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Hepatoprotective, antioxidant, and anti-inflammatory effects of *Ephedra alata* extract against bifenthrin-induced liver toxicity in rats

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This study aimed to evaluate the ameliorative, antioxidant, anti-inflammatory, and detoxifying effects of *Ephedra alata* extract (EAE) against bifenthrin-induced hepatotoxicity. Adult male rats (180–220 g) were randomly divided into four groups (n = 10) and treated orally for 30 days: (1) control; (2) EAE (120 mg/kg/day); (3) bifenthrin (8 mg/kg/day); and (4) EAE administered one hour prior to bifenthrin exposure. Bifenthrin significantly increased serum biomarkers of liver injury (LDH, ASAT, and ALAT), pro-inflammatory cytokines (IL-1β, TNF-α, and IFN-γ), caspase-3 activity, and oxidative stress markers (NO and MDA), while markedly decreasing antioxidant defenses (GSH, SOD, CAT, and GPx) compared to controls. Pre-treatment with EAE significantly restored these parameters toward normal levels and improved hepatic histoarchitecture. These findings indicate that *Ephedra alata* extract exerts potent antioxidant, anti-inflammatory, hepatoprotective, and detoxifying effects against bifenthrin-induced liver injury.

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

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 insecticides due to their harmful impacts on humans and animals; yet, they continue to be utilized today[1]. Bifenthrin, a third-generation type one synthetic pyrethroid, has garnered significant interest for its enhanced insecticidal efficacy and durability relative to earlier synthetic pyrethroids[2]. Moreover, bifenthrin exposure has been documented to have the neurotoxic consequences in mammalian brains, along with apoptotic and inflammatory reactions in mouse brains[2]. 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[3]. Bifenthrin has demonstrated significant toxicity, tumorigenicity, immunotoxicity, and hepatotoxicity in many mammalian species through oxidative stress mechanisms and/or hormone disruption effects[4]. 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[5]. 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[6]. 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[7]. 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* is one of the most often utilized plant species in medicinal applications[8].

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Ephedra 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. *Ephedra alata* has attracted worldwide attention in recent years[9]. 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 *Ephedra alata*) are powerful antioxidants that efficiently neutralize free radicals and diminish the production of nitric oxide free radicals[10,11]. Multiple investigations have demonstrated that exposure to bifenthrin adversely affects several organs, including the liver, lungs, kidneys, testes, and brain[12]. The aim of this research was to investigate the therapeutic potential of *Ephedra alata* methanolic extract and to evaluate its anti-inflammatory, antioxidant, and detoxifying properties against bifenthrin-induced liver injury.

Materials and Methods

Chemicals

Bifenthrin is a synthetic pyrethroid insecticide with the chemical formula $C_{23}H_{22}ClF_3O_2$, 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 Uscn 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 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±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[13].

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.*[14], with results expressed in micrograms of gallic acid equivalent per milligram (µg GAE/mg). The total flavonoid content (TFC) was measured using the aluminum chloride complexation method, as outlined by Topçu *et al.*[15], with modifications for 96-well microplate analysis. Quercetin was used as a reference standard at concentrations of 12.5, 25, 50, 75, and 100 µg/mL, and the results were expressed as micrograms of quercetin equivalent per milligram (µg 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.*[16]. ABTS scavenging activity was measured according to the protocol described by Antolovich *et al.*[17]. The cupric reducing antioxidant capacity (CUPRAC) was assessed using the method of Apak *et al.*[18] with slight modifications. Additionally, the ferric reducing power assay was conducted based on the methodology of Oyaizu *et al.*[19] 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 accommodated 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 ± 4 ℃ 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 were orally supplied EAE at a dosage of 120 mg/kg/day, dissolved in maize oil, in

accordance with the methodology outlined by Kumari *et al*.[20]. 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*.[21]. 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×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[22]. The levels of nitric oxide (NO) were measured using the method outlined by Green *et al*.[23]. The content of glutathione (GSH) was assessed utilizing the Ellman method, as modified by Davis *et al.*[24]. The total superoxide dismutase (SOD) activity was assessed by quantifying the suppression of pyrogallol activity. Catalase (CAT) activity was evaluated using Aebi[25] technique. Ultimately, the activity of glutathione peroxidase (GPx) was assessed utilizing the Flohé [26] method.

Determination of hepatic inflammatory and apoptotic markers

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

Histological studies

Subsequent to sacrifice, 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 ± 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 ± 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 *Ephedra alata* 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).

Antioxidant activity

The antioxidant activity of the *Ephedra alata* 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 µg/mL, indicating its potent ability to scavenge DPPH radicals. Similarly, the extract exhibited strong activity in the ABTS assay (IC_{50} < 3.125 µg/mL). The ferric reducing power of the extract was also assessed. The extract showed effective ferric reducing power with an EC_{50}

Table 1 — Total phenolic compounds and flavonoids contents in *Ephedra alata* extract (1mg/mL).

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

Values were expressed as means ±S.D. (n= 3).

Table 2 — Comparative analysis of antioxidant activity of *Ephedra alata* extract

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

Values expressed are means ±S.D. of three parallel measurements; a Values expressed in IC50 (µg/mL); b Values expressed in EC50 (µg/mL); nt: not tested.

Table 3 — Effect of *Ephedra 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.

value of 9.11 ± 0.14 µg/mL, compared to the standards α-tocopherol (EC_{50} = 34.93 ± 2.38 µg/mL) and ascorbic acid. Furthermore, the extract displayed strong antioxidant activity in the CUPRAC assay (EC_{50} = 1.12 ± 0.05 µg/mL).

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).

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) (Fig 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).

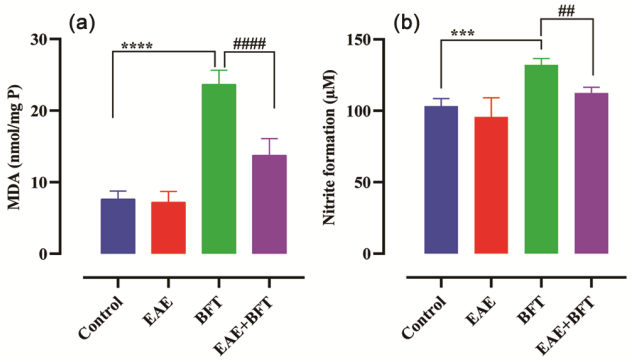

Fig 1 — Effect of *Ephedra alata* extract (EAE) on liver (a) MDA and (b) NO values in rats treated with bifenthrin. Value in mean ± SEM (n = 10/group) *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001: BFT-treated group compared with control group; ##P < 0.01, ####P < 0.0001: EAE+BFT group compared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

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-1β (IL-1β), 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-1β, 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.

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, perivenular 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.

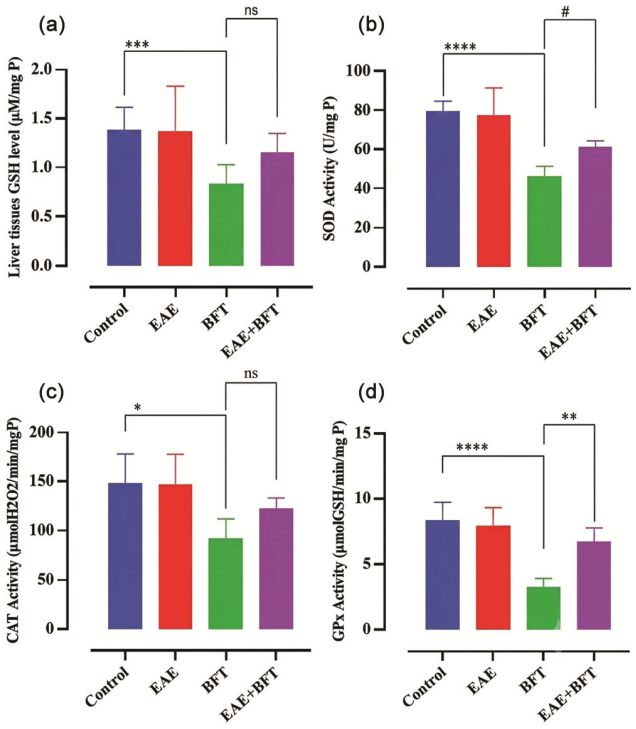

Fig 2 — Effect of *Ephedra alata* extract (EAE) on liver (a) Glutathione (GSH) activity; (b) superoxide dismutase (SOD) activity; (c) catalase (CAT) activity; (d) glutathione peroxidase (GPx) activity. in rats treated with bifenthrin. Value in mean ± SEM (n = 10/group) *$P < 0.05$, **$P < 0.01$, ***$P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

Discusson

Researchers have done a lot of work on the potential for pesticides to damage the liver using experimental animals. They have looked at biochemical markers and histopathological changes that are good signs of organ damage[27]. Among these, oxidative stress has emerged as a critical mechanism in pesticide-induced damage. Pyrethroid chemicals, especially bifenthrin (BFT), have been shown to cause oxidative stress-mediated cytotoxicity, which can damage the structure and function of several organs, including the liver[7,28]. An imbalance between the formation of reactive oxygen species (ROS) and the antioxidant defence mechanisms is a sign of these hazardous effects. Too much ROS can cause lipid peroxidation, protein oxidation, and DNA damage[29]. Many studies have shown that being exposed to BFT lowers the body's natural antioxidant defences, like glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). At the same time, it raises the levels of oxidative biomarkers like malondialdehyde (MDA) and nitric oxide (NO) in liver tissues. These results are in line with what Dar *et al.*[12] found, which showed that when xenobiotics are metabolised in the liver, notably pyrethroid-based pesticides, too many ROS are generated, which overwhelms the cells' ability to buffer antioxidants. This redox imbalance starts a chain reaction of oxidative stress and inflammatory responses that eventually cause hepatocellular degeneration, apoptosis, and tissue remodelling[12,29]. Also, long-term oxidative stress and inflammation are now known to be major causes of the development and progression of chronic liver disease. Oxidative

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

	Control	EAE	BFT	EAE+BFT
IL-1β (ng/g tissue)	121.4± 9.45	125.6± 8.77	274±12.08 ****	172.25±10.45 ####
TNF-α (µg/g tissue)	152.75± 11.46	154.73± 13.49	335.75±13.54 ****	221.05±13.35 ####
IFN-γ (pg/g tissue)	1090.57± 34.56	1111.04 ±39.73	2360.32 ±45.12 ****	1537.45 ±38.71 ###
Caspase- 3 (U/g tissue)	551.41± 18.56	528.23± 18.07	1291.45 ±23.2 ****	928.45 ±20.46 #

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.

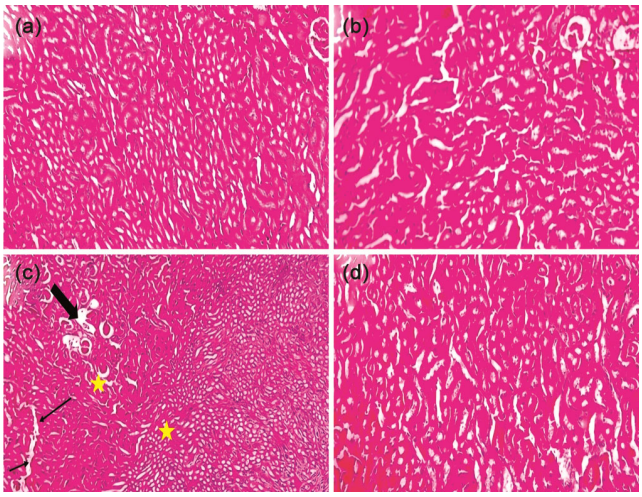

Fig 3 — Photomicrograph of liver of normal and treated animals' groups. *(a) is the image of the normal control group. (b) is the image of EAE administrated group. In contrast, (c) image is section of BFT-treated group; Finally, (d) is image of the EAE+BFT group. (Hematoxylin and Eosin, Magnification × 100).*

damage that lasts for a long-time damage's mitochondria, changes redox-sensitive transcription factors (such NF-κB), and increases the production of pro-inflammatory cytokines and fibrogenic mediators. All of these things together make hepatocellular dysfunction worse and speed up the growth of fibrosis and other liver problems[29]. The current results support what has already been found in the literature and show that oxidative stress is the main way that BFT exposure can cause liver damage.

Pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are key mediators of the innate immune response and are largely generated by activated monocytes and macrophages in response to pathogenic stimuli or cellular stress. Once secreted into the systemic circulation, these cytokines initiate and promote inflammation by altering vascular permeability, leukocyte recruitment, and the production of adhesion molecules and other inflammatory mediators[30]. TNF-α is among the initial cytokines generated during the acute phase of inflammation and operates as a critical upstream regulator of IL-1β production, especially under sustained oxidative stress conditions[7]. IL-1β, in turn, promotes the activation and proliferation of leukocytes and lymphocytes, operates as a potent endogenous pyrogen, and contributes to the amplification and maintenance of chronic inflammatory responses.

In the current investigation, exposure to bifenthrin (BFT) was related with a considerable elevation of inflammatory markers, including IL-1β, TNF-α, and interferon-gamma (IFN-γ), as well as the apoptotic marker caspase-3. These findings corroborate previous data reported by Magnuson *et al.*[31], who demonstrated that even sub-toxic doses of bifenthrin can provoke significant immune perturbations by elevating the transcription of pro-inflammatory cytokines (e.g., IL-6, TNF-α) in hepatic tissue, particularly following stimulation with lipopolysaccharides (LPS). The mechanism underlying these effects involves increased ROS production, which in turn activates redox-sensitive transcription factors such as NF-κB, leading to the elevated expression of inflammatory genes and disruption of antioxidant defense systems.

Moreover, the observed elevations in hepatic enzymes alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and lactate dehydrogenase (LDH) following BFT exposure are symptomatic of hepatocellular damage and impaired membrane integrity. These biochemical abnormalities are consistent with recent research[32], and imply that the hepatotoxic effects of BFT are mediated by oxidative and inflammatory processes. Excessive ROS generation can inflict oxidative damage on cellular macromolecules including membrane lipids, proteins, and nucleic acids ultimately resulting in mitochondrial malfunction, cell death, and local inflammatory responses[7]. This balance between oxidative stress and inflammation constitutes a crucial axis in the pathophysiology of pesticide-induced hepatic damage and may contribute to the progression of chronic liver diseases.

The research of the antitoxic and therapeutic potential of medicinal plant extracts has received growing attention in recent years, particularly in the context of oxidative stress and chemical-induced organ damage. This developing subject originates from the need to identify safer, natural alternatives to synthetic medications, which typically exhibit substantial side effects and higher economic expenses. Phytomedicine has various intrinsic advantages, including broad availability, lower toxicity, cost-effectiveness, and compatibility with human physiology[33]. Among these, *Ephedra alata* subsp. alenda has showed significant promise due to its rich phytochemical content and powerful bioactivities.

Quantitative phytochemical investigations of *Ephedra alata* extract have indicated a substantial presence of bioactive phenolic and flavonoid

components, which are fundamental to its antioxidant activity. The total phenolic content was determined at 317.05 ± 11.71 mg gallic acid equivalents (GAE)/g of extract, emphasising a strong ability for electron donation and hydrogen atom transfer mechanisms crucial in the neutralization of free radicals[34]. Phenolic chemicals serve a diverse role in cellular defence by halting lipid peroxidation chains, chelating transition metals, and altering endogenous antioxidant enzyme activity. Similarly, the total flavonoid concentration of the extract was determined to be 56.66 ± 2.06 mg quercetin equivalents (QE)/g. Flavonoids, as specialized secondary metabolites, perform antioxidant activities via radical scavenging, metal chelation, and inhibition of oxidative enzymes such as xanthine oxidase and NADPH oxidasem[35]. Their chemical structure, typified by numerous hydroxyl groups and conjugated double bonds, underpins their high redox potential and capacity to stabilize reactive species .

To further investigate the antioxidant properties of *Ephedra alata* a battery of in vitro experiments was performed. The DPPH assay produced a low IC_{50} value of 4.79 ± 0.28 µg/mL, showing a considerable potential to donate hydrogen atoms and quench free radicals. Similarly, the ABTS assay demonstrated significant antioxidant activity with an IC_{50} value below 3.125 µg/mL, suggesting the extract's efficiency in neutralizing $ABTS^{+}$ cation radicals. These findings show the extract's robust capacity to reduce oxidative damages across multiple radical systems Moussa *et al*.[36].

The ferric reducing antioxidant power (FRAP) assay further revealed the extract's reducing capacity, providing an EC_{50} of 9.11 ± 0.14 µg/mL significantly superior to that of α-tocopherol (EC_{50} = 34.93 ± 2.38 µg/mL) and comparable to ascorbic acid, a benchmark antioxidant Alam *et al*.[37]. In the cupric ion reducing antioxidant capacity (CUPRAC) assay, the extract exhibited exceptional reducing power with an EC_{50} value of 1.12 ± 0.05 µg/mL, demonstrating its capacity to reduce Cu^{2+} to Cu^{+} and suggesting its ability to interrupt redox cycling involved in oxidative tissue damage[38]. These convergent findings show the powerful antioxidant capacity of *Ephedra alata* which is likely owing to the synergistic activity of its various polyphenolic components.

The extract's improved performance compared to traditional antioxidants suggests its potential as a therapeutic candidate for preventing or attenuating oxidative stress-associated diseases. This natural antioxidant reservoir may play a significant role in hepatoprotection, nephroprotection, and immunomodulation when exploited in toxicological situations including xenobiotic exposure[39].

The present work indicated that the administration of *Ephedra alata* extract (EAE) alone greatly alleviated oxidative stress and restored redox equilibrium in hepatic tissues. Pre-treatment with EAE one hour prior to bifenthrin (BFT) exposure markedly elevated hepatic levels of critical endogenous antioxidant biomarkers, including glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while significantly reducing oxidative stress indicators such as malondialdehyde (MDA) and nitric oxide (NO). These findings are in agreement with those published by Medila *et al*.[40], who established that GSH plays a critical role in cellular defence by engaging in the GSH redox cycle, directly neutralizing ROS, and preserving protein thiol groups in their reduced form. Moreover, the restoration of enzymatic antioxidants highlights the modulatory influence of EAE on the cellular antioxidant defense system, hence boosting resilience to oxidative assaults .

The observed antioxidant effects of *Ephedra alata* may be attributed to its high content of polyphenolic compounds and flavonoids, which are well-documented for their ROS-scavenging, metal-chelating, and lipid peroxidation-inhibiting properties[34,35,36]. These phytochemicals exert their protective benefits through numerous pathways, including direct scavenging of reactive species, activation of antioxidant enzymes via the Nrf2/ARE signaling pathway, and stability of cellular membranes. Notably, EAE also boosts thiol-containing molecules, enhancing the intracellular buffering ability against oxidative stress and lipid peroxidation byproducts.

Consistent with these biochemical findings, bifenthrin exposure significantly elevated serum levels of liver injury markers alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and lactate dehydrogenase (LDH) reflecting hepatocellular membrane damage and increased enzyme leakage into the bloodstream[41]. These modifications may be connected to mitochondrial malfunction and increased amino acid catabolism under pyrethroid-induced stress, as previously

observed by Kumar *et al*.[42]. Remarkably, EAE supplementation ameliorated these biochemical abnormalities by returning enzymatic levels toward normal values, showing its hepatoprotective potential. This benefit

may be mediated by the ability of EAE to prevent membrane lipid peroxidation, retain mitochondrial integrity, and minimize oxidative stress to hepatocytes[39]. In addition to its antioxidant properties, EAE displayed powerful anti-inflammatory and anti-apoptotic activity. Co-administration of EAE with BFT resulted to considerable downregulation of major pro-inflammatory cytokines including interleukin-1β (IL-1β), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) as well as the apoptotic executioner caspase-3 in hepatic tissues. These data imply that EAE has immunomodulatory effects via inhibiting the oxidative stress–NF-κB axis, thereby attenuating the production of inflammatory mediators and avoiding apoptosis. This is in line with the findings of Abdel-Wahhab *et al.*[7], who also demonstrated that antioxidant-rich plant extracts attenuate inflammation and apoptosis through comparable molecular processes .

Histopathological examinations further validated the biochemical data, demonstrating that BFT generated substantial hepatic tissue abnormalities, including hepatocyte degradation, sinusoidal dilatation, and inflammatory cell infiltration. However, pre-treatment with EAE greatly relieved these histological abnormalities, conserving hepatic architecture and demonstrating efficient tissue-level protection. Interestingly, EAE also offered protective effects on renal tissue, as indicated by a reduction in pathological alterations relative to the BFT-only group highlighting the systemic protective impact of EAE beyond the liver .Altogether, these data underline the therapeutic promise of *Ephedra alata* extract as a strong natural agent capable of counteracting pesticide-induced oxidative and inflammatory damage. Its complex defensive actions rooted in its rich phytochemical profile include radical scavenging, metal chelation, prevention of lipid peroxidation, and regulation of inflammation and death pathways. These findings establish EAE as a good possibility for developing plant-based treatments against environmental toxin-induced organ toxicity.

Conclusion

The findings of the present investigation underline the significant protective role of *Ephedra alata* methanolic extract (EAE) against bifenthrin-induced liver damage. EAE displayed considerable antioxidant, anti-inflammatory, and hepatoprotective activities, which led to the restoration of liver function and mitigation of bifenthrin-induced histopathological alterations. These positive effects are primarily related to the presence of bioactive compounds, particularly flavonoids and phenolic constituents, which effectively prevent oxidative stress and inflammation. Taken together, the results imply that *Ephedra alata* extract offers promise as a natural therapeutic candidate for alleviating pesticide-induced hepatotoxicity. However, further mechanistic and preclinical studies are necessary to establish its safety profile and efficacy in broader toxicological contexts.

Ethical statement

All the animal experiment protocols were done under the guidelines and rules of the international animal experimentation chart, and the Helsinki Declaration of 2008.

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Conflict of interest

The authors have no conflicts of interest to declare.

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