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POSSIBLE PROTECTIVE EFFECT OF ALGERIAN *EPHEDRA ALATA* SUBSP. *ALENDA* (STAPF) TRAB EXTRACT AGAINST FENTHION-INDUCED HISTOPATHOLOGICAL AND BIOCHEMICAL ALTERATION IN RAT

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Abstract: *Ephedra alata* subsp *alenda* (Stapf) Trab, is an endemic plant of the Algerian Sahara. In this study, we sought to investigate the possible preventive effect of *plant* extract against hepatotoxicity induced by fenthion in male Wistar rats. For this purpose, 40 albinos male rats were randomly divided into four groups. The first group received orally 1.5 ml of corn oil serving as a control; the second group were treated by 1 mg/kg/d of fenthion; group 3 received 300 mg/kg/d of *E. alata* extract; and the 4th group received both fenthion and ephedra. 30 days later, rats were sacrificed, Blood samples were collected for hematological and biochemical estimation, and liver fragments were reserved to assess antioxidant activity and histopathological examination. Our results showed that exposure to fenthion caused decrease in body weight, and induced biochemical disturbances characterized by hyperglycemia, hyperlipidemia, leukocytosis, and significant increase in hepatic biomarkers accompanied by increase in liver relative weight. In the oxidative stress profile, we found that MDA activity increased, while GSH level, GST and CAT activities decreased. Furthermore, fenthion showed histological damage in liver tissue. Otherwise, addition of *E. alata* notably allows to restore most of the parameters measured in this study. These results suggest that the studied dose of *E. alata* had antioxidant activity and hepatoprotective effect against fenthion toxicity in male rats.

Keywords: *Ephedra alata alenda*, fenthion, oxidative stress, toxicity, liver.

INTRODUCTION

Pesticides belonged to a wide group of chemicals that are employed in agriculture in order to prevent agricultural pests, and to regulate plant growth. They are an important factor in world food security. Indeed, pesticides could be used as insecticides, herbicides, or fungicides (Sun et al., 2019). In spite of their wide benefits for preserving cultures, they are known as the most common factors leading to liver damage in developing countries (Abdou et al., 2012; El-Bini et al., 2015). In fact, fenthion is one of these phytosanitary products widely used. It is a systemic Organophosphate (OP) insecticide that is frequently used in urban and agricultural environments (Krieger, 2010). Fenthion possess a tremendous novices effects including inhibition of cholinesterase (AChE), flaccid paralysis, exophthalmia, diarrhea, vomiting and neurotoxicity—(Muralidharan, 2014). The liver is considered as an important site for fenthion's oxidative metabolism (Furnes and Schlenk, 2005).

Hence, natural and conventional remedies for preventing and treating liver diseases have gained global popularity in recent years. However, information about the biological activities and mechanisms of these remedies is still limited. Ephedra is one of the famous plants used in traditional remedies in Algeria. One of this species is *Ephedra alata* which belong to Ephedraceae family. Furthermore, it is primarily thriving in the Algerian desert and is locally known as alenda. *Ephedra alata* contains various phytochemicals, including alkaloids and flavonoids (Saidi et al., 2022). Some of these compounds may possess antioxidant and anti-inflammatory properties which are generally considered beneficial for liver health (Chouitah, 2019). We purposefully chose to research *Ephedra alata alenda's* preventive properties since its ephedrine content makes it a candidate for a preventative impact (Danciu et al., 2018;

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Mufti al ., 2023). Ephedrine has been shown to protect against neurotoxicity, ischemic damage, and depression (Shi and Li, 2021; Ramachandraih et al., 2011) While in the literature, all research has focused on the phytochemical screening and anticancer effects of Ephedra, there is some preliminary research suggesting that certain compounds found in *Ephedra alata* may have protective properties. The scientific evidence is not extensive, and more rigorous studies are needed to confirm these effects. In the highlight of this latter, this work is aimed at evaluating the antioxidant activity and hepatoprotective effect of *Ephedra alata alenda* extract against fenthion toxicity in rats.

MATERIALS AND METHODS

Chemicals

Fenthion is an organophosphate insecticide. Its chemical name is [O,O-dimethyl O-[3-methyl-4-(methylthio) phenyl]phosphorothioate] ($C_{10}H_{15}O_3$ PS_2). In this study, hepatotoxicity was induced using a commercial formulation of fenthion named ''Lebaycid 50 EC', Bayer Crop Science, East Hawthorn, Australia.

Plant collection, preparation, and decoction

Fresh harvested plant material (*Ephedra alata* subsp *alenda* (Stapf) Trab*)* was collected from El Oued (Algeria) in March 2022 (https://www.google.com/maps/place/33%C2%B014'07.8%22N+6%C2%B046'32.8%22E),and identified as *Ephedra alata subsp. alenda* (Stapf) Trab (Ephedraceae) by a botanist at Tebessa University. The aerial portion were dried at room temperature using a convection oven at 35°C for three days. It was grinded into fine powder using an electric blender (moulinex, France). The Ephedra decoction was obtained by boiling 100g of plant powdered leaves in 2L of distilled water for 25 min. The extract was filtered using Whatman paper and lyophilized (Saidi et al., 2022).

Animals

Forty adult Male Wistar rats weighing between 400-450g were obtained from Pasteur institute, Algeria.The experimental protocol was approved by the Local Animal Care Committee of TEBESSA University. All procedures were accomplished in accordance with laboratory guidelines for animal care, Algerian Executive Directive (18 March 2004, N_10–90 JORA) and Law No.88-08 of 26 January 1988 relating to veterinary medicine activities and the protection of animal health (NJORA: 004 of 27-01-198)

Forty rats were randomly divided into four groups of ten each:

Group C (n=10): Control rats received orally 1ml of corn oil by oral gavage route.

Group F (n=10): Fenthion treated group received 1mg/kg of fenthion dissolved in 1.5ml/kg of corn oil by oral gavage route.

Group E (n=10): Rats received 300mg/kg of *Ephedra alata* extract by oral gavage route (Ben Lamine et al., 2019).

Group E+F (n=10): Rats received 300mg/kg of *Ephedra alata* 30 min before fenthion administration (1mg/kg) by oral gavage route.

During experimental period (30 days) body weight of rats were measured every day at the same time . After 30 days, the rats were fasted overnight and then sacrificed. The blood samples were collected into heparin tubes. Then, the liver from each group were removed and washed with cold saline solution (0.9%) and fixed in 10% formalin for histopathological examination.

Oxidative Stress Parameters

Tissue Homogenate

Liver was immediately collected, washed using 0.9% NaCl solution and weighed;1g was put in 2 mL of TBS (Tris-buffered saline),Tris 50 mM, NaCl 150 mM, adjusted to pH=7.4 with HCl 1M.The mix was homogenized using a "SONICS,Vibra-Cell VX 130" sonificator, under ice-cold conditions. Homogenates were centrifuged at 3000×g for 30 min at 4°C. The supernatants were then aliquoted and stored at -20°C.

Malondialdehyde (MDA) evaluation

The evaluation of lipid peroxidation levels was accomplished by detecting the value of MDA in organ homogenates. MDA reacts with thiobarbituric acid as a reactive substance to generate a red-colored complex. The procedure involved combining 500L of tissue homogenate with 1 mL of TCA-TBA-HCI (15%, 0.375%, 0.25 N) and mixing thoroughly. The mixture was heated in a boiling water bath for 15 min. Then, the flocculent precipitate was removed by centrifuging at 1000×g for 10 min and the absorbance was measured at 535 nm (Draper and Hadley, 1990).

Reduced Glutathione (GSH) evaluation

Liver GSH was measured by employing a colorimetric technique based on the oxidation of GSH by DTNB, which generates a yellow color according to the Elman method (1959).Tissue homogenate (400 µL) was added to 100 uL of sulfosalicylic acid (0.25%) and left for15 min in an ice bath. After centrifugation at 1000 rpm for15 min, 250µL of supernatant was collected and added to 500 µL mL of Tris-EDTA buffer (0.4 M HCl, 0.02 M EDTA, pH 9.6) and 12.5 µL of DTNB (0.01 M). After shaking and incubating at 30ºC for 5 min, the absorbance was recorded at 412 nm using spectrophotometer UV.

Catalase (CAT) activity measurement

The Catalase (CAT) activity was assessed using the method described by Aebi (1984).The initiation of the enzymatic reaction was assured by adding 20 µl of the homogenized tissue and the substrate (hydrogen peroxide, H_2O_2) to a concentration of 0.5M in a medium containing 100 mM phosphate buffer at a pH of 7,4. The absorbance levels were measured at 240 nm using spectrophotometer,

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the enzyme activity was expressed as µmol H_2O_2 consumed per minute per milligram of protein.

Glutathione- S-transferase (GST) activity measurement

The activity of Glutathione-S-transferase (GST) was measure according to the method of Habig et al., (1974). The P-nitro benzyl chloride was used as substrate. The absorbance was measured using spectrophotometer UV at 340 nm wavelength at 60 second intervals for 5min.

Hematological evaluation

1mL of blood was collected in a test tube with an anti-coagulant substance (EDTA) for blood enumeration using ERMA INC full automatic blood cell counter model PCE-210N.

Plasma ALT and AST activities estimation

Plasma activities of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were assayed using RANDOX® assay kits following the manufacturer's procedure. ALT and AST activities were assayed by the method of Reltman and Frankel (1957). ALT activity was determined by observing the concentration of pyruvate hydrazone generated with 2,4-dinitrophenylhydrazine at a wavelength of 546 nm. Plasma AST activity was determined by monitoring the concentration of oxaloacetate hydrazone produced with 2,4-dinitrophenylhydrazine at a wavelength of 546 nm.

Plasma total cholesterol and triglycerides evaluation

The measurement of total cholesterol is based on an enzyme method determined by Allain et al. (1974) (Kit Biomaghreb, Algeria, Réf 20211). The intensity of the rose color is proportional to the initial concentration in cholesterol. The dosage of triglycerides is an enzymatic and colorimetric dosage based on the Fossati and Prencipe method (1982) (Kit Biomaghreb, Algeria, Réf 20231). The intensity of the rose color is proportional to the concentration of triglycerides. This coloration is evaluated by the spectrophotometer at the longest range of 505 nm.

Liver histopathological examination

Liver specimens were obtained from rats, and immediately fixed in 10% formalin for 24 hrs. and decalcification was occurred on formic acid then washed in tap water. Serial dilutions of alcohol (absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 hr. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by Hematoxylin & Eosin stain then examination was done through the light microscope (Banchroft et al., 1996).

Statistical analysis

Data were analyzed using one-way ANOVA, the significance of differences was checked using Tukey's test. (*** $p < 0.001$ = very highly significant, ** $p < 0.01$ = highly significant, * $p < 0.05$ = significant)

RESULTS

Physiological parameters

Effect of fenthion and Ephedra on body weight

The body mass control during 30 days of experiment period (Figure 1) indicated that fenthion and *E. alata* treatment induced a significant reduction in the body weight compared to control group.

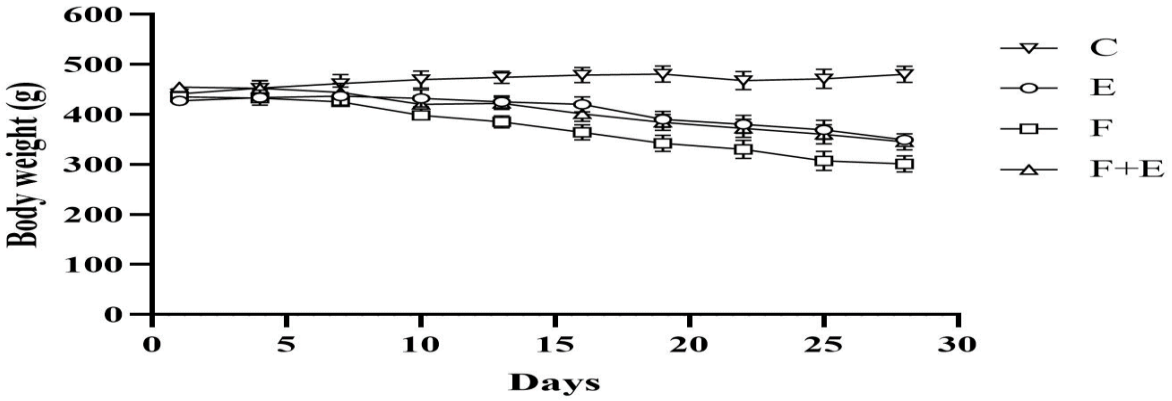

Fig 1. Effect of fenthion and *Ephedra alata alenda* on rat's body weight.

Effect of fenthion and Ephedra treatments on liver weight

The study of the absolute and relative weight of the liver (Table 1), shows a significant increase in fenthion-treated rats as compared to the control group. On the other side, *E. alata* supplementation ameliorated these settings.

Table 1.

Fenthion and *Ephedra alata alenda* treatment effect on absolute and relative liver weight

Parameters/Groups	C	F	E	E+F
Absolute Weight (g)	8.537±0.802	9.388±0.722**	8.580 ± 0.900^{ns}	7.338±0.647***
Relative Weight (g/100g)	1.958±0.221	2.259±0.187**	1.729±0.249^{ns}	1.753±0.165***

(*p < 0.05, **p< 0.01 , *** p< 0.001, ns: No significance)

Hematological parameters

Results in Table 2 revealed that fenthion exposition induced a significant increase in white blood cells (p<0,05), lymphocytes, monocytes and a decrease in eosinophil's, platelets, red blood cells and compared to control group. However, the addition of Ephedra to fenthion group restored some of the mentioned parameter.

Table 2.

Fenthion and *E. alata* treatment effects on Count Blood Cells

Parameters/groups	C	F	E	E+F
WBC10^3/µl	6.060 ±0.973	7.696 ±1.214 **	6.371± 0.725 ^{ns}	7.241±1.030 *
Lym 10^3/µl	7.013±1.027	9.303 ±2.318 *	7.06±1.915^{ns}	7.037±1.114 ^{ns}
Mon (10^3/µl)	0.528±0.052	0.680 ±0.08 **	0.582±0.05^{ns}	0.597± 0.070 ^{ns}
Eos (10^3/µl)	1.795±0.061	0.644 ± 0.342 **	1.60 ± 0.112*	1.016±0.303**
RBC(10^6/µl)	7.615 ±1.25	7.315 ± 1.562^{ns}	7.987 ± 0.917 *	8.137± 0.616 *
Hb (g/dl)	14.17±2.10	13.78 ±2.57 ^{ns}	15.31±2.046*	14.81± 0.775^{ns}
MCV (fl)	52.285± 2.89	54.142± 1.64**	53.87± 1.27ns	52.95±1.9 ^{ns}
MCHC(g/dl)	18.18± 1.93	18.95±1.03 ^{ns}	19.15± 0.72*	18.21± 0.78^{ns}
PLT (10^6/µl)	946.7±294.2	587.28±262.20***	1053±53.67 **	778±248**

(*p < 0.05, **p< 0.01 ,*** p< 0.001, ns: No significance)

Effect of fenthion and Ephedra on liver oxidative stress

Effect on lipid peroxidation

A significant increase in liver malondialdehyde (MDA) levels after oral administration of fenthion comparing with control rats. Interestingly, the treatment with Ephedra extract reduced significantly hepatic MDA elevation compared to rats treated with fenthion. While, no change in this parameter was observed when administrating Ephedra extract alone in comparison to control animals (Figure 2.A).

Effect on glutathione content

The reduced glutathione (GSH) form serves as one of the main organism detoxifiers. In this study we observed a decrease of liver GSH level after fenthion exposition, compared with control (Fig 2.C). However, administration of Ephedra extract to rats exposed to fenthion increased the level of GSH compared to fenthion group.

Effect on antioxidant enzyme activities

Liver antioxidant enzyme revealed that fenthion exposition induced a significant decrease in hepatic CAT and GST activities compared with controls (Figure 2B, 2D). However, administration of *Ephedra* extract to fenthion-treated group had significantly enhanced CAT and GST activities comparing with fenthion treated-group. Furthermore, the administration of *E. alata* extract alone improve the activities of hepatic antioxidant enzymes when compared to the control animals.

Effect of fenthion and *Ephedra alata* on biochemical parameters

Effect on Glucose level

Our findings show that daily treatment with fenthion at a rate of 1mg/kg for one month causes hyperglycemia. The addition of *E.alata decoction* to fenthion treated rats reduced insignificantly this elevation compared to fenthion group.

Effect on liver biomarkers

Liver injuries were observed in rats when they were received fenthion. Indeed, the levels of specific biomarkers related to liver impairments AST and ALT were significantly higher compared to the control group. On the other hand, when *E. alata* extract was administered in fenthion treated group, AST and ALT levels were decreased compared to fenthion-treated group.

Effect on lipid profile

Table 3 depict an important increase in the levels of triglycerides and total cholesterol in rats treated with fenthion. Yet, their levels were restored when *E. alata* was administered to fenthion-treated group. Interestingly, Ephedra decoction supplement were induced hypolipidemia compared to control group.

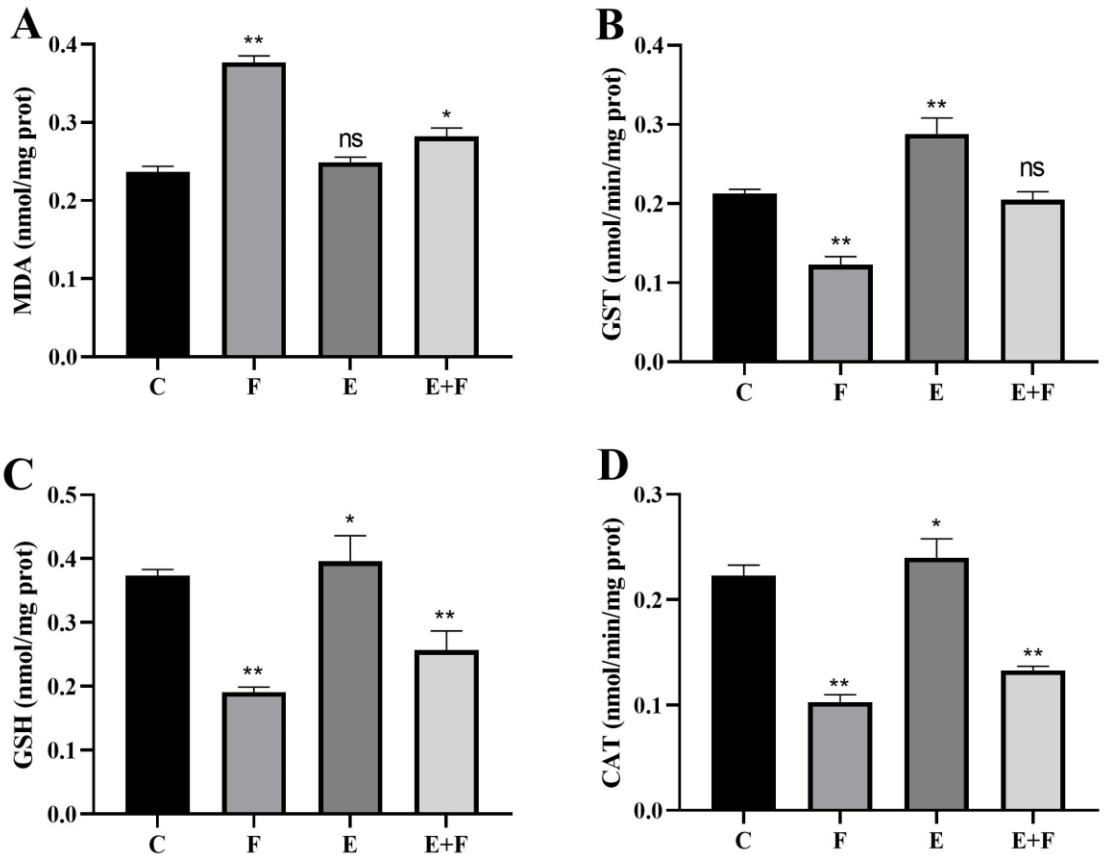

Fig 2. Effect of fenthion and *Ephedra alata alenda* on Lipid peroxidation (A), Glutathione-S-transferase (B), Reduced glutathione (C) and Catalase (D) activities of rat liver.

Table 3.

Fenthion and *Ephedra alata* treatment effects on biochemical parameters

Parameters	C	F	E	F+E
AST(Ul/l)	58,49 ± 7,99	79,13 ± 17,35**	54,28 ± 13,78^{ns}	61,20 ± 6,68*
ALT(Ul/l)	95,89 ± 9,01	137,43 ± 28,24**	84,10 ±7,36 *	101,11 ± 58,46*
Glycemia (g/l)	1,09 ± 0,05	1,24 ± 0,15*	1,00 ± 0,06 ^{ns}	1,12 ± 0,16 ^{ns}
Cholesterol (mg/dl)	0,740 ± 0,079	1,192 ± 0„375**	0,578 ± 0,07**	0,808 ± 0,19*
Triglycerides (g/l)	0.735±0.078	0.854 ± 0.212 **	0.645±0.076**	0.799 ± 0.075*

Liver histopathology assessment

Liver histology sections of control and *E. alata-treated* rats revealed a normal structure, characterized by well-defined hepatic cells; sinusoidal spaces were conserved and the central vein was normal (Figure 3A, 4D).

In contrast, the liver sections of rats treated with fenthion revealed disorganization of hepatocyte architecture; the central vein appeared dilated (CVD) and congested (CVC); inflammation cell infiltration (II); and sinusoidal vacuolization (black arrow) (Figure 3B, 3C). However, *E. alata*, was observed to minimize CVC and CVD but not other damages induced by fenthion (Figure 4E).

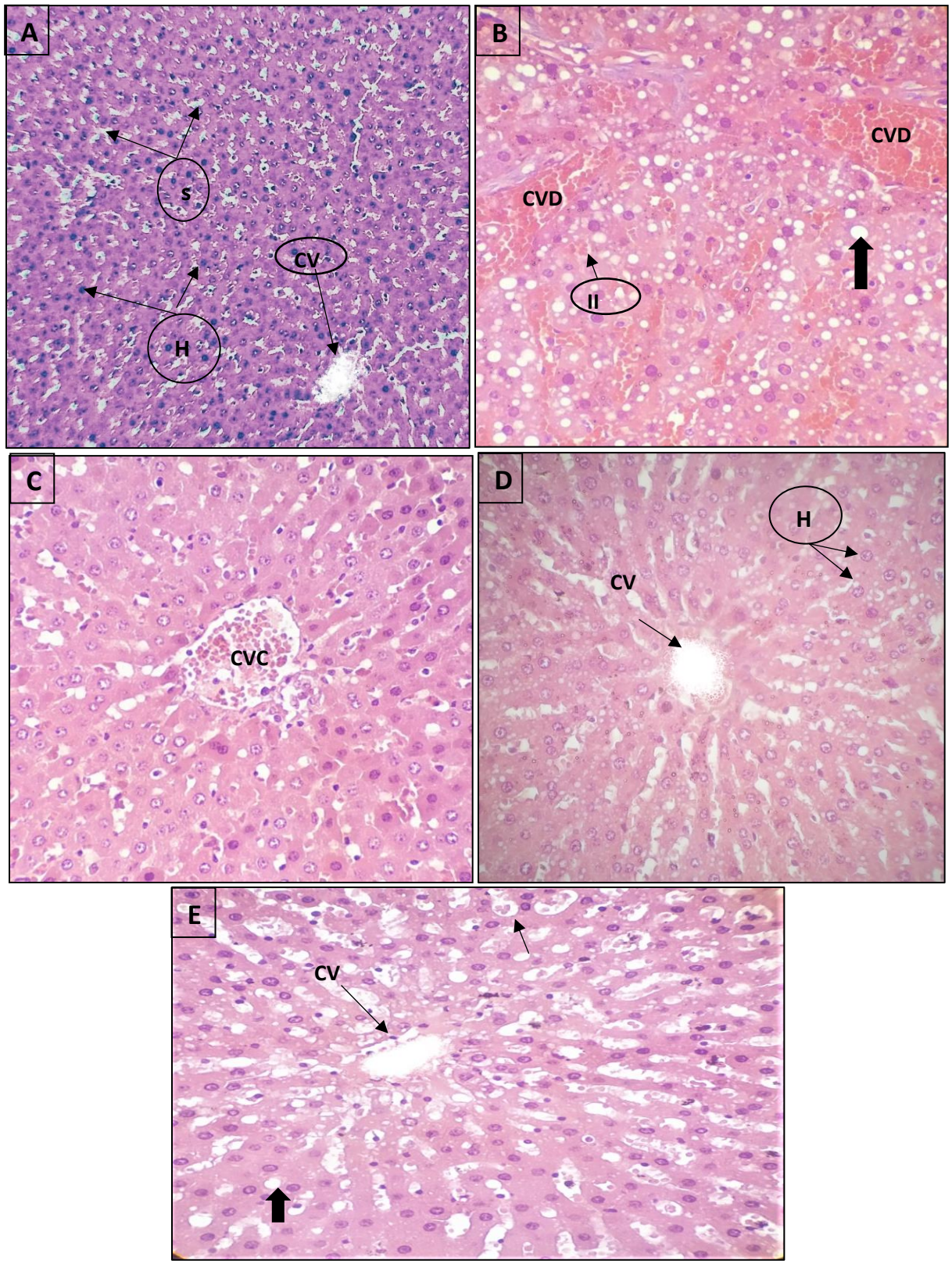

Fig 3. Photomicrographs of liver sections (H&E; Gr×40).(A):Control group: CV, Centrilobular vein; H, Hepatocytes; S, sinusoid, (B, C): Fenthion treated-group; CVC, CVD, and inflammation; (D), Ephedra-group; (E), fenthion-ephedra treated group.

DISCUSSION

This research was conducted to investigate the effect of *Ephedra alata alenda* decoction extract on the reduction of the harmful effects of fenthion's liver toxicity in Wistar rats. To our best knowledge, this research represents the first-time study highlighting the protective properties of *Ephedra alata alenda* in reducing liver damage caused by an organophosphorus compound such

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as fenthion. According to the obtained results, the body weight revealed a significant decrease in rats exposed to fenthion compared with the control group. These results are in agreement with other research (Emara et Draz, 2007; Zhang et al, 2008) which they found a similar impact on animals. Furthermore, the decline in body weight gain serves as an indicator of toxicity and the deterioration of animal wellbeing. The effect of pesticides on the gastrointestinal system might lead to a decrease in food and water intake. Thus, treated rats exhibit appetite loss and/or poor absorption of nutrients (Mairif, 2015). Furthermore, *Ephedra alata alenda* could promote weight loss, according to Boozer et al., (2002). Indeed, Ephedrine, the primary alkaloid in the Ephedra genus and a thermogenic agent, is assumed to be responsible for Ephedra's anti-obesity properties (Shekelle et al, 2003). This study illustrates a significant increase in both relative and absolute weight of the liver compared to the control group. This increase could be attributed to the liver tissue undergoing hypertrophy due to the accumulation of fenthion. In parallel, a reduction was observed in the absolute and relative weight of the liver in the fenthion-Ephedra-treated group; this could be attributed to the *E. alata* effect on lipid metabolism, responsible for lipid oxidation and metabolism activation.

Besides, the current data demonstrated fenthion treatment had induced hyperlipidemia, hyperglycemia, and a significant elevation in plasma transaminases activities (Table 3). These facts are a common sign of liver damage (Rao, 2006), especially in the case of increasing AST and ALT levels (Lasrama et al, 2014).Various enzymes typically found in the cytosol are released into the bloodstream following liver cell membrane disruption. Additionally, our data showed that rats exposed to fenthion and treated with *E. alata* extract had significantly reduced levels of transaminases compared to the control group. The liver biomarker damage was mitigated by the *E. alata* extract, suggesting its potential effect in facilitating the restoration of liver function. Furthermore, Sioud et al., (2021) demonstrated that the daily administration of alkaloids extracted from *E. alata* decreased serum levels of aminotransferase and creatinine in the Cisplatin-induced liver injury. In parallel, Karami-Mohajeri and Abdollahi (2011) found that pesticide exposure can lead to an increase in glucose levels due to two primary factors: decreased ability of target cells to take up glucose and suppression of insulin manufacturing in the Langerhans cells. Additionally, it's known that the lipophilic properties of pesticides allow their metabolites to accumulate in fatty tissues and progressively reach the bloodstream. These substances influence the differentiation of adipocytes and the mitochondrial functioning of Langerhans cells, by contributing to the development of insulin resistance (Azandjeme et al, 2013). In the highlight of this work, a notable increase in triglyceride and total cholesterol levels in fenthion-treated group was observed. According to Yousef et al., (2006), these elevation can be attributed to fenthion's impact on the permeability of the liver cell membrane (Kandil et al, 2006). Additionally, clogged hepatic bile ducts can limit or block the release of cholesterol into the duodenum, contributing to elevated levels of total cholesterol (Zaahkouk et al., 2000).

In the current research, fenthion exposition has induced various adverse effects on rats' blood parameters. This could be potentially attributed to the effect of stress hormones, like catecholamines, which cause neutrophil demargination. This process is frequently associated with lymphocytosis and can result in monocytosis and eosinophilia (Kandil et al., 2006). The rise in lymphocytes and monocytes may also be due to the stimulation of lymphopoiesis and the improvement of lymphocyte release from the lymph of myeloid tissue (Das and Das, 2007). Moreover, a significant decrease in the main inflammatory immune cells, such as white blood cells, monocytes, and lymphocytes, was also prevented by *E. alata* treatment. This may be attributed to the presence of flavonoids, which have both antioxidant and anti-inflammatory properties (Mufti et al., 2023). *E. alata* is rich in various secondary metabolites, including flavonoids, which have the potential to modulate the expression of genes coding for antioxidant and inflammatory molecules, either by enhancing or reducing their activity (Al-Rimawi et al., 2017). Also, Ephedra contains substances such as ephedrine and pseudoephedrine that have potent anti-inflammatory characteristics and are able to inhibit the synthesis of prostaglandin E2 (Kasahare et al., 1985).

On the other hand, oxidative stress represents another mechanism through which pesticides induce cellular damage and alterations in metabolic functions (Sioud et al., 2021).The mechanism by which fenthion and generally OP may cause oxidative stress has been demonstrated in recent study (Sharma et al, 2005). Othman and Abdel Hamid (2017) were established the relation between the increase of lipid peroxidation and the mechanism of pesticide toxicity observed in animals liver and brain . Importantly, our results demonstrated that oral fenthion administration increase MDA levels, which is in agreement with the research of Selmi et al, (2018). Also, our data demonstrated that fenthion exposure significantly reduced the activities of liver GSH, GST, and CAT (Figure 2), fenthion has been found to reduce the activity of antioxidant enzymes either directly or by generating free radicals that cause oxidative stress (Altuntas and Delibas, 2002; Ben Amara et al., 2014). Additionally, numerous experimental investigations have reported the therapeutic effects of the plant's extract, which is used in conventional medicine. Hence, it can help to hepatotoxicity medication and maintain a oxidant/antioxidant balance. Our findings support the previous research, in which the following study demonstrated that *E. alata alenda* extract induced an increase in the activity of the antioxidant enzymes GSH, CAT, and GST in rats (Bougrou et al., 2020; Djahara et al., 2023). Recent studies revealed that *Ephedra alata* and

its related flavonoids exert their activity by eliminating hydroxyl, superoxide, lipid peroxides (Al-Rimawi et al., 2017; Mighri et al., 2019) singlet oxygen, and peroxynitrite through a scavenging process (Sioud et al., 2021; Zhu et al., 2020) Furthermore, liver histology examination of rats treated with fenthion revealed a disorganization of hepatic architecture associated with an enlarged and clogged centrilobular vein. Besides, inflammatory and degenerative cells were observed in specific areas. These results are well supported by the study of Fidan et al. (2007), which showed that fenthion injection induced hepatocyte degradation, inflammatory cell infiltration and portal vein congestion. In our investigation, *E. alata* treatment of rats attenuated damages induced by fenthion.

CONCLUSION

This study has affirmed the hepatoprotective potential of Algerian *Ephedra alata subs alenda* (Stapf)Trab decoction extract, which modulate the side's effects of fenthion pesticide on oxidative stress, biochemical and hematological parameters, and mitigate liver damages. Ephedra extracts can serve as a potential candidate for pharmaceutical industry.

AUTHORS CONTRIBUTIONS

Conceptualization, ID and KG; methodology, ID, KD and FB; formal analysis and investigation, HT, AM and NA; writing original draft preparation, HS and SB; conceptualization, ID, KG and AM; writing review and editing, MA, KG and SG. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have not any competing financial, professional, or personal interests from other parties.

REFERENCES

Abdou, HM., Hussien, M., &Yousef MI. (2012). Deleterious effects of cypermethrin on rat liver and kidney: protective role of sesame oil. J Environ Sci Health B,47(4),306–314. https://doi.org/10.1080/03601234.2012.640913

Aebi, H. (1984). Catalase in vitro. Methods Enzymol,105,121-6. https://doi.org/10.1016/s0076-6879(84)05016-3.

Allain, CC., Poon, LS., Chan, CSG., Richmond, W., Fu, PC. (1974). Enzymatic Determination of Total Serum Cholesterol. Clinical Chemistry, 20(4):470-475.

Al-Rimawi, F., Abu-Lafi, S., Abbadi, J., Alamarneh, AA., Sawahreh, RA., & Odeh, I. (2017). Analysis of phenolic and flavonoids of wild ephedra alata plant extracts by lc/pda and lc/ms and their antioxidant activity. Afr J Tradit Complement Altern Med, 14(2),130–141. https://doi.org/10.21010/ajtcam.v14i2.14 Anal. 91.

Altuntas, R., & Delibas, N. (2002). The Effects of Fenthion on Lipid Peroxidation and Some Liver Enzymes: The Possible Protective Role of Vitamins E and C. Turk J Med Sci,32 (4)2,1–1.

Azandjeme, CS., Bouchard, M., Fayomi, B., Djrolo, F., Houinato, D., & Delisle, H. (2013). Growing burden of diabetes in sub-saharan Africa: contribution of pesticides? Curr. Diabetes Rev,9(6),437–49. https://doi.org/10.2174/15733998113099990078.

Ben Amara, I., Sefi, M., Troudi, A., Soudani, N., Boudawara, T., & Zeghal, N. (2014). Fenthion an organophosphorus pesticide, induces alterations in oxidant/antioxidant status and histopahtological disorders in cerebrum and cerebellum of suckling rats. Indian J Biochem Biophys, 51,293–301.

Banchroft, J.D., Stevens A., and Turner D.R. (1996). Theory and practice of histological techniques. Fourth ed. Churchil Livingstone, New York.

Ben Lamine, J., Boujbiha, MA., Dahane, S., Cherifa, AB., Khlifi, A., Chahdoura, H., Yakoubi, MT., Ferchichi, S., El Ayeb, N., & Achour L. (2019). α-Amylase and α-glucosidase inhibitor effects and pancreatic response to diabetes mellitus on Wistar rats of Ephedra alata areal part decoction with immunohistochemical analyses. Environ Sci Pollut Res Int,26,9739–9754. https://doi.org/10.1007/s11356-019-04339-3.

Boozer, CN., Daly, PA., Homel, P., Solomon, JL., Blanchard, D., Nasser, JA., Strauss, R., & Meredith, T. (2002). Herbal ephedra/caffeine for weight loss: a 6-month randomized safety and efficacy trial. Int J Obes Relat Metab Disord,26(5),593–604. https://doi.org/10.1038/sj.ijo.0802023

Bourgou, S., Ezzine, Y., Ben Mansour, R., Dakhlaoui, S., Selmi, S., Bachkouel, S., Msaada, K., Aidi-Wannes, W., Hiroko, I., & Megdiche-Ksouri, W. (2020). Preliminary phytochemical analysis, antioxidant, anti-inflammatory and anticancer activities of two Tunisian Ephedra species: Ephedra alata and Ephedra fragilis. South Afric J Botany,135,1–8. https://doi.org/10.1016/j.sajb.2020.09.033.

Chouitah, O. (2019). The essential oil of Algerian Ephedra alata subsp. alenda and its antimicrobial properties. J New Biol Rep, 8(3),190–193.

Danciu, C., Muntean, D., Alexa, E., Farcas, C., Oprean, C., Zupko, I., Bor, A., Minda, D., Proks, M., & Buda, V. (2018). Phytochemical Characterization and Evaluation of the Antimicrobial, Antiproliferative and Pro-Apoptotic Potential of Ephedra alata Decne. Hydroalcoholic Extract against the MCF-7 Breast Cancer Cell Line. Molecules, 24,13. https://doi.org/10.3390/molecules24010013

Das, S., & Das, DK. (2007). Anti-inflammatory responses of resveratrol. Inflamm. Allergy. Drug Targets,

6,168-173. https://doi.org/10.2174/187152807781696464

Djahra, AB., Zoubiri, F., Benkaddour, M., & Gouasmia, S. (2023). Antioxidant and Hepatoprotective Activity of Ephedra alata Extracts against Intoxication with Deltamethrin Pesticide in Male Rats. Pharmacophore,14(1):19-24. https://doi.org/10.51847/JvqlbdZpR6

Draper, HH., & Hadley, M. (1990). Methods Enzymol,86,421–431. https://doi.org/10.1016/0076-6879(90)86135-i.

El-Bini, D., Lasram, MM., Annabi, A., Gharbi, N., & Ei-Fazaa, SA. (2015). Comparative study on toxicity induced by carbosulfan and malathion in Wistar rat liver and spleen. Pestic. Biochem. Physiol,124,21–28. https://doi.org/10.1016/j.pestbp

Ellman,GL.(1959).Tissue sulfhydryl groups.ArchBiochem Biophys, 82(1),707. https://doi.org/10.1016/0003-9861(59)90090-6.

Emara, AM., & Draz, EI. (2007). Immunotoxicological study of one of the most common overthe-counter pyrethroid insecticide products in Egypt. Inhalation Toxicology,19(12),997–1009. https://doi.org/10.1080/08958370701533483.

Fidan, H., Sahin, O., Ela, Y., Kilbas, A., Bas, O., Yavuz, Y., Sahin, DA., & Altuntas, I. (2007). Influence of different atropine therapy strategies on fenthion-induced organ dysfunction in rats. Basic Clin Pharmacol Toxicol, 100(5), 308–15. https://doi.org/10.1111/j.1742-7843.2007.00047.x

Fossati, P., & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry, 28(10):2077-2080

Furnes, B., Schlenk, D. (2005). Extrahepatic metabolism of carbamate and organophosphate thioether compounds by the flavin-containing monooxygenase and cytochrome P450 systems. Drug Metab Dispos,33(2),214-8. https://doi.org/10.1124/dmd.104.000984.

Habig, WH., Pabst, MJ., & Jakoby, WB. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J Biol Chem, 249,7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8 Kandil, MA., EL-Kashoury, AA., EL-Said, MM., & EL-Herrawy, MA. (2006). Intractive effects of imidacloprid, profenofos and carbosulfan at low concentrations on homeostasis and haematological indices in male albino rats. J Egypt Soc Toxicol, 35, 69–78.

Karami-Mohajeri, S., & Abdollahi M.(2011). Toxic influence of organophosphate, carbamate and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: a systematic review Hum Exp Toxicol, 30(9),1119–1140. https://doi.org/10.1177/0960327110388959

Kasahara, Y., Hikino, H., Tsurufuji, S., Watanabe, M., & Ohuchi, K. (1985). Anti-inflammatory actions of ephedrines in acute inflammations. J Planta Med, 51,325–331. https://doi.org/10.1055/s-2007-969503.

Krieger, RI. (2010). Handbook of pesticide toxicology. New York (NY): Academic Press

Lasrama, MM., Bini Dhouiba, C., Bouzidb, K., Jrad Laminea, A., Annabia, A., Belhadj, NH., Ben Ahmed, M., El Fazaaa, S., Abdelmoulab, J., & Gharbi, N. (2014). Association of inflammatory response and oxidative injury in the pathogenesis of liversteatosis and insulin resistance following subchronic exposure to malathion in rats. Environ. Toxicol Pharmacol, 38,542–553. https://doi.org/10.1016/j.etap.2014.08.007

Mairif, S. (2015). Contribution to the study of the toxic effect of pesticides for domestic use used in Algeria. Thesis Doctoral. Guelma University, 82–95

Mighri, H., Akrout, A., Bennour, N., Eljeni, H., Zammouri, T., & Neffati, M. (2019). LC/MS method development for the determination of the phenolic compounds of Tunisian Ephedra alata hydro-methanolic extract and its fractions and evaluation of their antioxidant activities. South African Journal of Botany,124,102–110. https://doi.org/10.1016/j.sajb.2019.04.029

Mufti, A., del Mar Contreras, M., Gómez-Cruz, I., Alshamrani, A., Nahdi, S., Mansour, L., Alwasel, S., Harrath, AH., & Tlili, N. (2023). Ephedra alata Subsp. Alenda as a Novel Source of Bioactive Phytochemicals: Characterization Based on the Mass Spectrometry and Profiling of Antioxidant and Anti-Inflammatory Properties.Life,13,323. https://doi.org/10.3390/life13020323

Muralidharan, L. (2014). Chronic toxic impacts of fenthion on the profiles of enzymes in the freshwater fish Cyprinus carpio (Linn). Int J Fish Aquat Stud,1(4),51–56.

Othmann, AI., & Abdel-Hamid, M. (2017). Curcumin mitigates fenthion-induced testicular toxicity in rats: histopathological andimmunohistochemical study. African Zoology,52(4),209–215. https://doi.org/10.1080/15627020

Ramachandraih, CT., Subramanyam, N., Bar, KJ., Baker, G., & Yeragani, VK. (2011). Antidepressants: From MAOIs to SSRIs and more. Indian J Psychiatry,53,180–182. https://doi.org/10.4103/0019-5545.82567

Rao, JV. (2006). Biochemical alterations in euryhaline fish, Oreochromis mossambicus exposed to sub-lethal concentrations of an organophosphorus insecticide, monocrotophos. Chemosphere, 65(10),1814–1820. https://doi.org/10.1016/j.chemosphere

Reltman, S., Frankel, S.A. (1957). Colorimetric method for the determination of serum ALT and AST. *Am. J. Clin. Pathol*, *28*, 56–63

Saidi, SA., Al-Shaikh, TMA., Othman, A., & Hamden, K. (2022).Ephedra alata subsp alenda (Ephedraceae) leaf extracts: phytochemical screening, anti-diabetic, anti-obesity and anti-toxic activities on diabetic-induced liver-kidney-testes toxicities and inhibition of α-amylaseand lipase enzymes.Heliyon,8, https://doi.org/10.1016/j.heliyon.2022.e11954

Selmi, S., Rtibi, K., Grami, D., Sebai, H., & Marzouki, L. (2018). Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in mice prepubertal male. Toxicology Reports, 5,189–195

Sharma, YS., Bashir, M., Irshad, TC, Nag, TD., & Dogra, T. (2005). Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. Toxicology, 215,173-181. https://doi.org/10.1016/j.tox.2005.06.029

Shekelle, PG., Hardy, ML., Morton, SC., Maglione, M., Mojica, WA., Suttorp, MJ., Rhodes, SL., Jungvig, L., & Gagné J. (2003). Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis. JAMA, 26, 289(12):1537-45. https//doi.org/ 10.1001/jama.289.12.1537.

Shi, C., & Li, J. (2021). Ephedrine attenuates cerebral ischemia/reperfusion injury in rats through NF-B signaling pathway. Hum Exp Toxicol, 40,994–1002. https://doi.org/10.1177/0960327120975456

Sioud, F., Mangelinck, S., Lahmer, A., Bonneure, E., Chaabene, F., & Chekir, GL. (2021). Alkaloids Isolated from Ephedra Alata: Characterization and Protective Effects Against Cisplatin-Induced Liver and Kidney Injuries in Mice. J Sci Tech Res,36(3),28591-28602. https://doi.org/10.26717/BJSTR.2021.36.005861

Sun, S., Hu, R., Zhang, C., & Shi, G. (2019). Do farmers misuse pesticides in crop production in China? Evidence from a farm household survey. Pest Manag. Sci, 75,2133–2141. https://doi.org/ 10.1002/ps.5332.

Yousef, MI., Awad, TI., & Mohamed, EH. (2006). Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitaminE.Toxicology;2006;227:240–247.https://doi.org/ 10.1016/j.tox.2006.08.008

Zaahkouk, SAM., Helal, EGE., Abd-Rabo, TEI., & Rashed, SZA. (2000). Carbamate Toxicity and Protective effect of vitamin A and Vitamin E on some biochemical aspects of male albino rats. The Egyptian Journal of Hospital Medicine, 1, 60–77

Zhang, J., Zhu, W., Zheng, Y., Yang, J., Zhu, X. (2008). The antiandrogenic activity of pyrethroid pesticides cyfluthrin and beta-cyfluthrin. Reprod Toxicol, 25(4),491–6. https://doi.org/10.1016/j.reprotox.2008.05.054.

Zhu, B., Conlan, X., Cao, J., Meng, L., Zheng, K, Yang, D., & Yang, W. (2020). Case studies on illegal production of ephedrine/pseudoephedrine within Fujian China. Forensic Sci Int,312,110326. https://doi.org/10.1016/j.forsciint.2020.110326