

HEPATOPROTECTIVE, ANTIOXIDANT, AND ANTI-INFLAMMATORY EFFECTS OF *EPHEDRA ALATA* EXTRACT AGAINST BIFENTHRIN-INDUCED LIVER TOXICITY IN RATS

Brahim Benaicha^{*1}, Hichem Saker²

Address(es): Dr Brahim Benaicha,

¹ Laboratory of Toxicology and Ecosystems Pathologies, Echahid Cheikh Larbi Tebessi University, Constantine Road, 12002 Tebessa, Algeria.

² Faculty of Exact Sciences and Natural and Life Science, Echahid Cheikh Larbi Tebessi University, Constantine Road, 12002 Tebessa, Algeria.

*Corresponding author: correspondingauthor@mail.com

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ABSTRACT

This study aimed to evaluate the ameliorative, antioxidant, anti-inflammatory, and chemical detoxifying properties of *Ephedra alata* extract (EAE) in relation to bifenthrin-induced liver damage. Adult male rats (180-220 g) were divided into four groups (10 rats per group) and underwent oral therapy for a duration of 30 days as detailed below: (1) Normal control; (2) Healthy subjects received EAE (120 mg/kg/day) in maize oil; (3) Healthy subjects were administered bifenthrin (8 mg/kg/day) in maize oil; (4) Subjects were orally delivered EAE one hour before bifenthrin exposure. The findings demonstrated that bifenthrin administration in the subjects led to substantial elevations in serum levels of LDH, ASAT, and ALAT, as well as increased hepatic inflammatory markers (IL-1 β , TNF- α , and IFN- γ), an apoptotic marker (Casp-3), and oxidative stress indicators (NO and MDA), alongside a marked reduction in hepatic antioxidant markers (GSH, SOD, CAT, and GPx) relative to the normal control group. The administration of EAE prior to bifenthrin markedly ameliorated the previously described compromised parameters, nearing control values; furthermore, the extract substantially altered the liver histological structure. In summary, *Ephedra alata* extract demonstrates promising antioxidant, anti-inflammatory, hepatoprotective, and detoxifying effects against bifenthrin-induced liver damage in a rat model.

Keywords: *Ephedra alata*; Bifenthrin; Liver damage; Antioxidant; Anti-inflammatory

INTRODUCTION

Synthetic pyrethroids (SPs) represent the most commonly employed category of insecticides. They constitute around 25% of the global pesticide market. The Environmental Protection Agency (EPA) has limited the majority of residential uses of these herbicides due to their harmful impacts on humans and animals; yet, they continue to be utilized today (Meena *et al.*, 2022). Bifenthrin, a third-generation type one synthetic pyrethroid, has garnered significant interest for its enhanced insecticidal efficacy and durability relative to earlier synthetic pyrethroids (Adjidjonu, 2023). Moreover, bifenthrin exposure has been documented to exhibit neurotoxic effects in mammalian brains, along with apoptotic and inflammatory reactions in mouse brains (Farg *et al.*, 2021). Bifenthrin is claimed to be more detrimental than other synthetic pyrethroids due to its prolonged persistence in soil, rapid adherence to organic matter, and propensity to migrate into aquatic environments, resulting in ecological hazards and negative effects on aquatic organisms (Jagadeesh *et al.*, 2023). Bifenthrin has demonstrated significant toxicity, tumorigenicity, immunotoxicity, and hepatotoxicity in many mammalian species through oxidative stress mechanisms and/or hormone disruption effects (Marutescu and Chifriuc, 2017). Furthermore, its prolonged toxicity in animals leads to tremors, heightened sensitivity to stimuli, and aggressive behavior. Furthermore, prolonged exposure to its residues in food poses a significant risk to human health. Bifenthrin promotes chronic oxidative stress in animal models, leading to elevated oxidant markers and diminished antioxidant activity (Kassa *et al.*, 2020). Medicinal plants remain significant and potential sources of pharmaceuticals for the treatment of numerous ailments. Traditional medicine serves as the foundation that enhances scientific research to investigate novel therapeutic methods (Pan *et al.*, 2013). Extensive study is conducted on the pathophysiological-modulating properties of plants to improve their efficacy in disease prevention; thus, herbal treatments have been employed for decades owing to their effectiveness, safety, minimal adverse effects, and cultural acceptance (Abdel-Wahhab *et al.*, 2024). Species of the *Ephedra* genus are significant medicinal plants in the *Ephedraceae* family. They have been shown to exhibit inhibiting, antiproliferative, antimicrobial, and antioxidant properties. *Ephedra alata* (*E. alata*) is one of the most often utilized plant species in medicinal applications (González-Juárez *et al.*, 2020). *E. alata* is employed in multiple cases for the treatment of diverse maladies, including respiratory disorders (such as asthma and bronchitis), diabetes, obesity, and gastrointestinal issues. *E. alata* has attracted worldwide attention in recent years (Najja *et al.*, 2017). The plant extracts provide therapeutic benefits, such as anti-inflammatory, antioxidant,

hepatoprotective, and detoxifying properties. They can alleviate oxidative stress, modulate immune responses, and improve overall health by counteracting the adverse effects of environmental pollutants and disease-related processes. Ephedrine, pseudoephedrine, norephedrine, quercetin, and kaempferol (active ingredients of *E. alata*) are powerful antioxidants that efficiently neutralize free radicals and diminish the production of nitric oxide free radicals (Abou Seif, 2016; Saha *et al.*, 2019). Multiple investigations have demonstrated that exposure to bifenthrin adversely affects several organs, including the liver, lungs, kidneys, testes, and brain (Dar *et al.*, 2019). Therefore, the present study aims to investigate, for the first time, the protective effects of *Ephedra alata* extract against bifenthrin-induced hepatotoxicity in rats, focusing on biochemical, inflammatory, oxidative, and histopathological parameters.

MATERIAL AND METHODS

Chemicals

Bifenthrin is a synthetic pyrethroid insecticide with the chemical formula C₂₃H₂₂ClF₃O₂, and it is designated by the CAS number 82657-04-3. The experiment employed a commercial BFT formulation identified as Bifenthrin 10% EC. Chemical substances and reagents were acquired from Sigma (Germany). Caspase substrates were acquired from Beckman, USA, ELISA kits from Usnc Life Science Inc., China, and the BCA Protein Assay Kit from Thermo Scientific, Rockford, IL, USA.

Collection of plant material and extraction

The plant for the present study *Ephedra alata* subsp. Alenda (EA) was collected from Oued El Alenda, El Oued, Algeria, (<https://maps.app.goo.gl/vv6idYHQHtRthgWPA>). Plant material was cleansed in the laboratory using distilled water to eliminate clinging dust particles, thereafter shade-dried at ambient temperature (26 $\pm$ 2 °C). EA were pulverized into a fine powder utilizing an electric blender (Moulinex, France). One hundred grams of powdered leaves were extracted using methanol via the Soxhlet method for six hours. The extract underwent filtration and lyophilization at 85 °C for 72 hours. EAE was stored at -80°C until phytochemical assessments (Jones and Kinghorn 2012).

Phytochemical determination

The total phenolic and flavonoid contents of *Ephedra alata* extract (EAE) were determined using standard protocols with slight modifications. The total phenolic content (TPC) was quantified following the Folin-Ciocalteu reagent (FCR) method based on the microplate assay described by Müller et al (2017), with results expressed in micrograms of gallic acid equivalent per milligram ($\mu\text{g GAE/mg}$). The total flavonoid content (TFC) was measured using the aluminum chloride complexation method, as outlined by Topçu et al (2007), with modifications for 96-well microplate analysis. Quercetin was used as a reference standard at concentrations of 12.5, 25, 50, 75, and 100 $\mu\text{g/mL}$, and the results were expressed as micrograms of quercetin equivalent per milligram ($\mu\text{g QE/mg}$).

In vitro antioxidant capacity

The antioxidant potential of *Ephedra alata* extract (EAE) was evaluated through multiple assays. The free radical scavenging activity against DPPH radicals was determined following the method of Ben et al (2020). ABTS scavenging activity was measured according to the protocol described by Antolovich et al (2002). The cupric reducing antioxidant capacity (CUPRAC) was assessed using the method of Apak et al (2005) with slight modifications. Additionally, the ferric reducing power assay was conducted based on the methodology of Oyaizu et al (1986) with specific adjustments.

Animal treatments

Male rats weighing between 180 and 220 grams were acquired from the Pasteur Institute in Algiers, Algeria (PIA). Upon arrival, the animals were housed in groups of four per cage with full access to sustenance and hydration. The housing conditions were regulated under a 12-hour light/dark cycle, with a consistent temperature of $24 \pm 4^\circ\text{C}$ and a relative humidity of 60%. All experimental protocols complied with the standards established in the International Guidebook on the Care and Utilization of Laboratory Animals. Subsequent to a phase of acclimation, the animals were randomly allocated into four groups, each including 10 rats:

Group 1: Healthy control rats administered drinking water.

Group 2: Healthy rats received oral administration of EAE at a dosage of 120 mg/kg/day, dissolved in maize oil, in accordance with the methodology outlined by Kumari et al (2024). A constant concentration of EAE solution was formulated, and each rat was administered a dose proportional to its weight.

Group 3: Normal rats were orally supplied bifenthrin (8 mg/kg/day), dissolved in maize oil, in accordance with the approach developed by Pylak-Piwko et al (2021). A uniform concentration of bifenthrin solution was prepared, with each rat receiving a dosage commensurate with its weight.

Group 4: Rats were administered EAE (same dosage to Group 2) one hour before to exposure to bifenthrin (equal dosage to Group 3).

Biochemical analysis of the liver

Hepatic markers

Plasma separation and biochemical analyses were conducted as follows. Whole blood samples were collected in heparinized tubes and centrifuged at $2500 \times g$ for 10 minutes to isolate the plasma fraction. The obtained plasma samples were stored at -80°C for subsequent analyses. The enzymatic activities of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and lactate dehydrogenase (LDH) were quantified using commercially available colorimetric assay kits from Biomaghreb.

Determination of liver oxidative stress markers

The level of malondialdehyde (MDA) was assessed using the methodology outlined by Esterbauer (1993). The levels of nitric oxide (NO) were measured using the method outlined by Green et al (1982). The content of glutathione (GSH)

was assessed utilizing the Ellman method, as modified by Davis et al (1973). The total superoxide dismutase (SOD) activity was assessed by quantifying the suppression of pyrogallol activity. Catalase (CAT) activity was evaluated using Aebi's (1984) technique. Ultimately, the activity of glutathione peroxidase (GPx) was assessed utilizing the Gonzler (1984) method.

Determination of hepatic inflammatory and apoptotic markers

The levels of hepatic interleukin-1 β (IL-1 β), interleukin six (IL-6), tumor necrosis factor- α (TNF- α), and Caspase three were quantified utilizing an ELISA kit from Sino Gene Clon Biotech Co., Hang Zhou, China.

Histological studies

Following euthanasia, liver tissues were removed and preserved in a 10% formaldehyde solution. Subsequently, the fixed tissues were systematically treated and embedded in paraffin. Sections measuring five micrometers in thickness were excised and affixed to glass slides. The sections were deparaffinized and stained with hematoxylin and eosin (H&E) for histological analysis. A Leica® microscope paired with a Sony® digital camera was utilized to acquire photographs of the stained sections for histological examination.

Statistical analysis

Statistical analyses were conducted with GraphPad Prism 8.0 for Windows (GraphPad Software, San Diego, CA). Results are presented as mean $\pm$ standard deviation (SD). A one-way analysis of variance (ANOVA) was performed to analyze treatment effects, followed by Tukey's post-hoc test for multiple comparisons. The threshold for statistical significance was established at $p < 0.05$. Data for biological activity assays were derived from triplicate analyses and are expressed as mean $\pm$ SD. The student's t-test was employed to ascertain significant differences between means, with p values < 0.05 deemed statistically significant.

RESULTS

Total phenolic and flavonoid content

The total phenolic content of the *E. alata subsp. alenda* extract was determined to be 317.05 ± 11.71 mg gallic acid equivalents (GAE) per gram of extract. The total flavonoid content was found to be 56.66 ± 2.06 mg quercetin equivalents (QE) per gram of extract (Table 1).

Table 1 Total phenolic compounds and flavonoids contents in *E. alata subsp. Alenda* extract (1mg/mL).

Compounds	Content
Total phenolic content, (mg GAE/g)	317,05 $\pm$ 11,71
Total flavonoid content, (mg QE/g)	56,66 $\pm$ 2.06

Values were expressed as means $\pm$ S.D. (n= 3).

Antioxidant activity

The antioxidant activity of the *E. alata subsp. alenda* extract was evaluated using several assays (Table 2). In the DPPH radical scavenging assay, the extract demonstrated significant antioxidant activity with a low IC_{50} value of 4.79 ± 0.28 $\mu\text{g/mL}$, indicating its potent ability to scavenge DPPH radicals. Similarly, the extract exhibited strong activity in the ABTS assay ($\text{IC}_{50} < 3.125$ $\mu\text{g/mL}$). The ferric reducing power of the extract was also assessed. The extract showed effective ferric reducing power with an EC_{50} value of 9.11 ± 0.14 $\mu\text{g/mL}$, compared to the standards α -tocopherol ($\text{EC}_{50} = 34.93 \pm 2.38$ $\mu\text{g/mL}$) and ascorbic acid. Furthermore, the extract displayed strong antioxidant activity in the CUPRAC assay ($\text{EC}_{50} = 1.12 \pm 0.05$ $\mu\text{g/mL}$).

Table 2 Comparative analysis of antioxidant activity of *E. alata subsp. Alenda* extract

Extract and standards	Antioxidant activity			
	DPPH ^a	ABTS ^a	CUPRAC ^b	Ferric reducing power ^b
E alata subsp. Alenda	4,79 $\pm$ 0,28	<3,125	1,12 $\pm$ 0,05	9,11 $\pm$ 0,14
BHA	6.14 $\pm$ 0.41	1.29 $\pm$ 0.30	5.35 $\pm$ 0.71	Nt
BHT	12.99 $\pm$ 0.41	1.81 $\pm$ 0.10	8.97 $\pm$ 0.94	Nt
α -Tocopherol	13.02 $\pm$ 0.17	Nt	Nt	34.93 $\pm$ 2.38
Ascorbic acid	Nt	Nt	Nt	6.77 $\pm$ 1.15

Values expressed are means $\pm$ S.D. of three parallel measurements; a Values expressed in IC_{50} ($\mu\text{g/mL}$); b Values expressed in EC_{50} ($\mu\text{g/mL}$); nt: not tested.

Effect of EAE on liver functions in rats treated with BFT

Analysis of liver function markers revealed that administration of EAE to healthy rats did not significantly alter the levels of ALAT, ASAT, or LDH, suggesting that EAE does not have adverse effects on liver function. In contrast, BFT exposure

induced a significant elevation in these liver enzymes, confirming the hepatotoxic effects of BFT. Pretreatment with EAE one hour before BFT intoxication significantly decreased ASAT, ALAT, and LDH levels, demonstrating EAE's protective action against BFT-induced liver damage (Table 3).

Table 3 Effect of *E. alata* extract (EAE) on liver functions (ASAT, ALAT and LDH) in rats treated with bifenthrin.

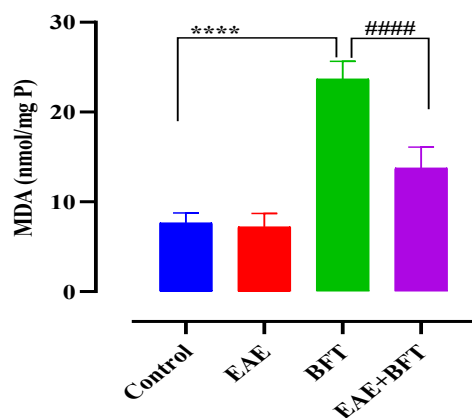
	Control	EAE	BFT	EAE+BFT
ALAT (UI)	32±2.20	33.66±5.53	46.45±6.68 **	38.2±5.34 ns
ASLT (UI)	107.5±6.55	109.2±7.82	131.5±14.64 **	125.5±12.78 ##
LDH (UI)	898.95±81.25	996.32±71.03	1769.75±37.60 **	1330.80±40.50 ns

Value in mean ± SEM (n = 10/group) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001: BFT-treated group compared with control group; ##p < 0.01, ####p < 0.0001: EAE+BFT group compared with BFT-treated group.

Effect of EAE on oxidative stress markers and antioxidant defense system in rats treated with BFT

The evaluation of hepatic oxidative stress indicated that EAE administration did not produce notable alterations in oxidative stress markers in healthy rats (Fig. 1). BFT intoxication led to a significant increase in hepatic oxidative stress markers, including malondialdehyde (MDA) and nitric oxide (NO) (Figure 1a-b), alongside a notable reduction in antioxidant enzymes such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Figure 1a-d). Prior pretreatment with EAE before BFT exposure significantly alleviated these effects, as demonstrated by elevated level of GSH, concentrations of CAT, SOD, and GPx, alongside decreased levels of MDA and NO (Fig. 2).

a



b

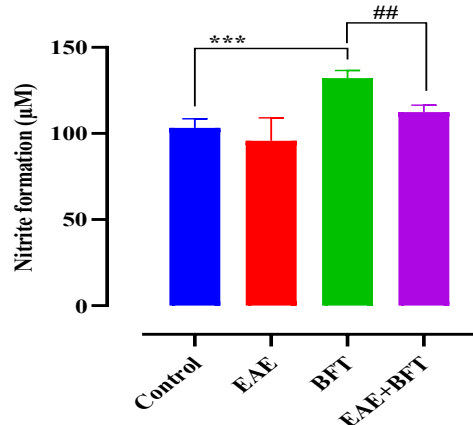
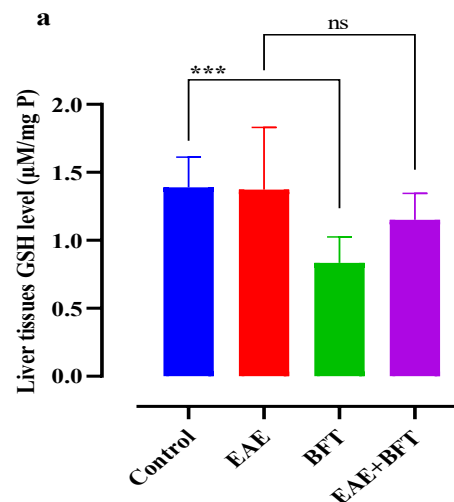
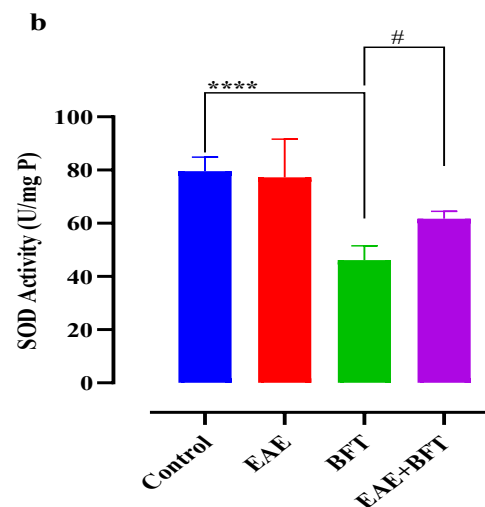


Figure 1 Effect of *E. alata* extract (EAE) on liver (a) MDA and (b) NO values in rats treated with bifenthrin. Value in mean ± SEM (n = 10/group) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001: BFT-treated group compared with control group; ##p < 0.01, ####p < 0.0001: EAE+BFT group compared with BFT-treated group.

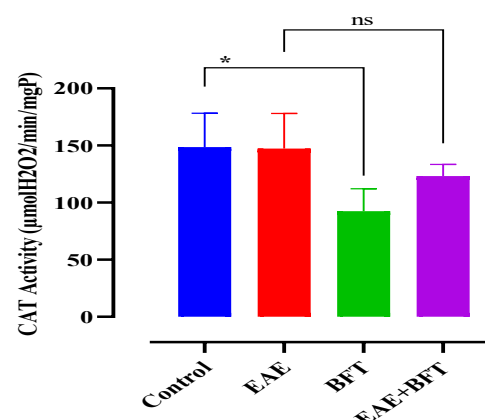
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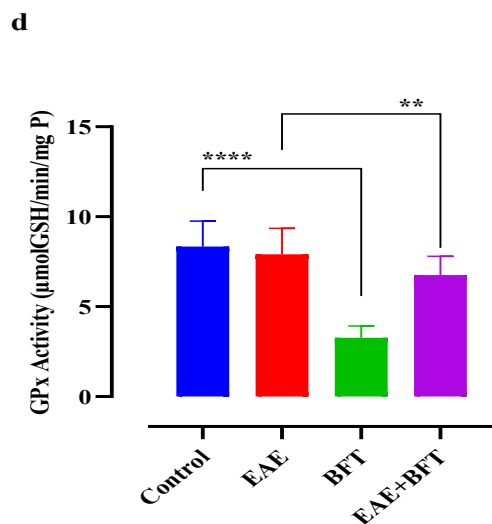


Figure 2 Effect of *E. alata* extract (EAE) on liver (a) Glutathione (GSH) activity; (b) superoxide dismutase (SOD) activity; (c) catalase (CAT) activity; (d) glutathione peroxidase (GPx) activity, in rats treated with bifenthrin. Value in mean $\pm$ SEM (n = 10/group) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001: BFT-treated group compared with control group; ##p < 0.01, ####p < 0.0001: EAE+BFT group compared with BFT-treated group.

Effect of EAE on inflammatory and apoptotic markers in rats treated with BFT

The assessment of hepatic inflammatory and apoptotic markers indicated that EAE administration in healthy rats did not result in significant alterations in the levels of interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), or caspase-3, hence reinforcing the safety of EAE (Table 4). BFT poisoning resulted in substantial elevations of several inflammatory and apoptotic markers relative to the control group. Nonetheless, pretreatment with EAE before BFT exposure markedly diminished these elevations, returning the levels of IFN- γ , TNF- α , IL-1 β , and casp-3 to nearly normal amounts (Table 4). The data indicate that EAE has anti-inflammatory and anti-apoptotic effects in relation to BFT-induced liver damage.

Table 4 Effect of *E. alata* extract (EAE) on liver IL-1 β , TNF- α , IFN- γ , and Caspase-3 values in rats treated with bifenthrin.

	Control	EAE	BFT	EAE+BFT
IL-1 β (ng/g tissue)	121.4 $\pm$ 9.45	125.6 $\pm$ 8.77	274 $\pm$ 12.08 ****	172.25 $\pm$ 10.45 ####
TNF- α (μ g/g tissue)	152.75 $\pm$ 11.46	154.73 $\pm$ 14.39	335.75 $\pm$ 13.54 ****	221.05 $\pm$ 13.35 ####
IFN- γ (pg/g tissue)	1090.57 $\pm$ 34.56	1111.04 $\pm$ 37.93	2360.32 $\pm$ 41.52 ****	1537.45 $\pm$ 38.71 ###
Caspase-3 (U/g tissue)	551.41 $\pm$ 18.56	528.23 $\pm$ 18.07	1291.45 $\pm$ 22.3 ****	928.45 $\pm$ 20.46 #

Value in mean $\pm$ SEM (n = 10/group) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001: BFT-treated group compared with control group; ##p < 0.01, ####p < 0.0001: EAE+BFT group compared with BFT-treated group.

Effect of EAE on histological sections in rats treated with BFT

Microscopic examination of hepatic tissue from the normal control and EAE-treated groups revealed a preserved histological architecture, devoid of discernible pathological alterations (Fig. 3a, 3b). Conversely, the BFT-intoxicated group exhibited significant hepatic damage, characterized by portal inflammatory cell infiltration, perivascular sinusoidal dilation, and focal hemorrhagic events (Fig. 3c). Notably, pretreatment with EAE prior to BFT exposure resulted in a marked attenuation of these pathological changes, with the liver tissue demonstrating a structure approaching that of the control group (Fig. 3d). These histological observations provide compelling evidence for the hepatoprotective efficacy of EAE against BFT-induced toxicity.

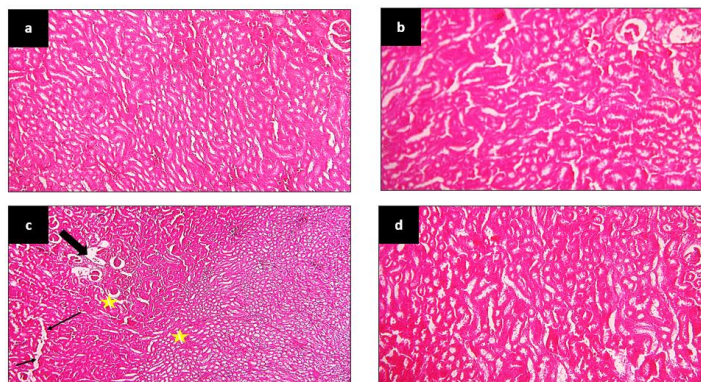


Figure 3 Photomicrograph of liver of normal and treated animals' groups. (a) is the image of the normal control group. (b) is the image of EAE administered group. In contrast, (c) image is section of BFT-treated group; Finally, (d) is image of the EAE+BFT group. (Hematoxylin and Eosin, Magnification $\times$ 100).

➡: Portal infiltration with inflammatory cells; ★: Perivascular sinusoidal dilation; ➡: focal hepatic hemorrhage.

DISCUSSION

The toxicity of insecticides has been thoroughly investigated in experimental animal models by evaluating metabolic markers and histoarchitectural changes as indicators of organ damage (Sharma and Sangha, 2014). Oxidative stress is a sensitive indication commonly utilized in the toxicological evaluation of pyrethroids, such as bifenthrin (BFT), and other insecticides as a likely cause for their adverse effects (Kassa et al., 2020; Abdel-Wahhab et al., 2024). Various pesticides have been shown to induce lipid oxidative stress in multiple tissue (Sule et al., 2022). The current investigation revealed a substantial decrease in antioxidant indicators (GSH, SOD, CAT, and GPx) and a notable rise in oxidative

products (NO and MDA) in the liver tissue of the BFT-exposed group. The findings correspond with the data presented by Dar et al., (2019), who demonstrated that reactive oxygen species (ROS) are generated during the metabolism of xenobiotics, including pyrethroids. Excessive production of reactive oxygen species (ROS) exceeds the body's antioxidant defenses, leading to oxidative stresses and inflammation responses that cause cellular and subcellular alterations (Aouey et al., 2017; Dar et al., 2019). Furthermore, the advancement of chronic liver disease (CLD) is closely linked to persistent inflammation and cellular oxidative stress, which are critical variables in many complications (Aouey et al., 2017).

IL-1 β and TNF- α are cytokines primarily produced by monocytes and macrophages, which are then discharged into the bloodstream, where they exert systemic effects. TNF- α is one of the initial cytokines generated during an inflammatory reaction (Abdel-Wahhab et al., 2024), and plays a key role in inducing IL-1 β synthesis under conditions of extended oxidative stress (Abdel-Wahhab et al., 2024). IL-1 β functions as a signaling molecule that regulates immune responses, enhances leukocyte and lymphocyte activity, acts as a pyrogen, and contributes to the progression of chronic inflammation (Malik and Kanneganti, 2018). In the current study, a significant elevation in the levels of inflammatory markers (IL-1 β , IFN- γ , and TNF- α) and apoptotic markers (casp-3) was observed in the BFT-exposed group. These findings are consistent with prior research (Magnuson et al., 2021), which indicated that little doses of bifenthrin can significantly increase the levels of inflammatory cytokines in the liver. The study elucidated the mechanisms of bifenthrin-induced immunotoxicity, which encompassed increased transcription of interleukin-6 and TNF- α after LPS stimulation, heightened ROS generation, and subsequent dysregulation of oxidative stress-related genes. Additionally, the observed increase in serum levels of ALAT, ASAT, and LDH following bifenthrin exposure indicates hepatic dysfunction, consistent with findings from recent studies (Nieradko-Iwanicka and Konopelko, 2020). The association between hepatotoxicity and oxidative stress may be explained by the damaging effects of ROS on liver macromolecules, including proteins, DNA, and lipids, result in reactions of inflammation, functional deficits, and structural damage (Abdel-Wahhab et al., 2024).

The investigation of the antitoxic potential of various medicinal plant extracts is a relatively recent area of research. Nevertheless, phytomedicinal treatments offer several advantages, including low cost, wide availability, and minimal health risks compared to synthetic pharmaceuticals (Khan and Ahmed, 2019).

The total phenolic content of *Ephedra alata* subsp. *alenda* extract was quantified at 317.05 $\pm$ 11.71 mg gallic acid equivalents (GAE) per gram of extract, signifying a considerable concentration of phenolic compounds, recognized for their function in antioxidant defense mechanisms (Rahman et al., 2021). Phenolic chemicals aid in neutralizing free radicals, hence diminishing oxidative stress (Rahman et al., 2021). The total flavonoid concentration was determined to be 56.66 $\pm$ 2.06 mg of quercetin equivalents (QE) per gram of extract. Flavonoids, classified as secondary metabolites, are acknowledged for their capacity to chelate metal ions and scavenge reactive oxygen species; hence augmenting the antioxidant efficacy of plant extracts (Kejik et al., 2021).

The antioxidant capabilities of the *E. alata* subsp. *alenda* extract were assessed by various assays, each demonstrating its notable efficacy. The DPPH radical scavenging experiment revealed significant antioxidant activity of the extract, indicated by a low IC₅₀ value of 4.79 ± 0.28 µg/mL. The low IC₅₀ value indicates a significant capacity to neutralize DPPH radicals, so establishing the extract as a formidable antioxidant candidate (Moussa *et al.*, 2024). The ABTS experiment demonstrated that the extract possesses significant antioxidant activity, with an IC₅₀ value under 3.125 µg/mL, thereby validating its efficacy in free radical scavenging. The ferric reducing power assay offered further understanding of the extract's antioxidant effectiveness, demonstrating an EC₅₀ value of 9.11 ± 0.14 µg/mL. This performance surpassed that of the typical antioxidant α -tocopherol (EC₅₀ = 34.93 ± 2.38 µg/mL) and was equivalent to ascorbic acid (Alam *et al.*, 2013). The extract exhibited significant antioxidant activity in the CUPRAC assay, attaining an EC₅₀ value of 1.12 ± 0.05 µg/mL. The notably low EC₅₀ value highlights the extract's significant capacity to decrease copper ions, a trait that signifies strong antioxidant potential (Djafarou *et al.*, 2025). These findings collectively highlight the strong antioxidant properties of *E. alata* subsp. *alenda* extract, likely attributable to its high phenolic and flavonoid content (Belazougui *et al.*, 2023). The superior performance compared to conventional antioxidants further emphasizes the extract's potential as a natural source of bioactive compounds (Belazougui *et al.*, 2023).

The current investigation shown that the administration of EAE alone diminished oxidative stress indicators and improved the antioxidant state in the hepatic tissue of rats. Moreover, pre-treatment with EAE one hour before BFT exposure dramatically elevated liver concentrations of essential antioxidant markers (GSH, SOD, CAT, and GPx) while markedly decreasing oxidative indicators (MDA and NO). The findings align with the observations of Medila *et al.*, (2021), who indicated that increased GSH levels in liver tissue safeguard proteins in cells from oxidation through the GSH redox cycle and facilitate the elimination of ROS. Furthermore, EAE was observed to influence antioxidant enzymes that together safeguard against reactive oxygen species (ROS). The protective effect was ascribed to the chelating capabilities of *E. alata*, which neutralizes free radicals and reactive lipid peroxidation byproducts while augmenting tissue thiol pools. The observed effects may be attributed to the plant's elevated levels of flavonoids and phenolic chemicals, which are extensively recognized for their antioxidant and anti-inflammatory activities (Rahman *et al.*, 2021; Kejik *et al.*, 2021; Moussa *et al.*, 2024; Alam *et al.*, 2013; Djafarou *et al.*, 2025; Belazougui *et al.*, 2023). Moreover, the serum levels of LDH, ASAT, and ALAT were markedly increased in BFT-treated subjects, signifying hepatotoxicity resulting from the efflux of these enzymes from compromised hepatocyte cytosol into the bloodstream (Firat *et al.*, 2011). The elevated transaminase activity may possibly result from the intensified catabolism of amino acids necessary to satisfy the immediate energy requirements during pyrethroid-induced stress (Kumar *et al.*, 2011). The administration of *E. alata* extract alleviated these effects, resulting in enhanced transaminase activity. The protective effect is due to the antioxidant characteristics of *E. alata*, which possesses phenolic and flavonoid components that inhibit lipid peroxidation, stabilize cell membranes, prevent oxidative damage to membrane lipids, and restore cellular integrity (Belazougui *et al.*, 2023). This study revealed that the co-administration of EAE with BFT significantly downregulated pro-inflammatory markers (IFN- γ IL-1 β , and TNF- α) and the apoptotic marker casp-3 in hepatic tissue, highlighting its substantial anti-inflammatory properties potential. Comparable results documented by Abdel-Wahhab *et al.*, (2024) were ascribed to the antioxidant characteristics of the phytochemical components found in *E. alata*.

The histopathological scoring results corroborated the earlier biochemical findings and revealed that BFT induced significant histological alterations in liver tissues. Similar hepatic histological changes have been reported in previous studies (Abdel-Wahhab *et al.*, 2024). However, animals treated with EAE exhibited improved renal tissue integrity compared to the BFT-exposed group, with EAE demonstrating a notable reduction in pathological changes.

The phytochemical constituents of *E. alata* extract exhibit antioxidant and anti-inflammatory properties suppress and stabilize free ROS generated by bifenthrin, while downregulating inflammatory pathways. This approach significantly diminished renal impairment, as seen by the notable reduction in serum levels of ALAT, ASAT, and LDH, along with pathological changes in bifenthrin-exposed rats. EAE supplementation substantially alleviated tissue damage induced by bifenthrin toxicity, showcasing its effectiveness in diminishing oxidative stress via its powerful antioxidant and chelating properties.

CONCLUSION

The findings of this study show that the methanolic extract of *Ephedra alata* (EAE) has notable ameliorative, antioxidant, anti-inflammatory, hepatoprotective, and detoxifying qualities against bifenthrin-induced liver damage. EAE successfully reinstated hepatic function and mitigated pathological impairments induced by bifenthrin exposure. The protective effect is attributed to the antioxidant properties of the EAE components. Notably, the administration of EAE at the tested dose exhibited no observable adverse effects in healthy rats, supporting its safety profile in this preclinical setting. Given these findings, the extract may be suggested as a

potential protective supplement for those exposed to bifenthrin, subject to further toxicological and pharmacokinetic investigations.

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Authors' Contributions

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