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ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *EPHEDRA ALATA* EXTRACTS AGAINST INTOXICATION WITH DELTAMETHRIN PESTICIDE IN MALE RATS

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ABSTRACT

The objective of this study is to evaluate the antioxidant and hepatoprotective activity of *Ephedra alata alenda* plant, against intoxication with Deltamethrin pesticide in rats. After preparation of plant extracts (macerated and decocted, powder), chemical profile and total phenolic compounds of plant were estimated. Furthermore, antioxidant activities In vitro were evaluated using the DPPH test, and to verify the antitoxic activity In vivo, male rats were exposed to Delamethrin (3.5 µl/kg/day) for 30 days and then treated for 14 days with plant preparations. The fourth group of rats is the positive control group exposed just to Delthmethrin, while the fifth group is the negative control group. 44 days later, rats were sacrificed, Blood samples were collected for serum biochemical estimation, and liver fragments were reserved to assess antioxidant activity. Results of plant extracts showed the presentation of many natural substances in two extracts. The concentration of total phenolic compounds was estimated at 13.72 mg EAG/g, 13.45 mg EAG/g for macerated and decocted extracts respectively. DPPH test was estimated at IC_{50}=0.901 mg/ml and IC_{50}=1.141 mg/ml for macerated and decocted extracts, respectively. Antioxidant activity results In vivo showed significant differences in blood biochemical such as ALT, ALP, Bilirubin and Urea, oxidative stress parameters (GSH), (GST), and lipid peroxidation (MDA), noting the reforms in the sections of some liver tissues that demonstrated the effectiveness of plant extract compared and its absence completely in the tissue of the liver treated with the powder. Finally, the liver tissue sections was confirmed with biochemical results.

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Introduction

In the world, pesticides are ranked as the third most significant source of pollution [1]. These pesticides pose a real public health problem, not only for most exposed users, but for the general population. However, numerous pesticides and other toxic compounds are found in our bodies due to the water, air, and food we consume [2]. Deltamethrin, is one of the most widely used pesticide molecules in agriculture in Algeria for its broad spectrum against insects activity and it belongs to the pyrethroid family. In Algeria, Deltamethrin registered in 2009 under the registration number R. 02. 144. 116, was marketed under the name Decis expert product from Bayer laboratories [3].

In general, the uses of medicinal plants to cure by humans, and Africans in particular, have existed for thousands of years. The development of modern medicine has been strongly influenced by medicinal plants, which have played a significant role in the history of medicine. Additionally, disturbances caused by phytosanitary products are treated with conventional medicine, also known as phytotherapy. It's the oldest way of healing in the world and found in all civilizations [4]. In fact, there are approximately 500,000 species of plants on earth, 80,000 of which have medicinal properties through many active substances [5]. Among those plants is *Ephedra alata alenda*, which is used to cure various ills such as fever, asthma, and acute bouts of rum or flu and relieves rheumatism [6].

Ephedra alata alenda Known and used in China for more than five thousand years under the name of Ma-Huang, it is very stimulating and holds an important place in the herbalist tradition of several countries. It is widely used and well-researched

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in the Saharan regions of southern Algeria, where its anticancer effects have been demonstrated in numerous studies. This study aims to evaluate the antioxidant and hepatoprotective activity *Ephedra alata alenda* plant aerial part (medicinal plant growth in desert of El Oued state-Algeria) against intoxication with Deltamethrin pesticide in rats [7-10].

Materials and Methods

Chemicals

All chemicals were analytical grade and were obtained from Sigma Chemicals Co., USA. Commercial kit from Spinreact, Spain, were used for biochemical parameters determinations.

Preparation of Plant Extract

10 g of plant were mixed with 100 ml distilled water for macerated extract, and 100 ml of boiled water for decocted extract, extracts kept at room temperature in the dark for 24 h. The distilled water was removed from two extracts with a rotary evaporator. After being, the extracts were kept in a refrigerator at 4°C. Extracts of *Ephedra alata alenda* percentage yield were 13% and 16% w/w of initial raw material for macerated and decocted extracts, respectively.

Phytochemical Analysis

Qualitative analysis were done to find the presence of the bioactive substances, phytochemical analysis of the plant extracts was evaluated using standard procedures [11, 12].

Determination of Total Phenolic Content

Total phenolic content were determined according to method of Singleton & Rossi [13], with the use of the Folin–Ciocalteu reagent.

DPPH Free Radical Scavenging Assay

DPPH is free radicals used to determine the antioxidant activity of plant extracts. A radical scavenging assay was conducted as described by Benhammou *et al*. [14].

Antitoxic Activity

The Animals were divided into five groups of six animals per group. For first 30 days of experiment, the animals were treated with Deltamethrin, than; for 14 days, animals were treated with Deltamethrin and followed by different preparations of plant: macerated extract and decocted extract (orally with the help of gastric canula), for powder plant is mixed with food. Groups treated as follow: Group I: Normal Control (rats treated with 1ml/day of normal saline). Group II: Deltamethrin (3.5 µl/kg /day) in water. Group III: Deltamethrin (3.5 µl/ kg/day) in water + macerated extract (30 mg/kg/day). Group IV: Deltamethrin (3.5 µl/kg/day) in water + decocted extract (30 mg/kg/day). Group V: Deltamethrin (3.5 µl/kg/day) in water + powder plant (0.1875 g/kg/day). Body weight was recorded periodically during the experiment weeks.

Determination of Serum Biochemical and Liver Homogenate Parameters

The AST, ALT, ALP, GGT, Bilirubin, Creatinine, Urea, Cholesterol, Triglycerides, and Albumin levels were determined by using the automatic analyzer. Total proteins in liver homogenates were determined using Bradford method (1976) [15]. Glutathione (GSH) content was estimated by the method of Weckberker and Cory [16]. GST activity was measured by the method of Habig *et al*. (1974), and Malondialdehyde (MDA) contents were determined using the methods published by Yagi, (1976) [17, 18].

Histopathological Study

After the sacrificed rats, the liver parts tissues was removed and preserved in formaldehyde (10%) for fixation until time to prepare the slices. Then, it was dehydrated in a series of ethanol (with deferent concentrations), after cleaning liver parts with toluene, it were immersed in paraffin to prepare paraffin blocks. By using of Leica Rotary Microtome (Wetzlar, Germany), 2~4 Mm Sections were prepared from paraffin blocks, and colored with hematoxylin and eosin after being dehydrated in an escalating graded series of ethanol. At the same time, a light microscope was used to evaluate histopathology.

Statistical Analysis

Data was expressed as mean ± standard deviation (M ± SD) of six animals. Statistical analysis was carried out using Student T-test to compare means between two groups. Results were evaluated using the Minitab and EXCEL software.

Results and Discussion

Phytochemical Analysis

The results of the chemical profile were represented in **Table 1**.

Table 1. Chemical profile of *Ephedra alata alenda* aerial parts extracts.

Bioactive substances	Alkaloids	Tannins	Flavonoids	Terpenoids	Saponins	Steroids
Macerated extract	(+)	(+)	(+)	(+)	(+)	(-)
Decocted extract	(+)	(+)	(+)	(+)	(+)	(-)

(-) Absence of bioactive substances (+) Presence of bioactive substances

Total Phenolic Content

Results of phenolic content are expressed in mg Gallic acid equivalent per mg extract (mg GAE/mg extract). The calibration curve is established with a correlation y = 0.0009x + 0.001 and coefficient R2 = 0.9926. The results were represented in **Table 2**.

Table 2. Total phenolic content of *Ephedra alata alenda* aerial parts extracts.

Total phenolic content (mg GAE/mg extract)	
Macerated extract	Decocted extract
13.72 ± 0.02	13.42 ± 0.06

DPPH Free Radical Scavenging Assay

The results of antioxidant activity were summarized in **Table 3**, and the DPPH assay of plant extracts was compared with standard (Ascorbic acid).

Table 3. Antioxidant activity of *Ephedra alata alenda* aerial parts extracts.

	Macerated extract	**Decocted extract**	**Ascorbic acid**
IC_{50} (mg/ml)	0.901	1.141	0.347

Weight Body and Relative Liver

The results of Body weight, body weight gain, and relative liver weight in control and experimental groups were summarized in **Table 4**.

Table 4. Body weight, body weight gain, and relative liver weight in control and experimental groups.

Groups / **Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
Body weight (g)	244.83±15.40	267.33±31.23	264.00±16.84	258.5±22.06	269.33±20.41
Body weight gain (g/day)	0.424±0.7	0.045±0.063**	0.193±0.090 *	0.159±0.115**[a]	0.53±0.13***[b]
Relative liver weight (g/100 g bw)	3.359±0.039	3.941±0.1**	3.084±0.046[a]	3.168±0.070*	2.908±0.11**[a]

Results are presented as the mean ± S.E. (Standard Error) (n = 6). (*/a) p≤0.05, (**/b)p≤0.01 and (***/c)p≤0.001. (NS)p > 0.05 as compared with the normal control group. (a,b,c) p < 0.05 as compared with the Deltametrine model group.

Serum Biochemical and Liver Homogenate Parameters

Levels of serum biochemical parameters and liver homogenate parameters in all groups of rats intoxicated by Deltametrine and then treated with *Ephedra alata alenda* extracts were presented in **Tables 5 and 6**.

Table 5. Effects of *Ephedra alata alenda* extracts on levels of serum biochemical parameters.

Groups / **Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
AST (U/L)	75,572±3,978	84,572±1,978**	74,548±2,049	71,506±4,641[b]	59,266±3,349[c]
ALT (U/L)	132,944±6,624	161,272±5,137**	142,472±8,704[a]	144,096±6,087[a]	145,118±5,349[a]
ALP (U/L)	223,104±1,820	75,184±2,876**	210,6±2,436[c]	123,704±2,983[a]	51,778±2,904[Ns]
GGT (U/L)	14,324±3,349	12,9416±2,439[Nc]	11,184±2,550[Nc]	11,806±4,317[Nc]	12,216±2,321[Nc]
Bilirubin (mg/L)	6,472±0,657	32,508±0,268**	4,034±0,290[b]	5,018±0,183[b]	4,124±0,457[b]
Creatinine mg/L	7,318±2,843	7,896±1,548[Ns]	8,014±3,332[Ns]	9,704±1,274[b]	9,358±1,120[b]
Urea (g/L)	0.19±0.043	0.29±0.022***	0.25±0.051[a]	0.28±0.076[Ns]	0.37±0.034[b]
Cholesterol g/L	0,884±0,238	0,750±0,297[Ns]	0,926±0,152[a]	0,716±0,102[Ns]	0,784±0,180[Ns]
Triglyceride g/L	0.88±0.01	0.91±0.008[Ns]	0.62±0.019[a]	0.55±0.015[b]	0.47±0.023[c]
Albumin (mg/L)	31,918±4,822	27,596±1,492[Ns]	28,214±5,682[Ns]	29,304±4,174[Ns]	28,828±3,133[Ns]

Results are presented as the mean ± S.E. (Standard Error) (n = 6). (*/a) p≤0.05, (**/b) p≤0.01 and (***/c)p≤0.001. (NS)p > 0.05 as compared with a normal control group. (a,b,c) p < 0.05 as compared with the Deltametrine model group.

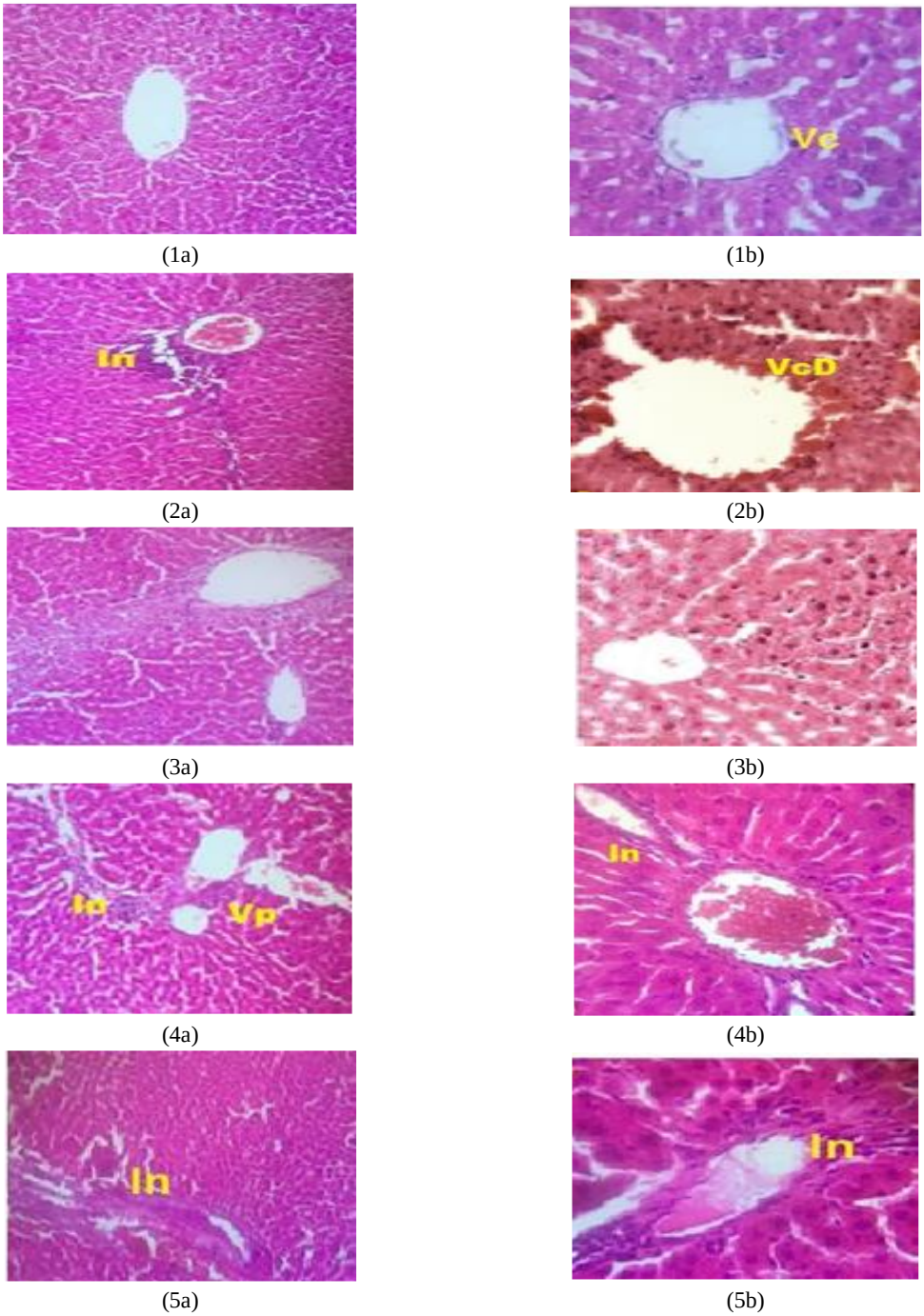
Table 6. Effects of *Ephedra alata alenda* extracts on levels of liver homogenate parameters.

Groups / **Parameters**	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
GSH (U/L)	0,413±3,978	0,273±1,978***	0,375±2,049[b]	0,354±4,641[b]	0,236±3,349[c]
GST (U/L)	4,414±3,624	1,972±2,137***	3,272±3,704[b]	2,796±2,087[a]	1,718±3,349[a]
MDA	0,624±1,519	1,314±2,951**	0,876±3,753[b]	1,214±1,654[a]	1,343±3,721[a]

Results are presented as the mean ± S.E. (Standard Error) (n = 6). (*/a) p≤0.05, (**/b) p≤0.01 and (***/c)p≤0.001. (NS)p > 0.05 as compared with the normal control group. (a,b,c) p < 0.05 as compared with the Deltametrine model group.

Histopathological Observation

Liver tissues in group 1 demonstrated the standard architecture of hepatic cells with typical central and portal area in the control and Ah groups **(Figure 1)**. In contrast, Deltametrine pesticide intoxicated rats (Group 2) revealed pathological changes manifested by congestion and dilatation of hepatic vasculature, inflammatory cell infiltrations, and clarified dystrophies. Whereas, for group (3,4,5), were contaminated with Deltametrine and treated by deferent preparation of *Ephedra alata alenda*, showed moderate liver damage especially group 3.



(1a) (1b) (2a) (2b) (3a) (3b) (4a) (4b) (5a) (5b)

Figure 1. Liver section from the control group and other experimental groups: (1a,2a,3a,4a,5a x10) and (1b,2b,3b,4b,5b x40). 1a and 1b: Group 1, 2a and 2b: Group 2, 3a and 3b: Group 3, 4a and 4b: Group 4, 5a and 5b: Group 5, In: Inflammation, Vc: Central Vein, Vp: Portal Vein. VcD : Central Vein Dilatation

Result of Chemical profile for *Ephedra alata alenda* extracts has shown the presence of several bioactive substances, such as: Flavonoids, Alkaloids, Tannins, absence of Sterols. These findings conform to the literature [19]. Total Polyphenols were estimated at 13.72 mg EAG/g in macerated extract and 13.42 mg EAG/g in decocted extract. An essential content (30.53 mg EAG/g) compared to ours was advanced by Kmail and these collaborators (2017) [20]. This variability in results could be related to the climatic conditions of the species' or to the different methods used during extraction [21].

Results of antioxidant activity by DPPH free radical scavenging method indicate that macerated extract activity (IC_{50} (mg/ml):

0.901) is higher than decocted extract (IC_{50} (mg/ml): 1.141). In a similar study on ethanol extract of *Ephedra procera*, the authors reported an IC_{50} equal to 0.056 mg/ml, deficient levels of IC_{50} indicating significant antioxidant activity [22].

Results of intoxication with Deltamethrin pesticide in rats show that administration orally of Deltamethrin (3.5 µl/ kg/day) causes disturbances in the serum biochemical and liver homogenate parameters of animals such as AST, ALT, ALP, Bilirubin and Urea. In fact, there were decreases in rats intoxicated with xenobiotics compared to controls. The protective effect of *Ephedra alata alenda* preparations is reflected by the decrease in these parameters levels in plant-treated groups compared with other exposed to Deltamethrin [23]. An increase in the lipid peroxidation marker MDA in the liver in the Deltamethrin group compared to controls, this indicates a decrease in antioxidants which play a critical role in inhibiting lipid peroxidation [24]. Our results show that hepatic GSH and GST activity decreased in rats exposed to Deltamethrin and rats who consumed the plant in powder. Cells can defend face several detoxification systems; the most important is that of glutathione which is a tripeptide playing a role at various levels in the fight against oxidative stress [25]. This enzyme system also contains glutathione-S-transferase GST that catalyzes the reaction between reduced glutathione and xenobiotic, forming glutathione-conjugated metabolites [26].

The study involving histopathology provided evidence to support biochemical analyses and oxidative stress. Our biochemical observations, which revealed an elevated amount of lipid peroxidation, are consistent with this hepatic injury. Recent researchers have noticed similar changes in the hepatic tissues under the toxic xenobiotic effect. Therefore, all abnormalities appearing in liver tissues are attributed to oxidative stress, which is the primary mechanism behind all these degenerations [27-29]. In contract, treatment with deferent preparations, in particular, *Ephedra alata alenda* macerated extract, give a significant recovery in tissues, which can be attributed to bioactive substances in plant with a high concentration of polyphenols, which are responsible for its antioxidant activity and tissues-protective effects [30].

Conclusion

This study has again affirmed the protective potential of natural compounds, particularly *Ephedra alata alenda* plant preparations, which have a more significant hepatoprotective effect on alteration of biochemical parameters resulting from oxidative damages after intoxication by Deltametrine pesticide.

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