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Immuno-antioxidative reno-modulatory effectiveness of Echinacea purpurea extract against bifenthrin-induced renal poisoning

Khaled Abdel-Wahhab

kgm194@yahoo.com

National Research Centre

Ghada Elqattan

National Research Centre

Laila Hassan

National Research Centre

Rehab Sayed

Regional Center for Food and Feed, Agriculture Research Centre

Doaa El-Sahra

Modern University for Technology and Information

Fathia Mannaa

National Research Centre

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Abstract

Echinacea purpurea is a precious medicinal herb. Many civilizations utilize it as a natural medicine. Its extracts provide antifungal, antiviral, antibacterial, and antioxidative activities and are utilized for treating the common cold in addition to respiratory and urinary illnesses. Using male albino rats (160-200g), an *in vivo* study was conducted to examine the ameliorative potential and investigate the anti-inflammatory, antioxidant, and chemical detoxifying activities of *Echinacea purpurea* ethanolic extract (EEE) against bifenthrin-induced renal injury. The animals were divided into four groups and orally treated for 30 days as follows: normal control; animals were treated with EEE (465 mg/kg/day) dissolved in water; healthy animals were given bifenthrin (7mg/kg/day) dissolved in olive oil and the last group was administered EEE one-hour prior bifenthrin intoxication. It was noticed that the administration of the animals with bifenthrin caused significant elevations in serum values of ALAT, ASAT, urea, creatinine, the renal inflammatory and apoptotic markers (IL-1β, TNF-α, IFN-γ and caspase-3), and the oxidative stress and antioxidant markers in kidney (MDA and NO) while, renal GSH, GPx, and SOD values showed about two-fold decrement in compared to normal control. EEE treatment resulted in a considerable restoring of all these parameters to near the control values. Moreover, the extract improved the histological architecture of the kidney. It was concluded that *Echinacea purpurea* extract has ameliorative potential and chemo-preventive efficacy against bifenthrin-induced renal injury, as well as the significant role of its anti-inflammatory, antioxidant, and chemical detoxifying activities.

Introduction

Plants' immunomodulating properties are being studied extensively in order to achieve the desired impact on disease prevention. As a result, herbal therapies have been used over decades for their efficacy, safety, minimal adverse effects, and cultural acceptance (El-Ashmawy et al., 2015).

Echinacea genus plants, popularly referred to as coneflower, are prominent medicinal plants of the *Asteraceae* family that originated in the Russia, Canada, Australia, and United States (Ahmadi et al., 2023). They are reported to have inhibitory, antiproliferative, antibacterial, and antioxidant activities, and *Echinacea purpurea* (*E. purpurea*) is one of the most often used plant species in medicine. In many circumstances, *Echinacea purpurea* L. is used as supplementation of herbs for the treatment of a variety of disorders, including symptoms of respiratory tract infections treatment, immune system strengthening, wound healing, and migraine headaches discomfort and anxiety relieve. It is available in teas, creams and lotions, tablets, capsules, and other forms (Temerdashev et al., 2022). It has attracted international attention in the past few years according to reports that it boosts the body's immune system (Ahmadi et al., 2021). The plant's extracts provide antifungal, antiviral, antibacterial, and antioxidative activities and are utilized for treating the common cold in addition to respiratory and urinary illnesses (Kumar and Ramaiah, 2011). Cichoric acid, echinacoside, chlorogenic acid, caffeic acid, cynarine, sitosterol, polyacetylene, alkylamides, alkaloids, glycosides, and carbohydrates are all active ingredients of *E. purpurea*. They are all strong antioxidants that can successfully eliminate free radicals as well as decrease the nitric oxide free radical generation (EL-Sahra et al., 2022). The plant's most prevalent

ingredients include lipoproteins, polysaccharides, caffeic acid derivatives, and alkylamides, although the active phytochemicals in it differ based on how old is the plant, section of the plant, growing circumstances, geographical region, and extraction process. Polysaccharides are often found at the highest levels in aqueous or freshly squeezed juice extracts, whereas alkylamides have a greater tendency to be key ingredients in ethanol extracts (Benson et al., 2010).

Synthetic pyrethroids (SPs) are the most frequently utilized type of insecticides today, representing over twenty-five percent of the worldwide pesticide industry. Because of the toxic effects of organophosphorus insecticides on humans and animals, the Environmental Protection Agency (EPA) restricted most residential applications of them in 2001; however, they are still utilized today (Farag et al., 2021).

Bifenthrin (BF), a third-generation type 1 SP, has received a widespread attention due to its superior insecticidal action and stability when compared to previous SPs (Yang et al., 2018). It is utilized for controlling flies, mosquitoes, cockroaches, and termites, by interference with ATPase enzymes and voltage-gated ion channels, resulting in hyperexcitability, tremors, convulsions, and death. Furthermore, BF exposure caused neurotoxic effects in mammals' brains as well as apoptotic and inflammatory responses in murine brains (Gargouri et al., 2018; Syed et al., 2018). It is more hazardous than other SPs because it stays longer in the soil, quickly attaches to organic materials, and flows into aquatic areas, causing ecological dangers and harmful impacts on aquatic life (Manzoor and Pervez, 2017). Because BF is used for pest control in agriculture and horticulture, there is definitely a danger of long-term exposure to residues of pesticides in food products for humans (Pylak-Piwko and Nieradko-Iwanicka, 2021).

Bifenthrin has been proven to be highly poisonous, with tumorigenic, immunotoxic, and hepatotoxic effects in species of mammals via oxidative stress pathways, as well as a hormonal-disrupting influence in mice and various kinds of fish (Xiang et al., 2019). Chronic BF poisoning in animals results in tremor, hypersensitivity to stimuli, and aggressive sparring (Pylak-Piwko and Nieradko-Iwanicka, 2021). Many studies have demonstrated that BF exposure is toxic to numerous organs of the body, such as nervous system, testes, kidneys, lungs, and the liver (Ikram et al., 2021).

Consequently, the key objective of this study was to explore the ameliorative potential and investigate the anti-inflammatory, antioxidant, and chemical detoxifying activities of *Echinacea purpurea* extract against bifenthrin-induced renal injury.

Materials and Methods

All the used chemicals were pure and of analytical grade and obtained from the stores of the National Research Centre, Egypt, while bifenthrin (purity 98%) was purchased from Sigma, MO, USA. Flowers of *Echinacea purpurea* were obtained from IMTENAN Company (Production and sale of nutritional and healthy products, Giza, Egypt) that collected and dried the flowers in accordance with relevant institutional, national, and international guidelines and legislation. The plant was examined by botanical

specialists (Faculty of pharmacy, Ain-Shams university, Cairo, Egypt) and was found carrying the taxonomic serial number 3728.

Herb Extraction:

The plant flowers were air-dried and then grinded. Two hundred grams from the powdered material were soaked in one liter of 70% ethanol for seven days. After filtration through Whatman 1 filter paper, the filtrate was evaporated using rotary evaporator. The remaining moisture residue was lyophilized by the freeze drier (Todd et al., 2015). The obtained dry extract (EEE) was stored at -20°C until further use.

HPLC analysis of phenolic ingredient:

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A) ; 15–16 min (82% A) and 16–20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40°C.

Experimental design:

Adult male albino rats (160-200g) were provided from the animal house colony, National Research Centre. They placed in suitable plastic cages and fed with standard laboratory diet and water *ad libitum* one week for acclimatization before starting the experiment. All animals received human care in compliance with the standard institution's criteria; the proposal was approved and certified by the ethics committee of the use of experimental animals, Faculty of Science, Al-Azhar University with a reference number (#AZHAR 9/2023, Feb 2023).

The animals were then divided into 4 groups of 10 rats each; group 1 (Control), group 2: Rats were orally given EEE dissolved in water at a daily dose of 465 mg/kg body weight, group 3: Rats were orally given bifenthrin dissolved in olive oil at a dose of 7mg/kg/day, group 4: Rats were administered EEE one-hour prior bifenthrin intoxication. The experiment lasted for 30 days. All animals received human care in compliance with the standard criteria of ethical committee of National Research Centre.

Blood and tissue sampling:

At the end of the experimental duration, the rats were fasted overnight, and anesthetized, then blood samples were collected, left to clot, and cool-centrifugated at 3000 rpm for 15 minutes; then, the clear sera were separated and stored at − 80˚C until analysis. After blood collection, the animals were sacrificed soon by sudden decapitation; then both kidneys of each rat were removed; the left kidney was washed in saline and dried, rolled in a piece of plastic sheet, and stored at -80°C for determination of inflammatory and oxidative stress markers, and the percentage of DNA fragmentation. The right one was immersed in 10% formalin-saline (v/v) buffer for histopathological examination.

Serum biochemical measurements:

Serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), urea and creatinine were estimated using reagent kits purchased from Bio Vision, Inc, USA.

Renal inflammatory and apoptotic markers:

The levels of renal interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNF-α) and caspase-3 were estimated using rats' reagent ELISA kits obtained from Sino Gene Clon Biotech Co., Hang Zhou, China, while the assessment of interferon-γ (IFN-γ) level was carried out using rats' reagent ELISA kits purchased from Ray Biotech Co., Norcross, Georgia, USA.

Estimation of oxidative stress markers:

Lipid peroxidation (Malondialdehyde, MDA), nitric oxide (NO) and reduced glutathione (GSH) levels as well as enzymatic activity of glutathione peroxidase (GPx), and superoxide dismutase (SOD) in renal tissue were determined spectrophotometrically using reagent kits obtained from Bio diagnostic Co., Giza, Egypt.

Determination of DNA fragmentation:

The degree of the DNA fragmentation was determined using the quantitative method used for grading the DNA damage as described by (Perandones et al., 1993). The degree of DNA fragmentation was determined by separating the cleaved DNA from the intact chromatin by centrifugation and measuring the amount of DNA present in the supernatant and pellet using the diphenylamine assay. The percentage of the fragmented DNA was calculated as follow:

$$\text{DNA fragmentation } \% = \frac{\text{A supernatant}}{\text{A supernatant} + \text{A pellet}} \text{ x } 100$$

Histopathological examination:

Kidney samples were hydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin. Sections of 5μm thick were cut and stained with hematoxylin and eosin (H&E) for histological examination under light microscopy (Drury et al., 1980).

Morphometric analysis:

An image analysis system consisting of a digital camera and a light microscope was used for histomorphometry. The evaluation focused on studying glomerular injury in H&E stained kidney sections (5μm thick). Digital images of thirty glomeruli per animal were captured using a light microscope. Then, a computerized morphometric analysis system (ImageJ; National Institutes of Health) was employed to trace and calculate the Bowman's capsule area (BCA), glomerular tuft area (GTA), and Bowman's space area (BSA) from the digitized images.

Statistical analysis:

Multiple comparisons between means were carried out using one way ANOVA followed by Duncan post hock test at $p \geq 0.05$ using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

Results

In vitro study

The *in vitro* analyses of the EEE revealed that the extract contains significant amount of total phenolic compounds and has a strong antioxidant capacity as a consequence of its strong capacity to remove DPPH radicals, which validates the consequent reducing power, which indicated a concentration-dependent relationship (Data under publication). In addition, the phytochemical analysis of the EEE by HPLC revealed the identification of 19 phenolic and flavonoid compounds. Among these, chlorogenic acid, naringenin, gallic acid coumaric acid, and caffeic acid were the major phenolic components while querectin, rutin, and apigenin were the major flavonoids compound present in the extract **(**Table 1 **and** Fig. 1**)**.

Table 1
Phenolic and flavonoid ingredients of EEE using HPLC analysis.

	Area	Conc. (µg/ml = 20mg/ml)	Conc. (µg/g)
Gallic acid	94.15	8.13	406.51
Chlorogenic acid	127.67	17.48	874.19
Catechin	4.91	1.21	60.74
Methyl gallate	39.96	2.18	109.05
Coffeic acid	74.76	5.75	287.44
Syringic acid	42.41	2.88	143.80
Pyro catechol	5.23	0.75	37.62
Rutin	89.65	10.41	520.50
Ellagic acid	5.46	1.01	50.63
Coumaric acid	214.12	6.76	337.75
Vanillin	14.68	0.64	32.13
Ferulic acid	57.33	3.92	195.86
Naringenin	124.86	15.06	752.83
Daidzein	33.05	2.04	102.08
Querectin	111.91	15.42	770.75
Cinnamic acid	76.35	1.41	70.33
Apigenin	118.19	9.00	449.84
Kaempferol	48.40	3.75	187.65
Hesperetin	72.99	4.25	212.75

In vivo Study

Our data illustrated that the levels of ALAT, ASAT, urea, and creatinine in the healthy rats weren't affected by the administration with EEE and this demonstrates EEE's non-toxicity to the liver and kidney; meanwhile, the administration of the animals with BF caused a significant increase in their values. In contrast, in the animal group orally administrated with EEE prior to BF administration the values of ALAT, ASAT, urea, and creatinine were noticeably almost near the normal control which prove the improvement effect of EEE on the intoxicated animals (Table 2).

Table 2
Effect of *Echinacea purpurea* ethanolic extract (EEE) on liver functions (ASAT and ALAT) and kidney functions (urea and creatinine) in animals treated with bifenthrin.

	ALAT (U/ml)	ASAT (U/ml)	Urea (mg/dl)	Creatinine (mg/dl)
Control	36.22 ± 1.81	33.55 ± 1.1	29.56 ± 1.95	0.783 ± 0.03
EEE	34.65 ± 1.75	32.15 ± 1.33	28.25 ± 1.27	0.724 ± 0.028
Bifenthrin	117.3 ± 3.26*	95.64 ± 2.88*	83.5 ± 3.65*	2.34 ± 0.054*
EEE + Bifenthrin	55.32 ± 2.11#	62.38 ± 2.10#	51.65 ± 2.71#	1.35 ± 0.041#

Data are presented as mean ± standard error. Within the same column, means with superscript symbol (*) is significantly different from that of normal control, while those with superscript symbol (**#**) is significantly different from bifenthrin intoxicated group at $p \leq 0.05$; EEE (Echinacea ethanolic extract).

In the current study, we noticed that the administration of the healthy rats with EEE didn't affect the renal inflammatory and apoptotic markers (IL-1β, TNF-α, IFN-γ, and caspase-3) reflecting its safe effect on the kidney of the healthy animals. In contrast, treating animals with BF caused significant increases in the levels of these markers. This highly increment was noticed to return to levels almost near the normal control in the group treated with EEE prior to BF administration proving that EEE can ameliorate the IL-1β, TNF-α, IFN-γ, and caspase-3 values in the intoxicated rats **(**Table 3**)**.

Table 3
Effect of *Echinacea purpurea* ethanolic extract (EEE) on IL-1β, TNF-α, IFN-γ, and caspase-3 in animals treated with Bifenthrin.

	IL-1β (ng/ g tissue)	TNF-α (µg/ g tissue)	IFN-γ (pg/g tissue)	Caspase-3 (U/g tissue)
Control	84.1 ± 6.4	112.2 ± 3.8	978 ± 8.8	503 ± 1.21
EEE	82.3 ± 7.1	109.4 ± 4.3	990 ± 7.9	522 ± 1.07
Bifenthrin	221.5 ± 9.8*	266.1 ± 8.2*	2175 ± 11.5*	1758 ± 12.3*
EEE + Bifenthrin	113.8 ± 7.7#	145.6 ± 6.9#	1354 ± 9.9#	844 ± 7.6#

Data are presented as mean ± standard error. Within the same column, means with superscript symbol (*) is significantly different from that of normal control, while those with superscript symbol (#) is significantly different from bifenthrin intoxicated group at $p \leq 0.05$; EEE (Echinacea ethanolic extract).

Oxidative stress and antioxidant markers (MDA, NO, GSH, GPx, and SOD) values were noticeably altered in the BF group in compared to normal control as the MDA and NO levels increased significantly by up to two times more than normal, while the GSH level and GPx, and SOD activities showed a significant

decrease. EEE treatment resulted in a considerable restoring of the antioxidant indicators (GSH, SOD, and GPX), as well as a notable downregulation of MDA and NO. Meanwhile, these values weren't affected in the healthy animals that treated with EEE alone **(**Table 4**)**.

Table 4
Effect of *Echinacea purpurea* ethanolic extract (EEE) on MDA, NO, GSH, GPx, and SOD in animals treated with Bifenthrin.

	MDA (nmol/g tissue)	**NO (µmo1/g tissue)**	**GSH (mmo1/g tissue)**	**GPx (U/g tissue)**	**SOD (U/g tissue)**
Control	146.5 ± 4.3	53.7 ± 2.1	4.82 ± 0.86	4873 ± 32.5	8254 ± 66.5
EEE	140.3 ± 3.2	49.8 ± 2.3	5.01 ± 1.11	4921 ± 41.2	8377 ± 73.2
Bifenthrin	387 ± 13.5*	216 ± 9.3*	1.97 ± 0.35*	2049 ± 23.7*	3746 ± 44.5*
EEE + Bifenthrin	194.6 ± 8.7$^{\#}$	74.5 ± 4.7$^{\#}$	3.42 ± 0.77$^{\#}$	3894 ± 29.8$^{\#}$	6668 ± 59.6$^{\#}$

Data are presented as mean ± standard error. Within the same column, means with superscript symbol (*) is significantly different from that of normal control, while those with superscript symbol ($^{\#}$) is significantly different from bifenthrin intoxicated group at $p \leq 0.05$; EEE (Echinacea ethanolic extract).

Renal DNA fragmentation is shown in **Figure (2**) and our data illustrated that normal animals treated with EEE didn't have any noticed damaging effect on the renal DNA while BF administration caused a highly significant increase in the renal DNA fragmentation compared to the normal animals. In the same time treating the intoxicated animals with EEE caused the renal DNA fragmentation value to be decreased to level almost near the normal control.

Histopathological and morphometric Evaluations

The assessment of kidney sections through histopathological examination was conducted in this study. The kidney sections from the control group displayed a normal architecture in the outer cortex and inner medulla upon examination with hematoxylin- and eosin-staining. The normal group (group 1) showed typical and healthy structures (Fig. 3a)., while the group treated with the ethanolic extract of Echinacea (group 2) displayed a histological framework almost similar to the control group; morphometric evaluations showed no significant differences in the glomerular, tuft, and Bowman's space areas between the normal and treated groups (Fig. 3b). In contrast, the group intoxicated with bifenthrin (BF) (group 3) showed severe renal damage and necrosis, with significant histopathological changes, including degeneration, dilatation of Bowman's capsule, glomerular atrophy, and disintegration of the tubular epithelium, pyknotic nuclei, and proteinaceous casts in the renal tubules. Morphometric evaluations revealed an increase in the glomerular and Bowman's space areas **(Fig. 3c&d**). Post-treatment of the BF-

intoxicated animals with EEE (group 4) resulted in a significant improvement in the microanatomical structure of the glomeruli, with the elimination of eosinophilic cast formations and concurrent regeneration of glomeruli in the basal cortical and juxtamedullary regions. Furthermore, a remarkable improvement in the renal capsules, displaying near-normal appearances as well as the renal tubules showing marked improvements (Fig. 3e**&f**).

Figure (3) exhibits photomicrographs that compare the histology and morphometry of kidneys in rats treated with various agents, showing both healthy and therapeutic effects, as well as severe nephrotoxic damage. The images of Group 1 (A) demonstrate normal renal structures; however, the kidney structures also are almost normal in Group 2 (B). In contrast, Bifenthrin-intoxicated group 3 exhibits severe renal damage and necrosis (C and D). Group 4 (E and F) demonstrates a significant improvement in the microanatomical structure of glomeruli, and in renal capsules, displaying normal glomeruli and Bowman's spaces. The abbreviations used in the figure include G for glomeruli, PT for proximal tubule, DT for distal tubule, b for Bowman's space, e for eosinophilic cast, ED for epithelial degeneration, and PK for pyknosis. (Hematoxylin & Eosin, Magnification power = x 200, Scale bare = 20 µm).

The morphometric analysis showed that the normal group (group 1) displayed healthy structures, such as typical tubules, glomerular capillaries, Malpighian corpuscles, and Bowman's capsule. Kidney sections from rats treated with the EEE (group 2) displayed almost normal histological features, akin to those of the control rats. In contrast, group 3, treated with the nephrotoxic agent bifenthrin (BF), exhibited severe renal damage and necrosis, as evidenced by marked changes in the glomerular and Bowman's space areas. The fourth group (group 4) showed a significant improvement in the microanatomical structure of the glomeruli, and a remarkable improvement in the renal capsules (Fig. 4**).**

Figure (4) depicts the examination of kidney tissue microanatomy in rats treated with various substances. The morphometric analysis showed significant increases in these areas compared to the healthy control group. However, group 4 demonstrated improvements in the glomerular and Bowman's space areas compared to the BF-treated group, indicating possible therapeutic effects. The symbol * is significantly different from that of the control, while those with the symbol # is significantly different from bifenthrin intoxicated group at $p \leq 0.05$; EEE (Echinacea ethanolic extract) Bifen (Bifenthrin).

Discussion

Insecticide toxicity has been extensively researched in experimental animals using metabolic and histo-architectural indicators of organ toxicity **(**Ahmad et al., 2015). Exposure to BF has been shown to increase renal pro-inflammatory cytokines (TNF-, IL-2, and IL-6), hepato-renal oxidative stress, LDL, LDL-apoB-100, oxidized-LDL, circulating cholesterol, and lipid peroxidation. All of these conditions of stress must be closely associated with glomerular and renal tubular damage **(**Dar et al., 2019; Feriani et al., 2020). The precise mechanism of BF and other pyrethroid pesticides induced renal toxicity is unknown. Despite this, animal studies have connected pyrethroid exposure to hyperglycemia and elevated plasma concentrations of catecholamines (noradrenaline and adrenaline). Higher catecholamine levels result in

higher cardiac output in addition to vasoconstriction of the glomerular arterioles. Moreover, higher catecholamine levels have been associated with renin release, which stimulates the renin-angiotensin system. Angiotensin II, which is generated in response to renin, stimulates the production of vasopressin (an antidiuretic hormone) (Ikram et al., 2021).

On the contrary, research into the antitoxic ability of diverse medicinal plant extracts, which include important phytochemicals that give natural antidotes opposing those noxious environmental toxins, is a somewhat recent field of study. The benefits of utilizing phytomedicinal remedies vary between their low cost, free availability, and lack of possible health concerns associated with synthetic medicines (Al-Sowayan and Mousa, 2014).

In our study, serum ALAT and ASAT levels in the BF-treated animal group were significantly higher, demonstrating hepatotoxicity resulting from the leakage of the enzyme from the degenerated hepatocyte cytosol into the circulation (Fırat et al., 2011). Transaminase activities can be increased as well because of aggressive catabolism of amino acids to meet the immediate requirement for energy during pyrethroid stress (Kumar et al., 2011). We also observed elevations of serum urea and creatinine levels after administration with BF, indicating renal impairments. Hepatotoxicity and nephrotoxicity endpoints modification may be supported by the idea that oxidative stress generated by reactive oxygen species (ROS) affects liver and kidney macromolecules such as lipids, DNA, and, protein producing functional and structural changes (Farag et al., 2022). These findings are consistent with those of Pylak-Piwko and Nieradko-Iwanicka (2021); Farag et al. (2022).

Because *Echinacea purpurea* extract comprises antioxidant ingredients such as phenolic and flavonoids components which are capable of suppressing lipid peroxidation, stabilizing cell membranes, preventing membrane lipids oxidation, and restoring cellular integrity, there is improvement in the liver and kidney markers and less leakage of hepatic ASAT and ALAT into the bloodstream (EL-Sahra et al., 2022).

The TNF-α and IL-1β are generated by macrophages and monocytes. They are released into the bloodstream, where they exert a systemic impact. TNF-α constitutes one of the first cytokines to be released during an inflammatory response (Gargouri et al., 2018). It promotes the synthesis of IL-1β that occurs during prolonged oxidative stress (Piłat and Mika, 2014). IL-1β is a signaling molecule that modulates immunological response, facilitates leukocyte and lymphocyte activity, functions as a pyrogen, and promotes the progress of prolonged inflammation (Banerjee and Saxena, 2012).

In our results, we noticed significant increases in the renal inflammatory and apoptotic markers (IL-1β, TNF-α, IFN-γ, and caspase-3) levels in the BF-treated group. This is consistent with the findings of Pylak-Piwko and Nieradko-Iwanicka (2021), which obtained similar results in mice and demonstrated that even low doses of bifenthrin might lead to a significant increase in IL-1β in mice kidneys, demonstrating that inflammation in the kidney can take place at very low doses. It is related to the fact that pyrethroid metabolites are removed by urine. Similarly, studies on the immunotoxic effects of bifenthrin on zebrafish embryos have been published. According to Jin et al. (2013), being exposed to bifenthrin elevated the concentrations of IL8, IL-1β, caspase 9 and 3 in embryos exposed to S-cis-bifenthrin.

Our findings are also similarly consistent with those of Wang et al. (2017 a), who performed an experiment on male mice exposed to bifenthrin for three weeks and validated the pyrethroid's immunotoxic impact. Wang et al. (2017 b) published a study that clarified our findings and gave light on the mechanisms of the immunotoxicity of bifenthrin in murine macrophages. Bifenthrin exposure restrained transcription concentrations of interleukin 6 and TNFα as a result of lipopolysaccharide stimulation. Bifenthrin intoxication elevated ROS and caused oxidative stress-related gene dysregulation. Treatment with EEE caused the highly increment in the renal inflammatory and apoptotic markers (IL-1β, TNF-α, IFN-γ, and caspase-3) levels by BF administration to return to levels almost near the normal control.

As numerous pyrethroids have been found to cause lipid peroxidative damage in different tissues (Khan et al., 2017), oxidative stress is a sensitive indicator that is frequently utilized for toxicological evaluations of pyrethroids, particularly bifenthrin, and other insecticides as a potential mechanism underlying their harmful effects (yang et al., 2018).

The values of the antioxidant markers (GSH, GPx and SOD) in the kidney tissues were reduced in BF-treated rats. This deterioration of the antioxidant defenses was associated with an elevation in oxidative stress products, such as increased NO and MDA concentrations in the kidney. These findings are consistent with the previous study of Dar et al. (2019). These results can be explained according to Talla and Veerareddy (2011) who reported that ROS are produced as a result of the metabolism of xenobiotics, which includes pyrethroids. The body's antioxidant defense is overwhelmed by the excessive creation of ROS, causing lipid peroxidation of cell membranes, which causes disruption of the integrity of cells and leads to cellular and subcellular abnormalities and the death of the cells. On the contrary of BF, treatment with EEE caused a significant restoration of the levels of the antioxidant markers (GSH, SOD and GPX), matched with remarkable down-regulation in MDA and NO. This effect could be related to the high content of flavonoids and phenolic compounds which were proven to have anti-inflammatory and antioxidant effects (Hong et al., 2016).These compounds can also prevent GSH depletion and protect cells by scavenging free radicals and inhibiting lipid peroxidation (Lee et al., 2003).

In the present study, BF increase percentage of DNA fragmentation compared to the control group. The detected damage in DNA in the present study could be due to the genotoxic effect of BF. Administration of EEE to BF -treated rats significantly decreased DNA fragmentation. The reduced level of DNA damage by EEE may be attributed to its antioxidant capacity that demonstrated from the data of reducing power ability and DPPH radical scavenging activity (Data under publication).

The results of the histopathological and morphometric evaluations confirmed the biochemical findings reported earlier and indicated that BF induced severe histological changes in the kidney tissues. Similar histological changes in the kidney have been documented previously (Tahoun et al., 2019) However, animals treated with EEE resulted in an improvement in renal tissues as compared with the group of BF, as EEE demonstrated improvement in the pathological changes. Morphometric assessment showed also an improvement in the glomerular and Bowman's space areas compared to rats treated with BF. These

findings suggest possible modifications in the function and structure of the kidney which occurred probably by preventing oxidative stress.

The reno protective ability of EEE may be due to the high content of phenolic and flavonoids compounds in the extract. In the present study, HPLC analysis revealed identification of 19 compounds in EEE which are characterized as having an antioxidative capacity including chlorogenic acid, naringenin, gallic acid coumaric acid, coffeic acid, querectin, rutin, and apigenin which were the major ingredients present in the extract. For examples, chlorogenic acid was found to improve hyperuricemia, alleviate renal inflammation, and ameliorate intestinal homeostasis in hyperuricemic mice (Zhou et al., 2021). Khan et al. (2020) reported the protective effect of naringenin against doxorubicin-induced renal injury by down-regulating the levels of nuclear factor-κB (NF-κB), TNF-α and prostaglandin-E2. Gholamine et al. (2021) demonstrated the ameliorative effect of the gallic acid against oxidative stress mediating renal and hepatic injuries induced by sodium arsenite toxicity. It improved also hematological and histopathological changing induced by sodium arsenite. Investigators have described the anti-renalinflammatory and antioxidant activity of p-coumaric acid. p-Coumaric acid has been found to attenuate oxidative stress and nephropathy in diabetic rats (Mani et al., 2022) and ameliorate the biochemical and histopathological changes induced by gentamicin (Hakyemez et al., 2022). Gu et al. (2021) reported that quercetin can protect the kidney cells from COVID-19 injury by inhibiting inflammatory and apoptosis-related signaling pathways. Kandemir et al. (2022) reported the protective effect of rutin against sodium valproate-induce renal and hepatic damage and concluded that rutin exerts its action by inhibiting inflammation, apoptosis and autophagy. Apigenin was also found to protect kidney against doxorubicin-induced renal injury through inhibiting inflammation and oxidative stress (Wu et al., 2021). Wang et al. (2016) discovered that EEE's caffeic acid is an additional biologically active component capable of inhibiting oxidative stress through elevating GSH and CAT values as well as suppressing inflammation by inhibiting TNF-α, IL-6, MAPK, p-p38, and NF-κB, that was able to hold apoptosis through controlling caspase-3 and p53 concentrations.

Previous studies also indicated other alkylamide compounds that may also play a role in the EEE activity. Akylamides are regarded to be the key ingredients responsible for *Echinacea* plants' anti-inflammatory effects (Hou et al., 2010). Several investigations have shown that alkylamide-rich Echinacea extracts can influence pro-inflammatory cytokines such as TNF-α (Woelkart and Bauer, 2007) and have been demonstrated to have immunomodulatory properties in both *in vivo* and *in vitro*. These immunomodulatory impacts are thought to be related to alkylamides' capacity for binding to cannabinoid receptor type 2 (CB2) (Bruni et al., 2018).

Despite the alkamide fraction does not have antioxidative activity by itself, it boosts the ability of cichoric acid, a caffeic acid derivatives, which has been demonstrated to be responsible for most of EEE's antioxidant activity since it is an effective scavenger of free radicals (Thygesen et al., 2007). This explanation agreed with Wang et al. (2017) and Mohamed et al. (2023) who demonstrated that EEE's chicoric acid reduced oxidative stress by tilting the balance of CAT, Nrf2, and GSH over MDA, resulting in reductions of apoptosis, neuronal damage, and inflammation.

Conclusion

In conclusion, the current study findings confirmed the ameliorative potential and chemo-preventive efficacy of *Echinacea purpurea* extract against bifenthrin-induced renal injury, as well as the significant role of its anti-inflammatory, antioxidant, and chemical detoxifying activities.

Declarations

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Author contributions

G.M.K, D.G.E and R.S.S: carried out experiments, and performed neurochemical, behavioral, and morphological analyses. F.A.M and L.K.H: analyzed the data and helped in writing the manuscript. K.G.A and F.A.M: conceived the idea, designed the experiments, and wrote the manuscript. K.G. M: gave final approval of the version for submission.

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Data availability

All data generated or analyzed during this study are included in this published article.

Ethics approval

All methods and the experimental animals were used in accordance with the standard criteria of the ethics committee, Faculty of Science, Al-Azhar University, Egypt that approved the used methods, experimental animals, and protocols with a reference number (#AZHAR 9/2023, Jan. 2023). The authors confirm that the study was carried out comply with the ARRIVE guidelines.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

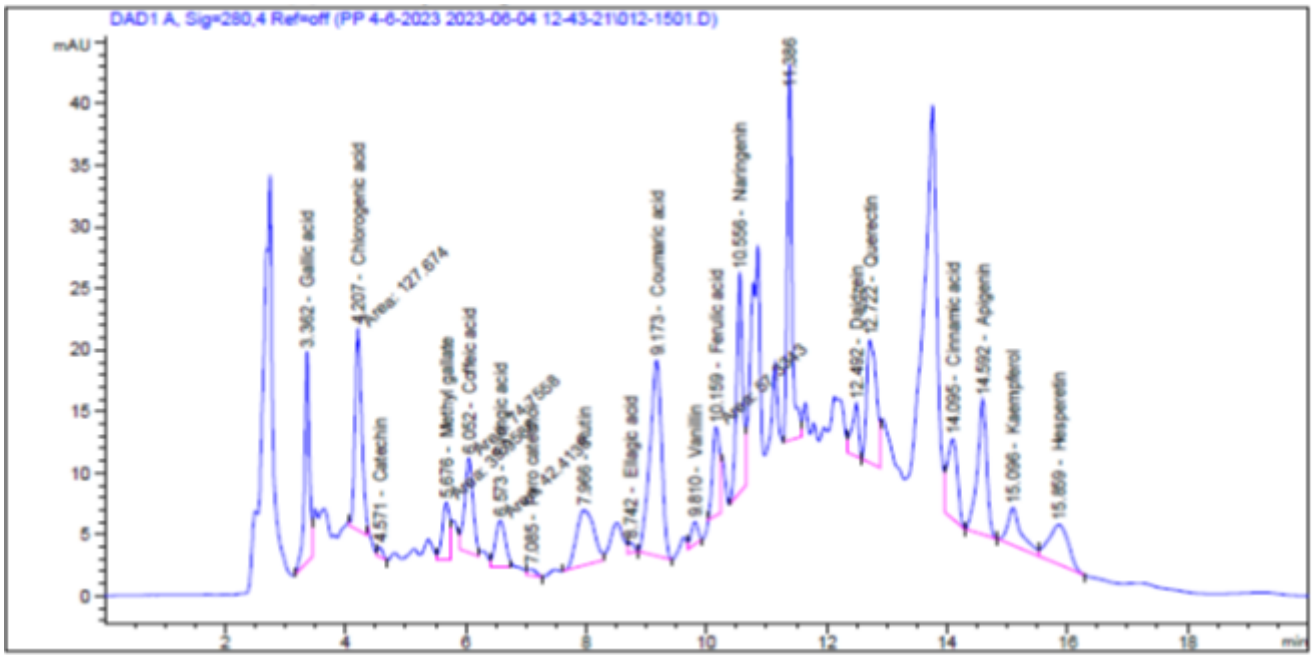

Figure 1

HPLC imprint profile of EEE. 19 components were identified, other peaks not identified.

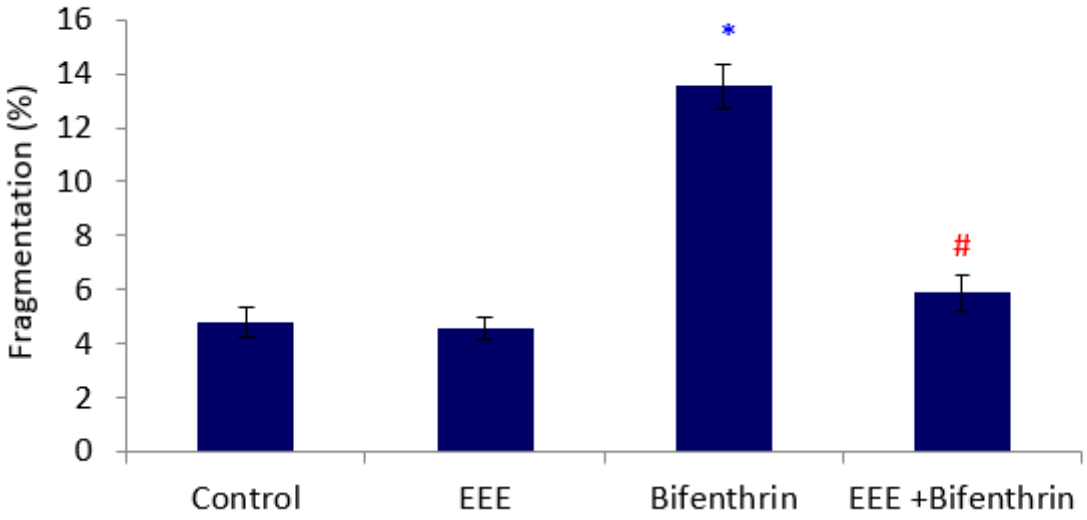

Figure 2

Effect of EEE on renal DNA fragmentation; Data are presented as mean ±standard error. The symbol * is significantly different from that of the control, while those with the symbol # is significantly different from bifenthrin intoxicated group at $p \leq 0.05$; EEE (Echinacea ethanolic extract).

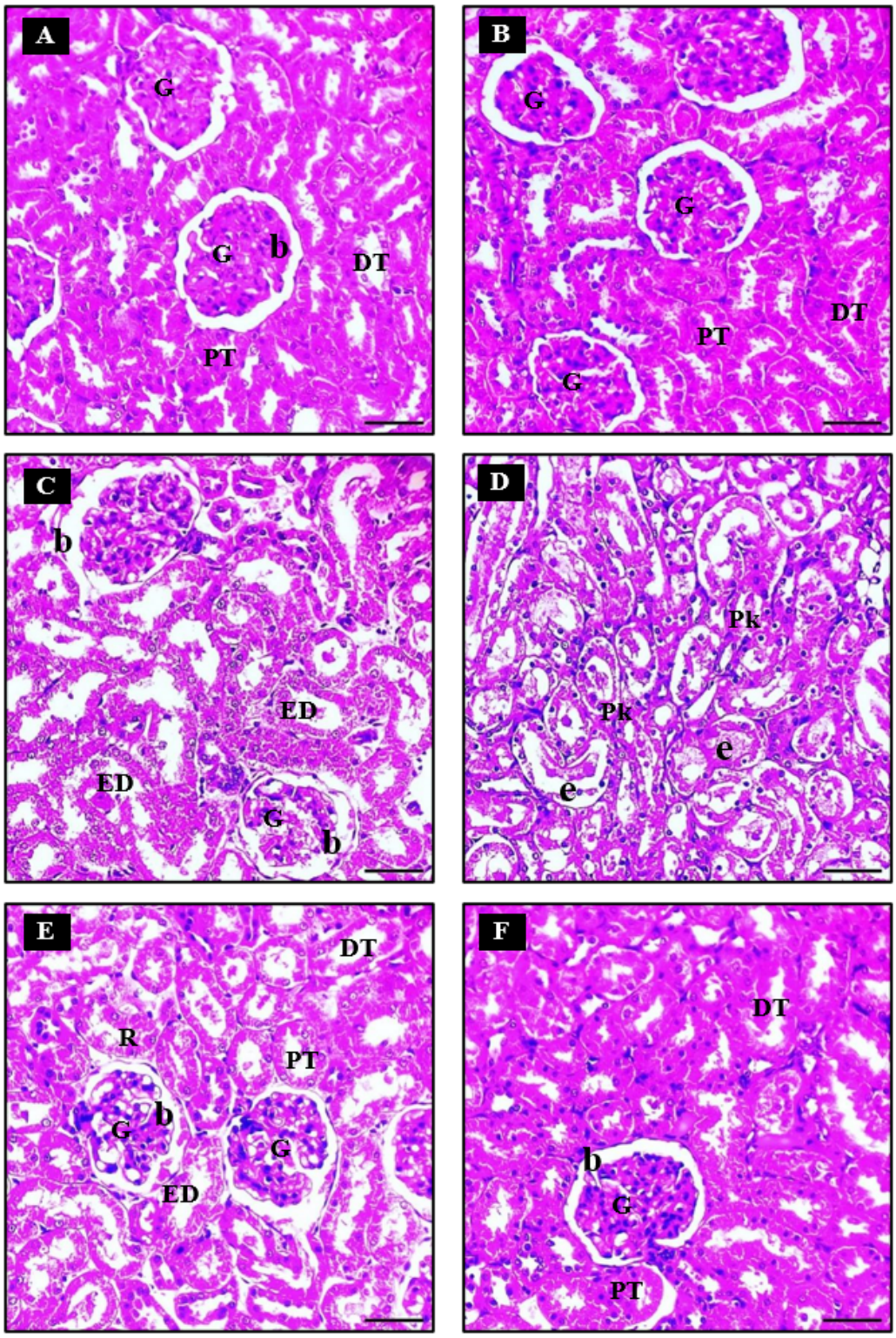

Figure 3

exhibits photomicrographs that compare the histology and morphometry of kidneys in rats treated with various agents, showing both healthy and therapeutic effects, as well as severe nephrotoxic damage. The images of Group 1 (A) demonstrate normal renal structures; however, the kidney structures also are almost normal in Group 2 (B). In contrast, Bifenthrin-intoxicated group 3 exhibits severe renal damage and necrosis (C and D). Group 4 (E and F) demonstrates a significant improvement in the

microanatomical structure of glomeruli, and in renal capsules, displaying normal glomeruli and Bowman's spaces. The abbreviations used in the figure include G for glomeruli, PT for proximal tubule, DT for distal tubule, b for Bowman's space, e for eosinophilic cast, ED for epithelial degeneration, and PK for pyknosis. (Hematoxylin & Eosin, Magnification power = x 200, Scale bare = 20 µm).

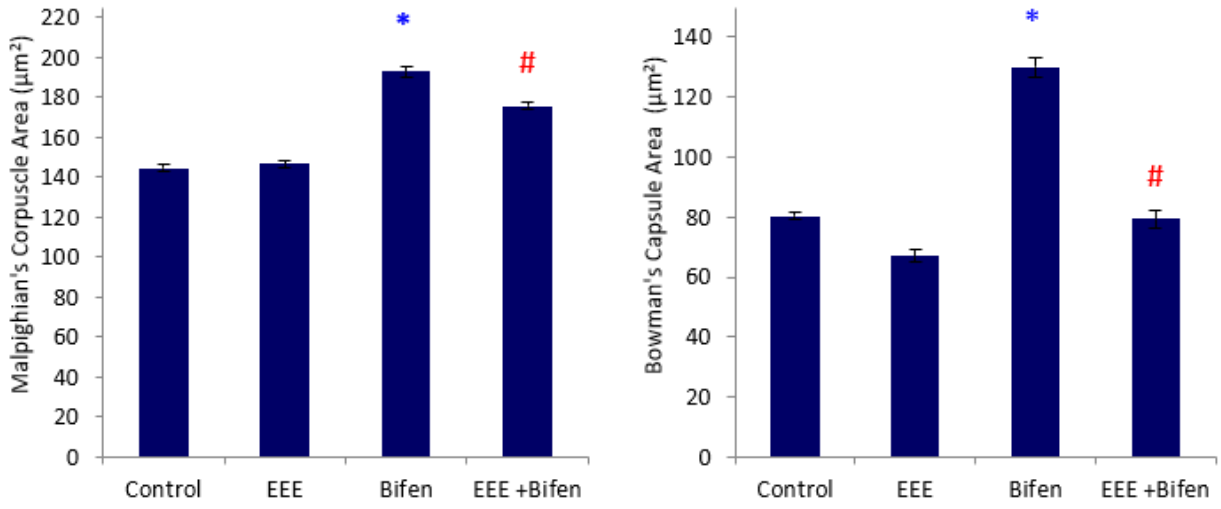

Figure 4

depicts the examination of kidney tissue microanatomy in rats treated with various substances. The morphometric analysis showed significant increases in these areas compared to the healthy control group. However, group 4 demonstrated improvements in the glomerular and Bowman's space areas compared to the BF-treated group, indicating possible therapeutic effects. The symbol * is significantly different from that of the control, while those with the symbol # is significantly different from bifenthrin intoxicated group at $p \leq 0.05$; EEE (Echinacea ethanolic extract) Bifen (Bifenthrin).