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Hepatoprotective effects of *Echinacea purpurea* against copper oxide nanoparticles-induced hepatic toxicity in albino rats

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ABSTRACT

The expanding use of copper oxide nanoparticles (CuO-NPs) in industries and their extensive applications raised potential hazardous effects. *Echinacea purpurea (E. purpurea*) possesses numerous medicinal benefits owing to its anti-apoptotic, antioxidant, and anti-inflammatory effects. This study aimed to assess the role of *E. purpurea* in mitigating CuO-NPs induced liver toxicity in rats. Twenty-eight adult male albino rats were divided into four equal groups. The first group (G1) received saline (1 ml per rat) daily via stomach tube. G2 was given *E. purpurea* at a dose of 150 mg/kg b.wt. daily via stomach tube. CuO-NPs were administered orally to G3 at a dose of 300 mg/kg b.wt. every day. G4 was given CuO-NPs and *E. purpurea* daily in the same dose and route. After four weeks of the experiment, liver specimens and serum samples were taken from rats in all groups. The findings showed that *E. purpurea* alleviated the hepatic damage induced by CuO-NPs through improvement of body weight, hematological parameters, serum ALT, AST, and ALP, also by restoring the antioxidant state, as evidenced by elevated catalase (CAT), reduced glutathione (GSH) and lowered malondialdehyde (MDA). Additionally, *E. purpurea* maintained liver tissue architecture, and decreased immunoreactivity of caspase-3 (Cas-3). This study concluded that *E. purpurea* administration attenuated the hepatotoxicity of CuO-NPs treated rats through its antioxidant, anti-inflammatory, and anti-apoptotic properties.

1. INTRODUCTION

Nanoparticle research is now a topic of tremendous scientific interest due to its vast range of applications in many disciplines (Joudeh and Linke, 2022). Nanoparticles are particles measuring between 1 to 100 nanometers in two or three dimensions. Nanoparticles possess distinctive physiochemical features, including an extremely tiny size and huge surface area to mass ratio (Naz et al., 2020). These qualities can be utilized to circumvent some limitations observed in traditional therapeutic and diagnostic drugs (Hsu et al., 2023).

Metal-oxide nanoparticles are defined by their unique morphology, aggregation capabilities, and surface structure, making them suitable for use in numerous industries (Hashim et al., 2024). One of the most developed metals-oxide nanoparticles is copper oxide nanoparticles (CuO-NPs). CuO-NPs are an economical and effective catalyst for a wide range of chemical processes due to their huge surface area, high reactivity, and low price (Devaraji et al., 2024). CuO-NPs offer a wide range of biomedical uses, diagnostic imaging, catalytic, optical characteristics, as well as their biocidal, antibacterial, and antifungal properties (Priya et al., 2023). CuO-NPs are also employed as feed additives in livestock and poultry production (El Bialy et al., 2020).

Liver, kidney, and spleen are the primary hazardous target organs of copper nanoparticles (Naz et al., 2023). Previous studies have revealed the hepatotoxic impact of CuO-NPs at various dosages and routes (Hashim et al., 2024). Upon entering the bloodstream, nano-copper induced oxidative reactions and triggered a series of adverse events, including genotoxicity, inflammation, fibrosis, and tumorigenesis (Fahmy et al., 2020).

Medicinal plant extracts have attracted widespread interest as natural therapy for various diseases, which might be related to the existence of several bioactive components that diminish oxidative stress and cell dysfunction (Xiong and Guan, 2017). In the past few years, experts have been particularly interested in the therapeutic benefits of *Echinacea purpurea* (purple coneflower) (Shalaby et al., 2021). Echinacea purpurea is commonly used to prevent or treat infections of the upper respiratory tract. This therapeutic benefits may be linked to the presence of many medicinal constituents, including alkylamides, caffeic acid derivatives, polysaccharides, glycoproteins, polyacetylenes, phenolic compounds and flavonoids (Ogal et al., 2021). Additional studies of the plant's root extract demonstrated antioxidant effects, likely due to its phenolic compounds and cichoric acid. Cichoric acid has strong radical scavenging action (Attarzadeh et al., 2020). Additionally, *E. purpurea* possesses immunostimulant, antiviral, antibacterial, antifungal, anti-inflammatory, antioxidant, and even anti-cancer effects (Pemmereddy et al., 2022).

Previous studies indicated that *E. purpurea* had a protective effect on the liver against the toxicity of Diethyl-nitrosamine (DEN), alcohol, and cadmium in rats (Rezaie et al., 2013; El-Demerdash et al., 2024). So, this study was conducted to

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investigate the role of *E. purpurea* against liver toxicity induced by CuO-NPs.

2. MATERIAL AND METHODS

Ethical approval

The guidelines and procedures used in this experiment were supplied by Benha University's Scientific Research Ethics Committee in Egypt. BUFVTM 15-09-23 is the protocol number that has been approved. Every procedure was carried out in compliance with the applicable rules and regulations. The report of the study follows the ARRIVE principles.

2.2. Chemicals

Copper oxide nanoparticles (CuO-NPs) powder was obtained from Nanotech Egypt for Photo Electronics, Al Giza, Egypt. CuO-NPs average size was 40±10 nm and Quasi-spherical shape. *Echinacea purpurea* capsules *(Echinacea* complex®*)* were purchased from Puritan's Pride company, USA.

2.2. Experimental animals

Twenty-eight mature male albino rats, with weights ranging between 150 and 170 g at twelve weeks of age, were acquired from the Institute of Veterinary Serum and Vaccine Research, Abasia, Cairo, Egypt and housed in solid bottom polypropylene cages and provided with a balanced diet, free access to clean water, and a hygienic environment and maintained in a natural 12-hour light/dark cycle and a room temperature of 23 ±3°C. Rats were accustomed to the environment for at least seven days before the experiment. Rats were buried after the experiment was completed and samples were collected.

2.3. Experimental design

The experimental rats were randomly assigned to four equal groups, each consisting of seven rats. The first group (G1) was preserved as a control that received normal saline (1 ml per rat) daily for 4 weeks. The second group (G2) was administered *E. purpurea* (dissolved in saline) by stomach tube at a dose of 150 mg/kg b.wt. daily for 4 weeks (Khalaf et al., 2019). The third group (G3) (intoxicated group) was administered oral CuO-NPs at a dose of 300 mg/kg b.wt. (dissolved in saline) daily for 4 weeks (Khalid et al., 2018). The fourth group (G4), which administered co-treatment, received CuO-NPs and *E. purpurea* by the same route and dose formerly mentioned. Throughout the trial, clinical symptoms and body weights were recorded for every group. At the end of the trial, the rats were euthanized by inhaling isoflurane.

Blood samples were obtained from the abdominal aorta, and liver specimens were collected from all rats in the experimental groups. These samples were used to assess hematological parameters, liver function tests, oxidative indicators, and histopathological and immunohistochemical changes.

2.4. Characterization of copper oxide nanoparticles

To investigate the morphology of nanoparticles, scanning electron microscopy (SEM) was used (JEOL-JSM 5910). The size and form of the nanoparticles were determined using transmission electron microscopy (TEM) (JEM-2100 HR, JEOL, USA) (Abudayyak et al., 2016). CuO-NPs were found to have a quasi-spherical form with an average size of less than 50 nm, as displayed in Fig. (1).

2.5. Preparation of liver tissue homogenate

The liver was homogenized using the techniques specified by Nasr et al. (2024). Total proteins and oxidative markers [reduced glutathione (GSH), catalase (CAT) and malondialdehyde (MDA) level were analyzed in the collected supernatant.

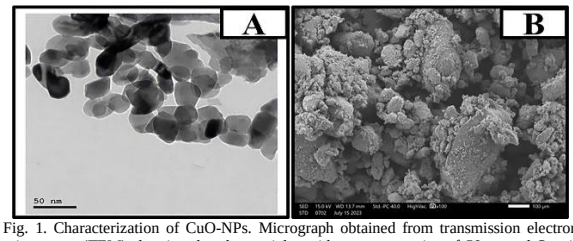

Fig. 1. Characterization of CuO-NPs. Micrograph obtained from transmission electron microscope (TEM) showing that the particles with an average size of 50nm and Quasi-spherical in shape (Fig. 1A). Scanning electron microscopy (SEM) image for CuO-NPs (Fig. 1B).

2.6. Assays methods

2.6.1. Body weight measurement

Body weights were determined weekly to study the toxic effect of CuO-NPs and protective effect of *E. purpurea* on body weight of rats.

2.6.2. Hematological profile

All hematology parameters in the blood samples were determined in an Advia 120 Hematology Analyzer (Siemens Health Care, Berlin, Germany) according to the manufacturer's instructions. Hematological parameters included red blood cells count (RBC), hemoglobin levels (Hb), packed cell volume (PCV), platelets count (PLT), white blood cells count (WBC) and differential leukocytic count (DLC).

2.6.3. Liver function tests

The activities of AST and ALT were evaluated using the procedures of Reitman and Frankel, (1957) and alkaline phosphatase (ALP) was assessed based according to Belfield and Goldberg, (1971) using reagent kits obtained from Bio diagnostic Co., Giza, Egypt

2.6.4. Oxidative markers in liver homogenate

GSH contents and CAT activity were measured according to the method of Ellman (1959) and Johansson and Borg (1988), respectively. MDA levels were determined following the procedures of Ohkawa et al. (1979).

2.6.5. Histopathological examination

Liver specimens were taken immediately from each group and preserved in 10% buffered neutral formalin for 24 hrs. After being washed with water, the specimens were subjected to dehydration using a series of ethyl alcohol dilutions. Specimens were then cleared in xylol, embedded in paraffin wax and dehydrated using different grades of ethyl alcohol. Tissue blocks of Paraffin wax were cut into 4 µm thick sections using an RM2235 Leica microtome (Biosystems, Nussloch, Germany). Staining of tissue sections with H&E (Bancroft and Layton, 2019). Then, a microscopic examination was carried out.

2.6.6. Immunohistochemical study

The paraffin sections were rehydrated and deparaffinized. Rabbit monoclonal antibodies against caspase-3 (Cas-3) ([EPR12012] (ab179800); 1/1000-1/5000 dilution; Abcam, UK) and primary rabbit polyclonal antibodies against cytochrome C (SAB4502234; 1:50-1:100 dilution; Sigma-Aldrich, St. Louis, Missouri, USA) were incubated with the buffer solution at a concentration of 2-4µg/ml. To stop nonspecific reactions, 10% hydrogen peroxide was used to block the sections initially. Citrate buffer (pH 6) was used for heat-mediated antigen retrieval prior to IHC labeling

with polyclonal antibody of rabbit against SERCA2 ATPase (ab3625, Abcam, UK, 1g/mL). After rinsing the cells with phosphate buffer, a secondary goat anti-rabbit antibody that had been biotinylated was applied. To localize the immunological response, sections were subjected to labeled avidin-biotin peroxidase treatment, which attaches to the biotin on the secondary antibody. Diaminobenzidine was converted to a brown precipitate by peroxidase, therefore it was utilized as a chromogen to show where the antibody attached (Suvarna et al., 2012).

Image analysis

Using a Nikon Eclipse E800 microscope (Melville, NY, USA), the area percentage (area %) occupied by brown color in Cas-3 immunostaining region was determined. The images were taken with an Olympus digital camera (E-620, United States). Digital image analysis and quantification were conducted via Scion Image Beta 4.03 (Scion Corporation, USA).

2.7. Statistical analysis

Statistical analysis was performed by SPSS (Version 16; SPSS Inc., Chicago, USA). One-way ANOVA was used to investigate the substantial divergence found through multiple group comparisons and the Duncan test was used as a post hoc assessment. It expresses all values as mean ±SE, with a significance considered at $P \leq 0.05$.

3. RESULTS

No symptoms of illness or mortality were observed in all groups throughout the study.

3.1. Body weight measurement

There was a significant ($P<0.05$) decline in body weights in CuO-NPs treated rats. Whereas *E. purpurea* in combination with CuO-NPs group revealed an increase in body weights compared to CuO-NPs group (Table 1).

3.2. Hematological profile

Hb concentration, RBCs, WBCs, lymphocytes, and platelets counts following CuO-NPs and/or *E. purpurea* treatment were displayed in Table (2). Significant decrease in RBCs, Hb concentration, and platelets count with noteworthy increase in the WBCs and lymphocyte counts following CuO-NPs intoxication were detected. However, *E. purpurea* administration significantly restored RBC and Hb levels close to normal levels in the group co-treated with CuO-NPs and *E. purpurea*. Moreover, *E. purpurea* markedly reduced the elevated WBCs and lymphocytes in the co-treated group.

3.3. Liver function tests

After the fourth week of administration, the levels of ALT, AST, and ALP of CuO-NPs-intoxicated group exhibited a substantial rise compared to other experimental groups. However, ALT, AST, and ALP levels were improved in the group that was given *E. purpurea* and CuO-NPs co-treatment (Table 3).

3.4. Liver oxidative markers

The liver tissue of CuO-NPs intoxicated group showed significantly reduced GSH content and CAT activity compared to the other groups being investigated. Despite that, after the fourth week of the study, the group that was administered *E. purpurea* and CuO-NPs co-treatment showed improvement in these activities compared to CuO-NPs intoxicated group. While the levels of MDA, an indicator of lipid peroxidation in liver tissue, were significantly higher in the CuO-NPs intoxicated group compared to the control group. However, after the fourth week of the experiment, the group received co-treatment with *E. purpurea*, and CuO-NPs exhibited a reduction in MDA levels compared to the control group (Table 4).

Table 1. Impact of *E. purpurea* and copper oxide nanoparticles (CuO-NPs) on body weights in different experimental groups (means± SE, n=7).

	Control	*E. purpurea*	CuO-NPs	CuO-NPs+ *E. purpurea*
Initial weight	158.57±2.83^{a}	165.71±2.54^{a}	162.86±3.76^{a}	168.57±3.57^{a}
First week	185.00±3.62^{a}	186.43±2.83^{a}	135.71±3.85^{c}	162.9±2.64^{b}
Second week	209.29±9.72^{a}	211.43±6.96^{a}	150.06±3.45^{c}	181.4±5.42^{b}
Third week	242.86±6.80^{a}	241.43±12.43^{a}	179.29±1.70^{c}	209.29±6.02^{b}
Fourth week	283.57±2.61^{a}	280.00±3.27^{a}	194.29±48.1^{c}	236.29±11.57^{b}

*Different superscript within the same row indicates significantly different mean values ($p\leq0.05$).

Table 2 Impact of *E. purpurea* and copper oxide nanoparticles (CuO-NPs) on hematological profile in different experimental groups (mean ±SE, n=7).

	Control	*E. purpurea*	CuO-NPs	CuO-NPs+ *E. purpurea*
Hb (g/dl)	10.96±0.31^{a}	11.1±0.38^{a}	7.60±0.32^{c}	9.03±0.34^{b}
PCV %	31.29±2.04^{a}	30.43±2.36^{a}	20.57±2.11^{c}	26.86±2.10^{b}
RBCs ($\times10^{6}$/mm^{3})	2.73±0.12^{a}	2.80±0.12^{a}	1.87±0.13^{c}	2.31±0.07^{b}
TLC ($\times10^{3}$/mm^{3})	23. 33±3.10^{c}	23.14±2.03^{c}	39.46±4.33^{a}	30.53±5.01^{b}
Lymphocytes (%)	48.57±3.85^{c}	50.29±2.34^{c}	77.38±4.90^{a}	62.38±5.09^{b}
Platelets	336±15.63^{a}	343.14±17.65^{a}	243.14±9.65^{c}	276.86±8.71^{b}

Hemoglobin (Hb), Packed cell volume (PCV), Red blood cells (RBCs) and Total leukocytic count (TLC). *Different superscript within the same row indicates significantly different mean values ($p\leq0.05$).

Table 3 Impact of *E. purpurea* and copper oxide nanoparticles (CuO-NPs) on liver function tests in different experimental groups (mean ±SE, n=7).

	Control	*E. purpurea*	CuO-NPs	CuO-NPs +*E. purpurea*
ALT (IU/L)	20.71±1.67^{c}	20.86±2.53^{c}	59.14±2.88^{a}	39.29±3.54^{b}
AST (IU/L)	91.71±4.25^{c}	90.14±5.28^{c}	168.14±3.86^{a}	134.71±6.90^{b}
ALP (IU/L)	293.14±11.80^{c}	318.86±11.72^{c}	476.33±13.14^{a}	383.43±10.00^{b}

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP). *Different superscript within the same row indicates significantly different mean values ($p\leq0.05$).

Table 4 Impact of *E. purpurea* and copper oxide nanoparticles (CuO-NPs) on oxidative markers in the liver homogenates in different experimental groups (mean ±SE, n=7).

	Control	*E. purpurea*	CuO-NPs	CuO-NPs+ *E. purpurea*
MDA (nmol/mg protein)	254.46±4.69^{c}	249.09±9.44^{c}	380.55±3.44^{a}	289.64±11.32^{b}
CAT (U/mg protein)	190.67±8.56^{a}	193.15±5.79^{a}	99.16±10.39^{c}	161.74±9.23^{b}
GSH (µmol/mg protein)	178.30±6.19^{a}	180.67±7.28^{a}	90.09±5.07^{c}	156.02±7.78^{b}

MDA (malondialdehyde), CAT (catalase) and GSH (reduced glutathione). *Different superscript within the same row indicates significantly different mean values ($p\leq0.05$).

3.5. Histopathological assessment of liver tissue

Examination of H&E-stained liver sections showed that CuO-NPs intoxicated rats revealed fibrin thrombus within the portal vein (Fig 2B), marked hydropic degeneration characterized by swollen and vacuolated hepatocytes with cytoplasm rarefaction; and coagulative necrosis of the hepatocytes (Fig 2C) and coagulative necrosis of shrunken hepatocytes with hyper-eosinophilic cytoplasm and pyknotic nucleus and focal interstitial aggregations of lymphocytes and macrophages (Fig, 2D). Co-treatment of rats with *E. purpurea* and CuO-NPs demonstrated improvement in the hepatocellular architecture with focal interstitial aggregations of inflammatory cells (Fig, 2E). The livers of control or *E. purpurea* treated rats showed normal histological appearance of hepatic architecture, portal areas, central veins, sinusoids and hepatocytes (Fig, 2A).

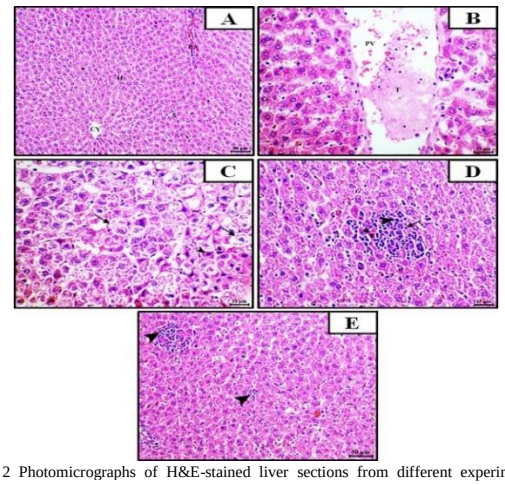

Fig. 2 Photomicrographs of H&E-stained liver sections from different experimental groups. (A) Control (saline) and *E. purpurea* -treated groups showing normal hepatic architecture, portal area (PA), central vein (CV) and surrounding hepatocytes (H). (B, C, D) CuO-NPs group showing (B) Fibrin thrombus (T) within the portal vein (PV), (C) Marked hydropic degeneration (arrow) and coagulative necrosis (arrowhead) of the hepatocytes, (D) Coagulative necrosis (thick arrow) of shrunken hepatocytes with hyper-eosinophilic cytoplasm and pyknotic nucleus and focal interstitial aggregation of lymphocytes (arrow) and macrophages (arrowhead). (E) CuO-NPs+ *E. purpurea* treated group showing normal hepatic architecture with focal interstitial aggregations of inflammatory cells (arrowhead).

3.6. Immunohistochemical assessment of liver apoptosis

Cas-3 immunohistochemistry staining sections of hepatic tissues from rats exposed to CuO-NPs revealed that Cas-3 protein exhibited increased expression in the walls of central vein, sinusoids, and between hepatocytes (Fig 3C). The Cas-3 area (%) was significantly higher in CuO-NPs intoxicated group after 4 weeks compared to the control (Fig 3E). Conversely, rats given *E. purpurea* and CuO-NPs showed reduced expression of Cas-3, and the number of positive cells is lower than CuO-NPs intoxicated rats (Fig, 3D). Following four weeks of the experiment, the group treated with *E. purpurea*, and CuO-NPs had a considerably reduced Cas-3 area (%) compared to the control (Fig 3E). The liver tissues of control, *E. purpurea* treated rats revealed negative to mild Cas-3 immunostaining (Fig, 3A&B).

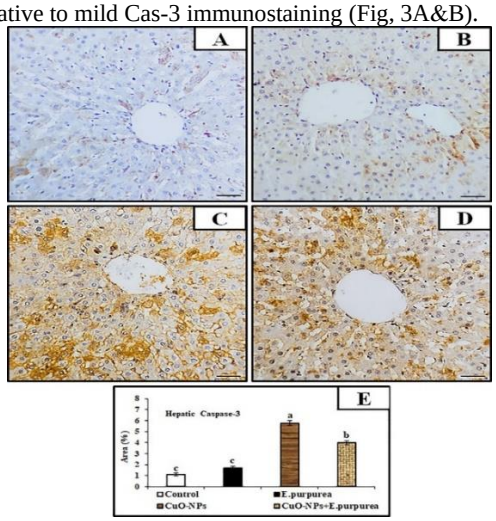

Fig. 3 Immunohistochemical expression of caspase-3 in liver tissues from different experimental groups. The brown color indicates positivity. (A) Control (saline) group showing negative to mild caspase-3 immunostaining in hepatocytes. (B) *E. purpurea* group exhibiting minimal caspase-3 staining, similar to the control (C) CuO-NPs group with marked caspase-3 staining around the central vein, sinusoids and hepatocytes (D) CuO-NPs+ *E. purpurea* treated group showing reduced caspase-3 staining compared to the toxic group. (E) Morphometric study of the percentage area of caspase-3 immunohistochemistry staining in liver sections of experimental rats. Data was used to assess the extent of caspase-3 immunohistochemistry staining. P<0.05 compared with different experimental groups. Bars represent mean ±SE, (n=7). (Scale bar=50 µm, ×50).

4. DISCUSSION

Copper oxide nanoparticles (CuO-NPs) are progressively used in various fields, including industrial catalysis, gas sensors, biomedicines, and environmental remediation due to their beneficial properties, specifically their huge surface area to volume ratio (Ahire et al., 2022). These extensive uses have raised human exposure, and hence the potential danger associated with their acute and chronic toxicity (Gakis et al., 2023).

CuO-NPs have received ~~a~~ significant attention due to their unique advantages and properties (Sicwetsha et al., 2021). Their small size improves their capacity to infiltrate and accumulate within several body tissues and organs, such as the liver, upsetting their usual structure and impairing the organs' regular function (Hashim et al., 2024). The present study's objective was to evaluate if *E. purpurea* could protect the liver from the hepatotoxicity caused by CuO-NPs or not. The current study demonstrated that the body weights of rats in CuO-NPs group were markedly decreased through the four weeks of the experiment. This could be related to reduced feed intake as a result of the negative effects of CuO-NPs, which in turn caused the stressed rats to consume less food, resulting in a loss of body weight (Emam et al., 2018). Rats that received both *E. purpurea* and CuO-NPs exhibited increased body weight compared to the intoxicated group. This might be attributable to enhanced feed consumption efficiency, as *E. purpurea* contains antioxidant compounds (Attarzadeh et al., 2020). The present study indicated that toxicity by CuO-NPs altered normal hematological parameters. CuO-NPs group showed a significant increase in WBCs, while RBCs, Hb, and platelets count decreased significantly. The increase in WBCs could be due to the activation of leucopoiesis by CuO-NPs, which might act as an immunosuppressive agent and due to the increased percent of lymphocytes (Abotaleb et al., 2021). There might be numerous reasons for the low RBCs and hemoglobin count. Exposure of rats to Cu nanoparticles causes red blood cell destruction, as a result of the oxidative stress triggered by these nanoparticles Ayaz et al. (2016) or abnormally elevated levels of Cu resulting from CuO-NPs intake might result in a Fe-deficient status, which eventually resulted in anemia (Zhang et al., 2010). These findings were in accordance with Khabbazi et al. (2014).

Meanwhile, CuO-NPs and *E. purpurea* treated rats presented a significant correction in all hematological parameters. This improvement is attributed to the contents of *E. purpurea* as cichoric acid and echinacin, that stimulates bone marrow and hematopoietic stem cells (Khattab et al., 2019).

The present study demonstrated that the levels of ALT, AST, and ALP were significantly elevated, suggesting that the liver tissue of the CuO-NPs-intoxicated group had been damaged after four weeks of oral administration. This could be the consequence of cellular hepatic injury and inflammation, which elevates the cell membrane permeability and leads to leakage of the functional intracellular enzymes (AST and ALT) and ALP (a membrane-bounded enzyme) into the bloodstream (Hashim et al., 2024). These findings agreed with Abdel-Azeem et al. (2023).

Significant reductions in serum ALT, AST, and ALP levels in rats co-treated with *E. purpurea* and CuO-NPs were recorded in correlation to CuO-NPs-intoxicated group. The reduction in these enzyme levels suggests that *E. purpurea* can protect hepatocyte membranes, reducing biomarkers leaking into the bloodstream. This might be attributed to *E. purpurea's* antioxidant capacities, total phenols, and caffeine derivatives (Sharif et al., 2021). Our findings were consistent with a previous study that reported the protective effects of *E. purpurea* against oxidative stress, inflammation, and apoptosis caused by potassium dichromate in rats (Karhib et al., 2022).

In the present work, rats administered with CuO-NPs exhibited a significant decrease in CAT activity and GSH contents following the fourth week of intoxication, and an increase in lipid peroxidation as evidenced by the increase in MDA levels. This impact is thought to arise from cellular component damage and membrane lipid peroxidation (El-Guendouz et al., 2020). The basic mechanism of CuO-NPs-induced hepatic injury is oxidative stress induction, which causes necrosis of liver cells, impairment of liver functions, and deterioration of hepatic structure due to the generation of free radicals by CuO-NPs toxicity (Moschini et al., 2023). ROS are responsible for the depletion of naturally produced antioxidants and the alteration of cellular lipids, resulting in lipid peroxidation (Naz et al., 2020). These findings were partially supported by those of Bugata et al. (2019), who found that MDA levels were significantly increased, but GSH activity was decreased in liver of rats receiving CuO-NPs.

Rats that received both *E. purpurea* and CuO-NPs concurrently exhibited higher GSH contents, CAT activity, and lower MDA levels. *E. purpurea* possesses antioxidant and free radical scavenging characteristics, which alleviated the negative consequences of CuO-NPs on the liver and effectively maintained the hepatocytes (Karhib et al., 2022). The present results were confirmed by histopathological findings that revealed several abnormalities in the liver of CuO-NPs-intoxicated group which showed fibrin thrombus within the portal vein, marked hydropic degeneration characterized by swollen and vacuolated hepatocytes with cytoplasm rarefaction and coagulative necrosis of the hepatocytes with hypereosinophilic cytoplasm and pyknotic nucleus and focal interstitial aggregations of lymphocytes and macrophages. These findings were supported by the findings of Tohamy et al. (2022), who revealed deteriorated bile ducts, thickening of the duct epithelial lining, and mononuclear inflammatory cells infiltration after intoxication with CuO-NPs. While rats treated with *E. purpurea* and CuO-NPs showed maintained normal hepatic architecture, with few localized interstitial aggregations of inflammatory cells. This finding revealed that *E. purpurea* has antioxidant activity by scavenging free radicals (Mohamed et al., 2023).

These results corroborated the Cas-3 immunohistochemistry features of the CuO-NPs-intoxicated rats, which showed that Cas-3 protein was strongly expressed in the region of the portal tract, the walls of central vein and sinusoids as well as the hepatic cells, indicating apoptotic alterations. CuO-NPs triggered mitochondrial destruction and apoptosis by interacting with the undissolved NPs directly with ROS-derived lipid peroxides, resulting in loss of integrity of membrane, release of the apoptotic enzymes, and activation of caspases such as caspase 3 (Liu et al., 2021). These findings were consistent with Goma et al. (2021) who found an increase in Cas-3 positive cells in the brain of rats exposed to CuO-NPs.

Rats treated with *E. purpurea*, and CuO-NPs showed a reduced Cas-3 expression, and the number of positive cells is lower than CuO-NPs intoxicated rats. This might be attributed to *E. purpurea*, which is an excellent source of natural antioxidants and has the ability to eliminate free radicals, due to its high amounts of bioactive phenolic compounds (Paudel et al., 2023).

5. CONCLUSIONS

The findings of the current study implied that *E. purpurea*'s anti-inflammatory, antioxidant, and anti-apoptotic properties mitigated CuO-NPs induced liver damage through amelioration of hematological parameters, hepatic enzymes, oxidative stress markers, histopathological findings, and caspase-3 immunoexpressing.

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