, Vol. 62, pp.19180716 (2019).
35. **A. E. Mohamed, M. A. El-Magd, K. S. El-Said, et al**., "Potential therapeutic effect of thymoquinone and/or bee pollen on fluvastatin-induced hepatitis in rats", *Scientific reports*, Vol. 11, pp. 110–117 (2021).
36. **K. M. Ahmed and S. J. P. Parsuraman,** "*Urtica dioica* L., (Urticaceae): a stinging nettle", *Systematic Reviews in Pharm*, Vol. 5, pp. 6 (2014).
37. **M. Hussain,** "Medicinal plant genus Urtica traditional uses phytochemical and pharmacological review", *Int J of Sci Engin Res*, Vol. 10, pp. 557–607 (2019).
38. **A. S. Choudhari, P. C. Mandave, M. Deshpande, et al.,** "Phytochemicals in cancer treatment: from preclinical studies to clinical practice", *Front Pharmacol*, Vol. 10, pp. 1614 (2020).
39. **W. M. Ibrahim, S. M. Foad, and A. Y. Eltoukh,** "Biochemical study in Ehrlich carcinoma cells-bearing mice treated with arsenic trioxide and cisplatin", *Advances in Environm Life Sci*, Vol. 1, pp. 40–47 (2022).
40. **A. Amuthan, V. Devi, C. S. Shreedhara, et al.,** "Vernonia cinerea regenerates tubular epithelial cells in cisplatin induced nephrotoxicity in cancer bearing mice without affecting antitumor activity", *Trad Complement Med*, Vol. 11, pp. 279–286 (2021).
41. **N. Saleh, T. Allam, R. M. S. Korany, et al.,** "Protective and therapeutic efficacy of hesperidin versus cisplatin against Ehrlich ascites carcinoma-induced renal damage in mice", *Pharmaceut*, Vol. 15, pp. 294 (2022).

42. **M. A. Hashem, S. B. Shoeeb, Y. M. Abd-Elhakim, et al.,** "The antitumor activity of Arthrospira platensis and/or cisplatin in a murine model of Ehrlich ascites carcinoma with hematinic and hepato-renal protective action", *Functional Foods*, Vol. 66, 103831 (2021).
43. **D. S. Morsi, S. H. El-Nabi, and M. A. Elmaghraby,** "Anti-proliferative and immunomodulatory potencies of cinnamon oil on Ehrlich ascites carcinoma bearing mice", *Sci Rep*, Vol. 12, 11839 (2022).
44. **E. Tousson, Hafez, E., and M. M. Abo Gazia,** "Hepatic ameliorative role of vitamin B17 against Ehrlich ascites carcinoma–induced liver toxicity", *Environ Sci Pollut Res*, Vol. 27, pp. 9236–9246 (2020).
45. **M. S. Nafie, K. Arafa, N. K. Sedky, et al.,** "Triaryl dicationic DNA minor-groove binders with antioxidant activity display cytotoxicity and induce apoptosis in breast cancer", *Chem Biol Interact*, Vol. 324, 109087 (2020).
46. **H. S. Eldamaty,** "Effect of adding nettle leaves (Uritca dioica L.) powder on basal diet to lower diabetes in rats", *Egypt J Food*, Vol. 46, pp. 141–151 (2018).
47. **M. S. Kataki, V. Murugamani, and A. Rajkumari,** "Antioxidant, hepatoprotective, and anthelmintic activities of methanol extract of *Urtica dioica* L. leaves", Pharmac Crops. Vol. 3, pp. 38–46 (2012).
48. **R. M. Pérez Gutiérrez, A. Muñiz-Ramirez, A. H. Garcia-Campoy,** "Evaluation of the antidiabetic potential of extracts of *Urtica dioica*, *Apium graveolens*, and *Zingiber officinale* in mice, zebrafish, and pancreatic β-cell", *Plants*, Vol. 10,1438 (2021).
49. **S. Esposito, A. Bianco, R. Russo, et al.,** "Therapeutic perspectives of molecules from *Urtica dioica* extracts for cancer treatment", *Molecule*, Vol. 24, 2753 (2019).