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In-depth hepatoprotective mechanistic study of *Echinacea purpurea* flowers: *In vitro* and *in vivo* studies

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ABSTRACT

Introduction: *Echinacea purpurea* is a flowering plant commonly used as an herbal medicine despite insufficient scientific bases to validate its usage. The present study aimed to examine *in vitro* and *in vivo* hepatoprotective effects of aqueous and alcoholic extracts of *E. purpurea* flowers.
Methods: *In vitro* protection against hepato-cytotoxicity was carried out on human HepG-2 cells using colorimetric tetrazolium (MTT) assay, while the *in vivo* hepatoprotective activity was studied against carbon-tetrachloride (CCl_4) induced acute hepatotoxicity in rats.
Results: The results revealed that the extracts of *E. purpurea* induced discernable *in vitro* protection on HepG-2 cells and *in vivo* against CCl_4 induced hepatotoxicity. Both extracts were significantly able to restore the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin to normal levels compared to the CCl_4 intoxicated group. In addition, the extracts markedly mitigated the oxidative stress by decreasing Malondialdehyde (MDA) and increasing superoxide dismutase (SOD) and glutathione (GSH) markers compared to the CCl_4 intoxicated group. It was also associated with the down-regulation of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels in liver tissues. Histopathological examination revealed a decrease in hepatocytes' degenerative changes and noticeable improvement of the liver damage by extracts of *E. purpurea*.
Conclusion: These findings have proven that aqueous and alcoholic extracts of *E. purpurea* flowers have a significant hepatoprotective effect, probably owing to antioxidant, anti-inflammatory activities, and regulating apoptotic-related genes. This confirms the ethnomedicinal uses of *E. purpurea* in patients suffering from liver diseases.

Implication for health policy/practice/research/medical education:
Echinacea purpurea flowers exhibited good hepatoprotective, anti-oxidant and anti-inflammatory activities. Thus, this plant might be considered a candidate for bioassay-guided and isolation of bio-active compounds, which could possibly be developed into new lead structures for drug development programs against hepatic disorders.
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Introduction

The soaring number of liver injuries has been an issue of concern worldwide. The liver's metabolic functions and its other pivotal roles, amongst which the purification of exogenous and endogenous threats, such as xenobiotics, medicines, bacterial infections, and chronic alcoholism (1), make liver injury one of the serious health hazards. Forms of liver damage have been attributed to numerous factors, amongst which are: environmental pollutants, excessive dose of drugs, and long-lasting cholestatic disease (2,3). Chronic liver diseases have severe repercussions starting with steatosis to chronic hepatitis, fibrosis, cirrhosis, and finally hepatocellular carcinoma (4). Hepatocarcinogenesis starts via persistent inflammation with the liberation of free radicals and lipid peroxidation. These free radicals interact directly with DNA and destroy specific genes that control cell growth and differentiation (5). The continual chronic liver injury and cellular degenerative events could lead to mutations of tumor-suppressor genes resulting in the development of hepatocellular carcinoma (6).

The lack of safe drugs to prevent and treat liver diseases has revealed an expressive affair in finding the natural

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products from medicinal plants (7). Medicinal plant extracts have received wide attention as natural therapy for many diseases, which could be attributed to the presence of many bioactive components that relieve oxidative stress and cell damage. Moreover, phytochemicals with anti-inflammatory, antibacterial, anti-oxidant, and anti-mutagenic effects have proven effective for disease prevention (8).

In recent decades, scientists are exceptionally interested in *Echinacea purpurea* (purple coneflower) efficacy, as they find it useful in treating the common cold and infectious diseases owing to its immunostimulant, anti-oxidant, and anti-inflammatory effects. Commercially available preparations of *E. purpurea* are widely used in the prevention or treatment of upper respiratory tract infections (9) due to the presence of various medicinal components (such as alkylamides, caffeic acid derivatives, glycoproteins, polysaccharides, polyacetylenes, phenolic compounds, cinnamic acids, essential oils, and flavonoids) to which its therapeutic effects could be, for the most part, attributed. The present study aimed to investigate the *in vitro* and *in vivo* hepatoprotective activities of aqueous and alcoholic extracts of *E. purpurea* flowers.

Materials and Methods

Rats

Thirty-five male Wistar strain rats weighing 150-160 g were used in this study. Animals were housed under hygienic laboratory conditions (12 hours/12 hours light/darkness cycle, 45%-50% relative humidity, and 23-25°C temperature). Animals were fed on standard rat pellets, and water was provided *ad libitum* throughout the experiment period.

Drugs and chemicals

Flowers of *E. purpura* herb were obtained from a local market of medicinal plants and herbs (Cairo, Egypt) and authenticated at the Herbarium of Botany Department, Faculty of Science, Cairo University, Egypt. A voucher specimen was deposited there (Voucher number: Hr513). The flowers were air-dried and pulverized using a metal grinder. Cold extraction was done by soaking one kilogram of the flower powder in three liters of 99% alcohol for three days at room temperature with intermittent shaking daily. The alcoholic extract was then concentrated using a rotatory evaporator at 50°C (10). Each one kg of pulverized flowers yielded 300 g semisolid extract. The extract was stored at 4°C until used. Aqueous extract was obtained as a commercially lyophilized powder from the Arab Company for Pharmaceuticals and Medicinal Plants (Cairo, Egypt). All kits were purchased from Spectrum Co (Cairo, Egypt).

Fractionation of aqueous extract of *Echinacea purpura* flowers

The aqueous extract was prepared from the dissolution of powder preparation from Arab Company for Pharmaceuticals and Medicinal Plants, Egypt, in distilled water. Fractionation was carried out at the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt. Fifty grams were loaded to a dianion HP-20 AG (250 g) packed in water. Elution was carried out with distilled water as an aqueous fraction (100%), followed by methanol-water (50:50), methanol (100%), and acetone (100%) to give four fractions as follows: 32 g, 6 g, 5 g, and 4 g, respectively.

Evaluation of hepatoprotective activity

In vitro hepatoprotective activity

The hepatoprotective activity evaluation was based on the ability of tested compounds to protect human liver-derived HepG2 cells against CCl_4-induced cytotoxicity. This was determined by the MTT cell viability test at 570 nm (11,12). The hepatoprotective effect on the HepG2 cell line was expressed as the hepatic cell viability percent. It was calculated as (ODt/ODc x100), where ODt is the optical density of the treated cells with the tested sample, and ODc is the optical density of the untreated cells. The more viable cells, the more integrity mitochondria able to reduce MTT solution to blue formazan. Data are presented as means ± SD of percent of different groups.

In vivo hepatoprotective activity

Thirty-five rats were allocated into seven groups of five animals in each. The groups were as follows: Group (1) was negative control, received only the vehicle (intraperitoneal injection of sterile corn oil, 2 mL/kg) for two consecutive days per week for two weeks. The other groups were intoxicated by injecting CCl4 intraperitoneally (dissolved 1:1 in corn oil) in a dose of 2 ml/kg for two consecutive days/week for two weeks to induce hepatotoxicity (13). Group (2) was served as CCl4 intoxicated group, while group 3 (positive control) was orally given silymarin (25 mg/kg) daily as a standard drug for two weeks (14). Groups (4) and (5) were given *E. purpurea* aqueous extract in doses of 50 and 100 mg/kg daily for two weeks, respectively. Groups (6) and (7) were given *E. purpurea* alcoholic extract in doses of 50 and 100 mg/kg daily for two weeks, respectively. By the end of the study, blood samples were collected, left to clot, and centrifuged for serum separation at 3000 g for 10 min. Serum samples were then kept frozen at −20°C until biochemical analysis. Livers were dissected out and washed in ice-cooled physiological saline and used to estimate oxidative stress parameters in liver homogenates. For histopathological examination, the liver was soaked in 10% formalin for paraffin sectioning and H&E staining.

Effect of aqueous and alcoholic extract of *Echinacea purpurea* on liver and oxidative stress biomarkers

Levels of serum liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) (15), and alkaline

phosphatase (ALP) were determined as previously described. Total protein was determined using Folin phenol reagent by Lowry method (17). Serum albumin (18) and total bilirubin (19) were also determined. Liver tissue oxidative stress markers (MDA, SOD, and GSH) were measured as previously described (20), (21), and (22), respectively.

Effect of aqueous and alcoholic extracts of *Echinacea* purpurea on TNF-α and IL-6 using reverse transcriptional polymerase chain reaction (RT-PCR)

Total RNA was isolated by using TRIzol (Invitrogen), according to the manufacturer's instructions. Expressions of mRNAs for the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were quantified by real-time RT-PCR. According to the manufacturer protocol, total RNA was isolated from approximately 30 mg liver tissues by RNAEasy kit (Qiagen, Germany). The extracted RNA was dissolved in 30 µL nuclease-free distilled water and stored at -20°C. The concentration and purity of RNA were determined by a spectrophotometer (Genway, UV/VIS 6305). Real-time PCR was performed using 5 µL template in a 20 µL reaction containing 0.25 µM of each primer and 10 µL SYBR Green Real-time PCR Master Mix. Each run consisted of 50°C for 2 minutes and 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds, 60°C for 20 seconds, and 72°C for 60 seconds, in a real-time qPCR machine, the CFX96 Instrument (Bio-Rad, USA). GAPDH was used as a housekeeping gene for normalizing the expression data. Table 1 shows primer sequences of two genes selected for RT-PCR.

Table 1. Primer sequences

Gene name primers	Primers (5′→3′)
IL-6	F: CTTCCAGCCA GTTGCCTTCT R: ATGTGTGTGGAGAGCGTCAACC
TNF-α	F: CGAGTGACAA GCCCGTAGCC R: GGATGAACAC GCCAGTCGCC

Histopathological examination

Liver specimens were collected from all rats and fixed in 10% neutral buffered formalin solution, and dehydrated in ascending concentrations of ethyl alcohol (70%-100%). Tissue specimens were routinely processed and stained by hematoxylin and eosin (H&E) as described (23).

Statistical analysis

Data were expressed as the mean ± SEM of six determinations. The significant difference between means was determined using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test (24). Statistical significance was established at $P \leq 0.05$.

Results

In vitro hepatoprotective activity

The obtained results presented in Table 2 showed that the percent of hepatocytes viability for the tested extracts (aqueous and ethanol), silymarin, and fractions (aqueous, methanol, methanol: water, and acetone) had the ability to protect HepG2 against CCl_4-induced cytotoxicity, and the ethanol extract was more effective in HepG2 protection compared to other groups.

Table 2. The effect of aqueous and ethanol extracts and different fractions of *Echinacea purpurea* flowers on the viability of HepG2 cells challenged with 1% CCl_4

Extracts of silymarin or fractions (200 µg/mL)	Means± SD of hepatocytes (viability %)
Aqueous extract	7.3 ± 1.5^{e}
Ethanol extract	20.6 ± 1.8^{b}
Silymarin (Standard drug)	86.8 ± 2.6^{a}
Aqueous (100%) fraction	15.4 ± 2.4^{c}
Methanol (100%) fraction	16.2 ± 2.2 ^{c}
Methanol: water (1:1) fraction	14.1 ± 2.1^{c}
Acetone (100%) fraction	11.1 ± 1.8^{d}

In vivo hepatoprotective activity

The results revealed a significant decrease in AST, ALT, and ALP serum levels compared to the CCl4 intoxicated group. There were no significant alterations ($P \leq 0.05$) in serum levels between aqueous extract and positive control group (silymarin as standard drug). The activity of serum liver enzymes was demonstrated in Figure 1.

The effects of CCl_4, aqueous and alcoholic extracts of *E. purpurea* flowers on total protein, albumin, and total bilirubin in rats are present in Table 3. Oral administration of both extracts significantly increased total protein and albumin but decreased total bilirubin compared to CCl_4 intoxicated group. There were no significant changes between aqueous and alcoholic extracts of *E. purpurea* flowers compared to the negative control group.

The effects of CCl4, aqueous and alcoholic extracts of *E. purpurea* flowers on malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) in carbon tetrachloride (CCl_4)-intoxicated rats are presents in Table 4. Oral administration of both extracts significantly ($P \leq 0.05$) decreased hepatic MDA and increased SOD and GSH compared to the CCl4-intoxicated group.

Effects of aqueous and alcoholic extracts of *Echinacea purpurea* on the expression level of TNF-α and IL-6 by RT-PCR

The expressions of IL-6 and TNF-α mRNA are shown in Table 5. In order to exclude variations due to RNA quantity and quality, the data were corrected to GAPDH expression. The expression levels of the IL-6 gene and TNF-α gene were markedly increased in the CCl_4 group compared with the negative control group that can indicate acute liver injury in rats. Their expression levels were significantly ($P \leq 0.05$) decreased in groups treated

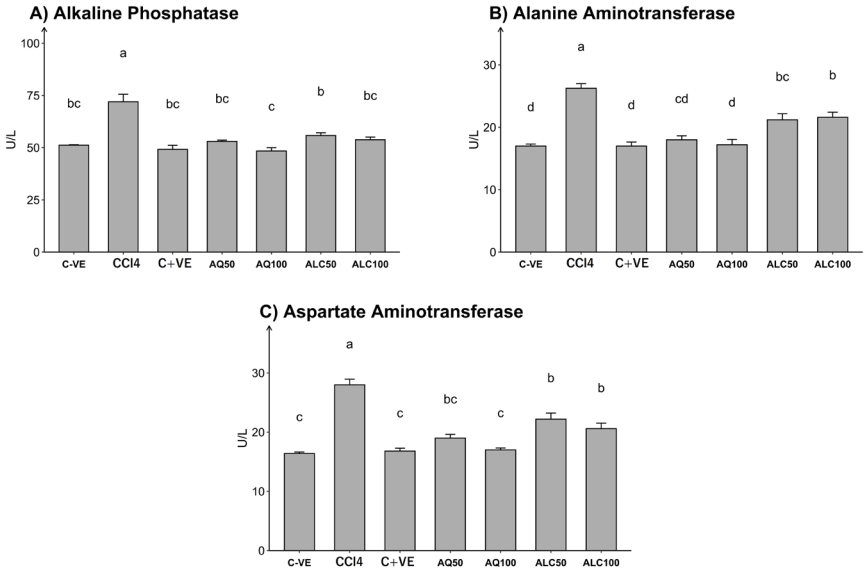

Figure 1. Effect of aqueous and alcoholic extracts of *Echinacea purpurea* flowers at 50 and 100 mg/kg on serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in carbon tetrachloride (CCL_4)-intoxicated rats (N= 5). Different letters on the columns indicate significant difference at $P \leq 0.05$. C-VE: negative control group; C+VE: positive control group treated with silymarin; NC: Negative control group; CCl4: Carbon tetrachloride intoxicated group; PC (sil): Positive control Silymarin treated group; Aq: Aqueous extract group; AlC: Alcoholic extract group.

with the high doses of alcoholic and aqueous extracts of *E. purpurea* and silymarin groups compared to the CCl4-intoxicated group. Administration of 50 mg/kg of either aqueous or alcoholic extracts of E. *purpurea* flowers did not alter TNF-α gene yet significantly altered the IL-6 gene expression.

Histopathological Findings

Figure 2 illustrates the results of histopathological examination. The negative control group (Figure 2a-d) revealed a normal liver structure; the hepatic capsule was thin and free from the inflammatory reaction. The hepatic parenchyma and the portal areas appeared normal as well. The CCl4 intoxicated rats (Figure 2e-i) showed thickening of the hepatic capsule by proliferating fibrous tissue and mononuclear inflammatory cell infiltration. The portal areas exhibited intense mononuclear inflammatory cell infiltration with the presence of periportal fibroplasia. Fibrous septa were extending to connect the portal areas in some instances. Steatosis was observed in the hepatocytes that were represented by well-circumscribed vacuoles occupying the cytoplasm. The positive control (silymarin treated) group (Figure 2j-n) showed mild improvement; the hepatic capsule showed mild thickening. Portal areas exhibited moderate mononuclear inflammatory cell infiltration. The hepatocytes were apparently normal in all examined sections except for few individuals that showed hepatocellular vacuolation. The group treated using the aqueous extract (Figure 2o-s) exhibited apparently normal hepatic capsule and parenchyma. Few sections showed mild limited mononuclear inflammatory reaction at the portal areas. Concerning the group treated using the

Table 3. Effect of aqueous and alcoholic extracts of *E. purpurea* flowers at 50 and 100 mg/kg on total protein (TP), albumin (Alb), and total bilirubin (TBil) in carbon tetrachloride (CCl_4)-intoxicated rats

Treatment		Dose (mg/kg)	TP (g/dL)	Alb (g/dL)	TBil (mg/dL)
Negative control		0	7.20 ± 0.57^{a}	4.12 ± 0.42^{a}	0.19 ± 0.025^{c}
CCl_4 intoxicated		2*	3.25 ± 0.54^{d}	2.12 ± 0.12^{c}	0.68 ± 0.04^{a}
Positive control (silymarin)		50	5.98 ± 0.73^{b}	4.0 ± 0.57^{a}	0.22 ± 0.05^{c}
CCl_4 intoxicated	Aqueous extract	50	4.56 ± 0.64^{c}	3.25 ± 0.44^{b}	0.47 ± 0.044^{b}
		100	6.86 ± 0.22^{a}	4.20 ± 0.45^{a}	0.24 ± 0.02^{c}
	Alcoholic extract	50	4.90 ± 0.53^{c}	3.35 ± 0.10^{b}	0.41 ± 0.05^{b}
		100	7.02 ± 0.97^{a}	4.1 ± 0.40^{a}	0.21 ± 0.02^{c}

One-way ANOVA + Dunnett's post hoc test (n = 5). Mean ± SD with different letter superscripts within a column are significantly different at $P \leq 0.05$ compared to the control group.

* CCl4 (dissolved 1:1 in corn oil) intraperitoneally injected at 2 mL/kg for two consecutive days/week for two weeks to induce hepatotoxicity.

Table 4. Effect of aqueous and alcoholic extracts of *Echinacea purpurea* flowers at 50 and 100 mg/kg on hepatic MDA, SOD, and GSH in CCl_4-intoxicated rats

Treatment		Dose (mg/kg)	MDA (µmol/g protein)	SOD (µmol/g protein)	GSH (µmol/g protein)
Negative control		0	19.82 ± 0.52^{b}	56.04 ± 1.9 ^{a}	1371.43 ± 59.7^{a}
CCl4 intoxicated		2*	34.42 ± 1.19^{a}	28.60 ± 1.22^{d}	527.77 ± 37.9^{d}
Positive control (silymarin)		50	24.27 ± 0.83^{b}	48.85 ± 2.87^{c}	1357.32 ± 71.1^{c}
CCl_4 intoxicated	Aqueous extract	50	18.45 ± 0.29^{b}	47.52 ± 1.55^{c}	1228.38 ± 15.2 ^{b}
		100	19.20 ± 1.30^{b}	50.69 ± 1.25^{b}	1285.50 ± 16.3 ^{b}
	Alcoholic extract	50	19.70 ± 1.09^{b}	50.99 ± 0.80^{b}	1299.52 ± 22.3 ^{b}
		100	20.88 ± 1.07^{b}	52.09 ± 0.40^{b}	1320.32 ± 71.1^{b}

MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathione; CCl4: Carbon tetrachloride.
One-way ANOVA + Dunnett's post hoc test (n=5). Means ± SD with different letter superscripts within column are significantly different at $P \leq 0.05$ compared to the control group.
* CCl4 (dissolved 1:1 in corn oil) intraperitoneally injected at 2 ml/kg for two consecutive days/week for two weeks to induce hepatotoxicity.

Table 5. Effects of aqueous and alcoholic extracts of *Echinacea purpurea* on TNF-α and IL-6

Treatment		Dose (mg/kg)	TNF-α (Pg/g)	IL-6 (Pg/g)
Negative control		0	1.00 ± 0.29^{d}	1.00 ± 0.38 ^{e}
CCl_4 intoxicated		2*	5.5 ± 0.38^{a}	6.43 ± 0.41^{a}
Positive control (silymarin)		50	5.7 ± 0.25^{a}	5.7 ± 0.25 ^{b}
CCl_4 intoxicated	Aqueous extract	50	5.22 ± 0.56 ^{b}	5.22 ± 0.56^{c}
		100	4.08 ± 0.18 ^{c}	4.08 ± 0.18^{d}
	Alcoholic extract	50	5.15 ± 0.11^{b}	5.15 ± 0.11^{c}
		100	4.56 ± 0.41^{c}	4.56 ± 0.41^{d}

TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; CCl_4, carbon tetrachloride.

alcoholic extract (Figure 2t-x), all the examined sections revealed normal hepatic parenchyma with mild portal inflammatory cells infiltration in sporadic cases.

Discussion

The current study aimed to examine the *in vitro* and *in vivo* hepatoprotective effects of both aqueous and alcoholic extracts of *E. purpurea* flowers on human HepG-2 cell lines using colorimetric tetrazolium (MTT) assay and in CCl4-induced hepatotoxicity in a rat model, respectively.

Liver damage and hepatotoxicity induced by intraperitoneal injection of CCl_4 (25) are due to the accumulation of CCl4 in hepatic parenchyma cells. The CCl_4, in turn, is actively metabolized by cytochrome P450 to form a trichloromethyl (CCl_3) radical. The CCl_3 radical is considered a highly reactive intermediate metabolite that initiates excessive lipid peroxidation, leading to hepatocytes' functional and structural distraction (26,27). In this study, intraperitoneal injection of CCl4 induced hepatotoxicity, which was confirmed by increasing AST, ALT, ALP, and total bilirubin, which are liver injury biomarkers associated with increase oxidative stress by increasing MDA and decreasing GSH and SOD in addition to alteration in normal histological architecture as shown in the positive control group (25). Therefore, antioxidant capacity or free radical generation embarrassment is important for protection against CCl_4-induced hepatotoxicity and liver lesions (28).

In this study, the oral administration of reference drug silymarin decreased the liver damage induced by CCl_4 with a consequent recovery towards healing. This suggests an enhanced regeneration of hepatocytes' parenchyma and protection against membrane fragility with reduced seepage of serum liver enzymes into the circulation. Silymarin achieves its hepatoprotective effect via several mechanisms, including regulation of cell membrane permeability (29), inhibition of lipid peroxidation and malondialdehyde production (30), increasing SOD activity (31), elevating reduced GSH (32), inhibition of Kupffer cell functions and nitric oxide production (33), mast cell stabilization (34), or reduction of inflammation by reducing neutrophil migration and inhibition of 5-lipoxygenase pathway (35).

Numerous studies have examined the immunomodulatory, anti-oxidant, anti-inflammatory, and antibacterial effects of *E. purpurea*, but scarce research is available on the hepatoprotective effect *E. purpurea*. The present study aimed to investigate the possibility of using *E. purpurea* extracts to counteract CCl_4 liver intoxication. The results showed that both aqueous and ethanolic extracts of *E. purpurea* flowers significantly decreased the serum liver biomarkers of AST, ALT, and ALP. In addition, both extracts were able to alleviate liver toxicity by reducing inflammatory cell infiltration, necrosis, and damage in hepatic cords with

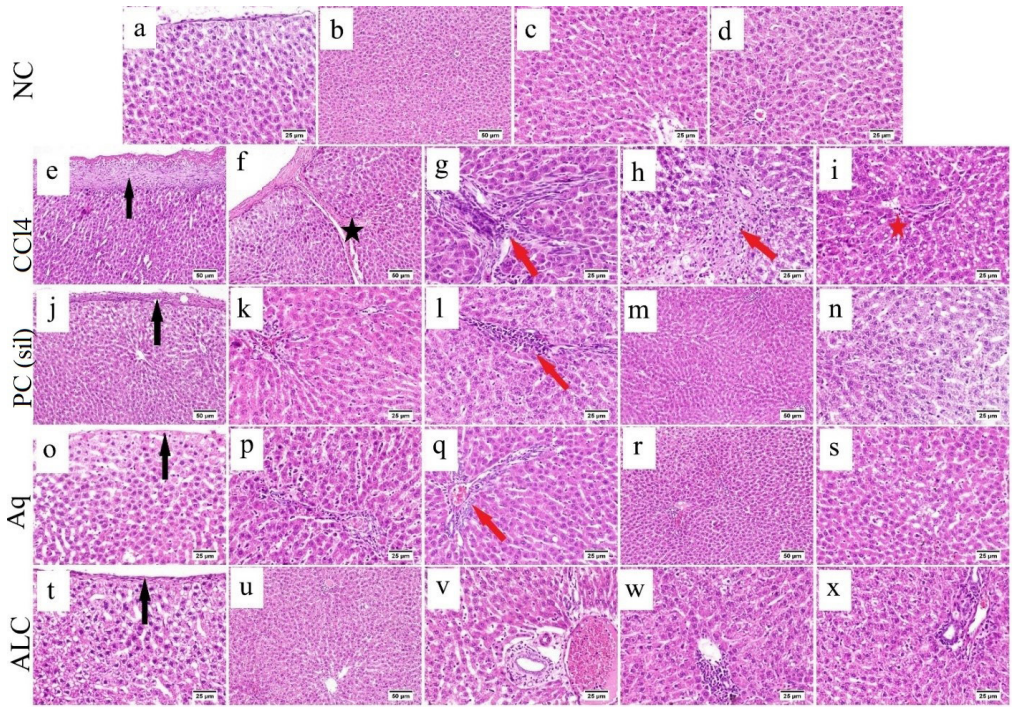

Figure 2. Photomicrographs of the liver (H&E); (**a-d**) negative control group showing normal hepatic capsule and parenchyma, (**e-i**) CCl4 intoxicated group showing thickened hepatic capsule (black arrow) with the presence of fibrous tissue strand (black star) extending into the hepatic parenchyma, mononuclear inflammatory cells at the portal area with fibroplasia (red arrow) and steatosis in the hepatocytes (red star), (**j-n**) PC(sil): positive control (Silymarin treated) group showing moderate thickening of the hepatic capsule (black arrow), mild mononuclear inflammatory cells infiltration at the portal area (red arrow), with apparently normal hepatic parenchyma and mild vacuolation of the hepatocytes, (**o-s**) Aqueous extract group showing mild apparently normal hepatic capsule (black arrow), mild mononuclear inflammatory cells at the portal area (red arrow) and apparently normal hepatic parenchyma, and (**t-x**) Alcoholic extract group, showing apparently normal hepatic capsule (black arrow), apparently normal hepatic parenchyma, mild portal and pericentral mononuclear inflammatory cells infiltration. NC: Negative control group; CCl4: Carbon tetrachloride intoxicated group; PC (sil): Positive control silymarin treated group; Aq: Aqueous extract group; AlC: Alcoholic extract group.

loss of intercellular border in the liver compared to the positive control group. These findings were in agreement with the results of previous researches (36). Moreover, the histopathological findings of the current study revealed remarkable concordance with the reported biochemical results, denoting a notable hepatoprotective effect of the extracts of *E. purpurea* flowers.

Oxidative stress, which induces hepatocyte dysfunction, is the key player for acute liver injury. Medicinal plants with bioactive anti-oxidant compounds have been considered a source for liver-curing drugs (37). The anti-oxidant activity of *E. purpurea* flowers could be owed to polyphenolic components such as total flavonoids, phenolic acids, or phenolic diterpenes (38). Hence, the hepatoprotective effect of *E. purpurea* flowers may be attributed to its anti-oxidant activity. This hypothesis was potently supported by our results, including restoring MDA, SOD, and GSH in liver tissue to approximately the normal levels compared to the negative control group.

TNF-α and IL-6 are cytokines implicated in various physiological and pathological disorders (39). In the present investigation, the TNF-α and IL-6 levels were increased in the CCl_4- positive control group, which is following the finding of Simeonova et al (40). TNF-α seems to be responsible for regulating products that stimulate inflammation and fibrosis in CCl_4-induced hepatotoxicity (41). Treatment with the high doses of aqueous or alcoholic extracts of *E. purpurea* down-regulated the expression of TNF-α and IL-6 compared to the positive control group. This may suggest that *E. purpurea* attenuated CCl_4-induced inflammatory cascade in the liver. Considerable evidence suggested that TNF-α and IL-6 contributed to the pathogenesis of liver inflammatory diseases by activating the NF-κB signaling pathway (42). In this respect, NF-κB p65 was activated in the CCl_4 group, and this activation was partially prevented by silymarin (43,44). The authors have found that aqueous extract of *Phyllanthus niruri* also decreased TNF-α, NF-KB, IL-6, IL-8, IL10, and COX-2 expression, and significantly antagonized the effect of CCl_4 on the anti-oxidant enzymes SOD, CAT, glutathione reductase, and glutathione peroxidase.

Conclusion

The present study suggests that the extracts of *E. purpurea* flowers exhibit a good hepatoprotective effect against CCl_4 induced hepatotoxicity in rats and in CCl4 induced HepG2 toxicity *in vitro*, possibly through anti-oxidant and anti-inflammatory activities. These results suggest that intake of *E. purpurea* flowers as a drink may be useful for patients who suffer from liver diseases.

Authors' contributions

SMA and EHA conceived the research idea and designed the work and wrote the first draft of the manuscript. SMM, GAA, and EAH carried out the experiments and GAA performed the statistical analysis. All authors contributed to the editing of the revised manuscript, and approved the final manuscript.

Conflict of interests

Authors declare no conflict of interests.

Ethical considerations

The protocol for this study was confirmed by Animal Research Ethical Committee, Faculty of Veterinary Medicine, Cairo University (Vet. CU. IACUC 16072020176, dated 16 July 2020) and the authors of this manuscript observed ethical issues. Animals were handled according to the International Guidelines for Care and Handling of Experimental Animals.

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