ORIGINAL ARTICLE

PHYTOCHEMICAL PROFILE, ANTIOXIDANT, ANALGESIC AND HYPOLIPIDAEMIC EFFECTS OF *EPHEDRA ALATA* DECNE. FEMALE CONES EXTRACT

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Abstract

The aim of this study is the estimation of the antioxidant, analgesic and hypolipidemic effects and the chromatographic analysis of the extract of female cones of *Ephedra alata* which is growing in South East Algeria. The extract of *E. alata* showed better inhibition of DPPH• radical, the highest reductive activity and gave the best protection of erythrocytes. The HPLC analyses has identified eight phenolic compounds: gallic acid, chlorogenic acid, vanillic acid, vanillin, p-coumaric acid, rutin, naringenin and quercetin. The results of the protective effect of hyperlipidaemia caused by Triton X-100 showed that there are noticeable changes in the biochemical and lipid parameters, also for the parameters of oxidative stress in the group exposed to Triton X-100. Also, the treatment with the methanol extract of *E. alata* yielded similar results to the control batches by normalizing and decreasing the biochemical, stress and lipid parameters. The histological study confirmed the biochemical results. According to the results of the analgesic activity, the extract of *E. alata* induced a significant decrease in abdominal writhings compared to the control group and the values obtained are very close to those obtained with indomethacin.

Rezumat

Scopul acestui studiu a fost evaluarea efectelor antioxidante, analgezice, hipolipemiante și analiza cromatografică a extractului de conuri femele de *Ephedra alata*, din sud-estul Algeriei. Extractul de *E. alata* a arătat o inhibiție mai bună a radicalului DPPH •, cea mai ridicată activitate reducătoare și a oferit cea mai bună protecție asupra eritrocitelor. Analizele HPLC au identificat opt compuși fenolici: acid galic, acid clorogenic, acid vanilic, vanilină, acid p-cumaric, rutină, naringenină și quercetină. Rezultatele efectului protector asupra hiperlipemiei cauzate de Triton X-100 au arătat că există modificări vizibile ale parametrilor biochimici și lipidici, de asemenea, pentru parametrii stresului oxidativ din grupul expus la Triton X-100. De asemenea, tratamentul cu extract metanolic de *E. alata* a scăzut parametrii biochimici, stresul oxidativ și parametrii lipidici. Studiul histologic a confirmat rezultatele biochimice. Acțiunea analgezică a extractului de *E. alata* a indus o scădere semnificativă a contorsiunilor comparativ cu grupul martor și valorile obținute sunt foarte apropiate de cele obținute în cazul administrării de indometacin.

Keywords: *Ephedra alata*, HPLC, hyperlipidaemia, analgesic activity, antioxidant activity

Introduction

The therapeutic use of medicinal plants is very present in certain countries of the world and especially in the developing countries, in the absence of a modern medical system [48]. The abundance of active ingredients gives the plant remarkable pharmacological properties, which could justify its multiple therapeutic indications and for which it is used in traditional therapy [27].

In Africa, where herbal medicines are still used by many populations in health care [26]. In Algeria, *E. alata* is used against influenza, whooping cough and general weakness in herbal tea and by inhalation as well as in the form of nasal drops against colds [37]. The stem of *Ephedra alata* contains alkaloids: ephedrine and pseudo ephedrine which are used for the treatment of cancer, inflammatory diseases, asthma and viral diseases. The use of this plant is also known to be associated with gastrointestinal and psychiatric manifestations [40].

These effects are the reasons why the use of *Ephedra* is recommended only for acute situations in traditional Chinese medicine and contraindicated for long-term use [9].

On the other hand, the *Ephedra alata* has been shown to have high antiviral activity against HSV (*Herpes simplex* virus). The aqueous extract of this plant which collected from Egypt has significant inhibition potential *in vitro* and *in vivo* against the growth and production of aflatoxins by *Aspergillus flavus* [3, 4].

The objective of this work is to evaluate the anti-hyperlipidaemic, analgesic and antioxidant activities and chromatographic analysis by HPLC of the female cones extract of *Ephedra alata* to avoid the toxicity of stems of this plant and search for less toxic and effective organs.

Materials and Methods

Materials
Plant material. The female cones of *Ephedra alata* subsp. *alenda* were collected on 24 March 2018 Hassi Khalifa (El Oued, Southeast Algeria, 33°40'42"N and 7°13'35"E). The drying was performed at room temperature, protected from light and moisture. After drying, the female cones were crushed and stored in a dry place.

Animals. The study is carried out on male rats weighing 140 ± 160 g, purchased from a farm of the central pharmacy of Tunis (SIPHAT). They are housed at a temperature of 22 ± 1°C with an alternation of 12 hours of lighting and 12 hours of darkness, the relative humidity being close to 40%. The rats received a balanced diet of proteins, lipids, carbohydrates, vitamins and trace elements of the animal nutrition company (SNA, Sfax, Tunisia). Throughout the experimental period (15 days), the animals were treated according to experimentation procedures and experimental protocols of the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Chemicals. Triton X-100, ascorbic acid, gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide ($K_4Fe(CN)_6$), quercetin, chlorogenic acid, p-coumaric acid, vanillic acid, rutin, vanillin and naringenin were procured from Sigma-Aldrich (USA). Aluminium chloride ($AlCl_3$), ferric chloride ($FeCl_3$), sodium carbonate (Na_2CO_3), acetic acid and trichloro-acetic acid were purchased from Prolabo (USA). Folin-Ciocalteu reagent (FCR), methanol (99%) and hydrogen peroxide H_2O_2 (30%) were obtained from Biochem Chemopharma Co (France). All other reagents used were of analytical grade, acetonitrile of HPLC gradient grade was purchased from Sigma Aldrich (USA).

Preparation of the extract. Ten grams of the dry plant were macerated with 150 mL of methanol (99%) at room temperature in dark for 24 hours. After filtration, the solvent was evaporated to dryness under reduced pressure in a rotary evaporator at 50°C [10], for obtaining the dry extract.

Spectrophotometric assays
Determination of total phenolic contents. The total phenolic contents of the crude extract were determined according to the Folin-Ciocalteu's phenol reagent method [46] with some modification; we mixed 0.2 mL of the extract with 1 mL of Folin-Ciocalteu reagent (10%), then we added 0.8 of sodium carbonate solution (7.5%). After stirring the test tubes, the absorbance was measured at 765 nm, after 30 min, using the spectrophotometer UV. The calibration curve was prepared with gallic acid solutions in the concentration range 0.02 - 0.12 mg/mL. The total phenolic content was expressed as mg of gallic acid equivalents in 1 g of extract.

Determination of flavonoids contents. According to [6], we blended 1 mL of the sample solution with 1 mL of $AlCl_3$ solution (2%). After stirring the test tubes, after 10 - 15 min at room temperature, the absorbance was measured at 430 nm. The calibration curve was prepared with quercetin solutions in the concentration range of 0.03 - 0.1 mg/mL. The content of flavonoids in the extract was expressed as mg of quercetin E/g of extract.

Evaluation of Antioxidant activity
DPPH scavenging assay
The DPPH scavenging activity of the extracts was measured by using the modified method of [7]. A volume of 1 mL of extract at different concentrations (2 - 50 µg/mL) was added to 1 mL of DPPH solution (0.1×10^{-3} M) in methanol. After incubation for 15 min at room temperature, the absorbance of the reaction mixture was measured at 517 nm.

The percentage of inhibition was calculated by using the following formula:

$$DPPH \text{ radical scavenging inhibition } \% = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100,$$

The IC_{50} values were calculated from the linear equation of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical 50% [25].

Haemolysis assay
This test is used to determine the ability of the plant extracts to protect the erythrocyte blood cells from damage or disruption of the cell membrane after exposing them to oxidative stress and free radicals by measuring the percentage of dissolved erythrocytes [12].

According to [11], a volume of 40 µL of human erythrocytes of was mixed with 2 mL of different concentrations (0.25 - 1 mg/mL) of extract and conserved for 5 min at 37°C. Then, we added 40 µL of H_2O_2 (30×10^{-3} M), 40 µL of $FeCl_3$ (80×10^{-3} M) and 40 µL of ascorbic acid solution (50×10^{-3} M) respectively.

After 1 hour of incubation at 37°C, the mixture was centrifuged with 700 rot/min for 10 min. The absorbance of the supernatant was read at $\lambda = 540$ nm.

The percentage of haemolysis was determined using the following formula:

$$Haemolysis\% = (Abs_{control}/Abs_{sample}) \times 100,$$

Reducing power assay
The presence of antioxidants in the extracts resulted in reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}) [15].

A volume of 0.5 mL of the different concentrations (0.01 - 0.1 mg/mL) of the extract was added to 1.25 mL of a phosphate buffer (0.2 M, pH 6.6) mixed with 1.25 mL of potassium ferricyanide (1%). The mixture was incubated in a hot bath at 50°C for 20 min, the reaction is stopped by adding 1.25 mL of trichloro-acetic acid (10%), the tubes were then centrifuged at 3000 rpm for 10 min. 1.25 mL of the supernatant, was mixed with 0.25 mL of $FeCl_3$ (0.1%) and 1.25 mL of

distilled water, and the optical density was read at 700 nm [43].

Chromatographic analysis by High-Performance Liquid Chromatography (HPLC)

A high performance liquid chromatography (HPLC) system, type Shimadzu LC 20 AL equipped with the universal injector (Hamilton 25 μL), an analytical column used was a Shim-pack VP-ODS C18 (4.6 mm × 250 mm, 5 μm), type (Shimadzu). UV-VIS detector SPD 20A (Shimadzu) was used [49]. 20 μL of plant extract solution was injected into the flow of mobile phase [25]. The separated compounds were determined using the column for 40 - 50 min, with detection at $\lambda = 268$ nm.

In this study the quantification of separated peaks was performed by calibration with standards.

Acute toxicity

Thirty rats were fasted for 24 hour and divided into 4 groups, each group containing 6 animals. All the groups were orally fed with the extract of *E. alata* suspended in 1% carboxy methyl cellulose in increasing doses of 10, 50, 100 and 200 mg/kg. The animals were observed for 2 h for any behavioural changes, neurological and autonomic profiles, or cases of death after 24 h and 72 h [54].

Evaluation of the protective effect of methanol extract of Ephedra alata due to hyperlipidaemia caused by Triton X-100

Acute hyperlipidaemia in animal was induced with Triton X-100 [42].

Experimental protocol

Thee rats were divided into 4 groups of 6 individuals each: The first group received distilled water by gavage (control group). The second group received by gavage a solution of the methanol extract of *Ephedra alata*, 50 mg/kg of body weight (EME. *alata* group). The third group received a single dose of Triton X-100 (100 mg/kg bw) (Hyp group). The fourth group was pre-treated daily with both methanolic received both extract of *E. alata* 50 mg/kg bw and Triton X-100 (100 mg/kg bw) (EME. *alata* + Hyp group).

Blood samples

The blood was collected in tubes with EDTA and then centrifuged for 15 min at 4000 rpm. The plasma was collected and frozen at -80°C until it was used for the determination of certain biochemical and enzymatic parameters.

Organs samples

The liver was removed and weighed. Part of this organ was fixed in alcoholic Bouin to carry out histological studies. The other part was frozen at -80°C for the study of oxidative stress.

Oxidative stress assessment

The parameters assessing the oxidative stress were the extraction of the cytosol, the determination of total tissue proteins and the tissue MDA; for the determination of the activity of tissue antioxidant enzymes we determined the superoxide dismutase (SOD) activity, catalase activity (CAT) and glutathione peroxidase activity (GPx) [39].

Determination of biochemical parameters of plasma

The parameters assessing liver function measured in this study in plasma were aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT). The parameters estimate of lipid plasma parameters were the total cholesterol, HDL and LDL-cholesterol, tri-glyceride [19].

Histological study

During this study, we used a technique previously described [16]. The preparation of cuts was performed using a microtome (Leica) to a thickness of 6 μm and the sections were frozen.

The staining of the cuts was haematoxylin-eosin, the haematoxylin colouring the nucleus in blue/purple. On the contrary, eosin is colouring the cytoplasm in pink and the other basic cellular elements in pink-red. The second staining with red-oil O coloured all lipids [51].

Evaluation of the analgesic power (writhing test)

The acetic-acid writhing test was used to evaluate the analgesic activity of the methanol extract of *E. alata*. Four groups of rats (n = 3 *per* group) were injected with acetic acid (10 mL/kg bw), and the intensity of nociception was quantified by counting the total number of writhes over 30 min. The animals received a dose of methanol extract of *E. alata* extract (50 mg/kg bw) or sterile saline (control group, 0.9%, w/v), 30 min before acetic acid injection. Indomethacin (10 mg/kg bw) was used as a reference substance (positive control). The numbers of writhes were counted 5 min after acetic acid injection for 30 min [29].

The inhibition percentage of writhing was calculated using the following formula:

% inhibition of writhing = [(Mean number of control - Mean number of test)/Mean number of control] × 100,

Statistical analysis

The results obtained are expressed as mean ± SEM. Data analysis was performed by using Student's T-test, using SPSS Statistics (version 23.0) at significance level $p < 0.05$.

Results and Discussion

Determination of total polyphenols and flavonoids in a crude extract

According to the histogram illustrated in Figure 1, we noted that the total polyphenols content 17.65 ± 0.36 mg gallic acid E/g extract and 13.05 ± 0.78 mg quercetin E/g extract expressing the flavonoids.

The content of polyphenols and flavonoids in the methanolic extract is linked to the high solubility of phenols in polar solvents [18].

The variability of the polyphenol content is due to several factors: the drying and extraction conditions in terms of method, time, temperature, particle size,

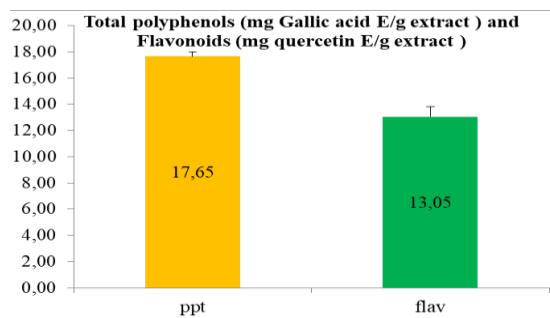solvent, number of extraction steps, expression of results and geographic origin [33].

Figure 1.
Polyphenols contents and Flavonoids in methanol extract of *Ephedra alata*

On the other hand, this variation can be linked to the distribution of secondary metabolites, which change during plant development, environmental stress (drought, poor soil nutrients) as well as intense sunlight [50].

Antioxidant activity

In the DPPH free radical trapping test, ascorbic acid was used as standard. The results are shown in Table I; we noted that the IC_{50} values were 31.08 µg/mL and 5.41 µg/mL for crude extract and ascorbic acid respectively.

We can see in Table I a reduction of iron for the extract studied, as we have seen that the ascorbic acid has the highest iron-reducing activity.

In our study, the protective effect of erythrocytes at a concentration of 1 mg/mL is similar in the extract of *E. alata* and ascorbic acid (Table I).

Table I

Value of antioxidant activity of different tests of the crude extract of *Ephedra alata* and ascorbic acid

	IC_{50} (µg/mL) DPPH free radical test	Reducing power absorbance with concentration (mg/mL)				% Haemolysis with concentration (mg/mL)			
		0.01	0.005	0.075	0.1	0.25	0.5	0.75	1
Ephedra alata extract	31.08	0.16	0.448	0.705	0.963	55.46	43.68	35.12	22.27
Ascorbic acid	5.41	0.196	0.576	0.790	0.91	54.06	41.68	29.29	16.38

Regarding the DPPH test, ascorbic acid had a very potent anti-radical activity compared to our extracts. Also, we noticed a remarkable anti-radical activity for *Ephedra alata* extract. There is a direct correlation between antioxidant activities and the reducing power of the components of some plants [55].

The extract reduces and discolours the DPPH• radical due to their ability to yield hydrogen to the free radicals produced during peroxidation [24, 56]. Therefore, the antioxidants are considered to be reducers and activators of oxidants [45]. Some previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity [24, 30].

The rate of haemolysis is much higher when red blood cells are treated with hydrogen peroxide. This could be attributed to the oxidative nature of hydrogen peroxide and its ability to destroy the cell membrane and consequently the release of haemoglobin from cells. Hydrogen peroxide can also cause toxicity by the hydroxyl radical [28]. H_2O_2 can cause the degradation of haemoglobin thus releasing Fe^{2+} ions. The anti-haemolytic activity of the plant extracts may be due to the inhibition of the radical by the bioactive compounds in the extract which neutralize H_2O_2 [11].

Chromatographic analysis by HPLC

The result of the separation of methanol extract from *Ephedra alata* by HPLC is illustrated in the chromatogram (Figure 2).
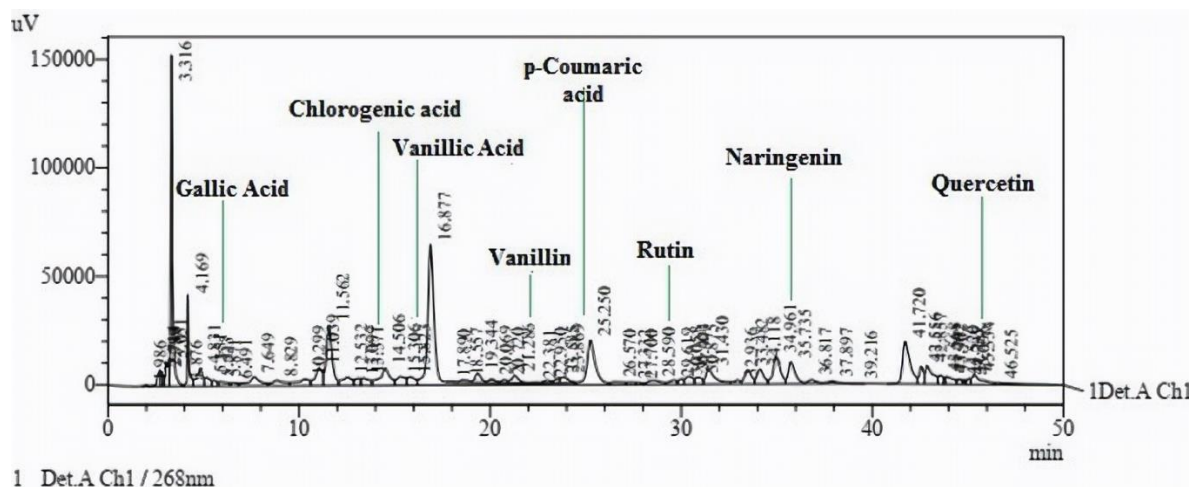

Figure 2.
HPLC chromatographic profile of the crude extract of *Ephedra alata*

Table II
Retention time and the concentration of some phenolic compounds identified

Retention Time (min)	Calibration Curve	Compounds	Concentration (µg/mg extract)
5.151	$y = 54681x$ $R^2 = 9956$	Gallic acid	0.289
13.371	$y = 21665x$ $R^2 = 9853$	Chlorogenic acid	0.926
15.306	$y = 56077x$ $R^2 = 9921$	Vanillic acid	0.392
21.286	$y = 58930x$ $R^2 = 9966$	Vanillin	0.276
23.869	$y = 49495x$ $R^2 = 9961$	p-Coumaric acid	0.278
28.59	$y = 28144x$ $R^2 = 9869$	Rutin	0.637
34.961	$y = 19379x$ $R^2 = 9968$	Naringenin	3.507
45.096	$y = 45378x$ $R^2 = 9962$	Quercetin	0.166

HPLC identified eight phenolic compounds out of 63 peaks in the crude extract of the *Ephedra alata* are gallic acid, chlorogenic acid, vanillic acid, vanillin, p-coumaric acid, rutin, naringenin and quercetin. The retention time of these compounds and their concentration is recorded in Table II.

Many studies identified the phenolic compounds in the extract of *Ephedra alata* by HPLC analysis [23, 34]. These studies are in agreement with our results. Despite the geographic differences of the studied sites, we found that the plant *Ephedra alata* has the same phenolic profile. This shows that there is no significant qualitative difference. We can explain some of our results as follows: gallic acid allowed the plans to adapt to the climatic conditions, vanillin accelerated the maturity of fruits and ρ-coumaric acid contributes to the antioxidant activity [11].

Acute toxicity
In acute toxicity, it was found that *E. alata* extract was safe at a dose of 100 mg/kg with no mortality of studied individuals.

Assessment of the protective effect caused by Triton X-100 (hyperlipidemia)
Changes in liver enzyme biomarkers in plasma
The effects of Triton X-100 and *Ephedra alata* on the liver balance in the different studied groups are shown in Figure 3.
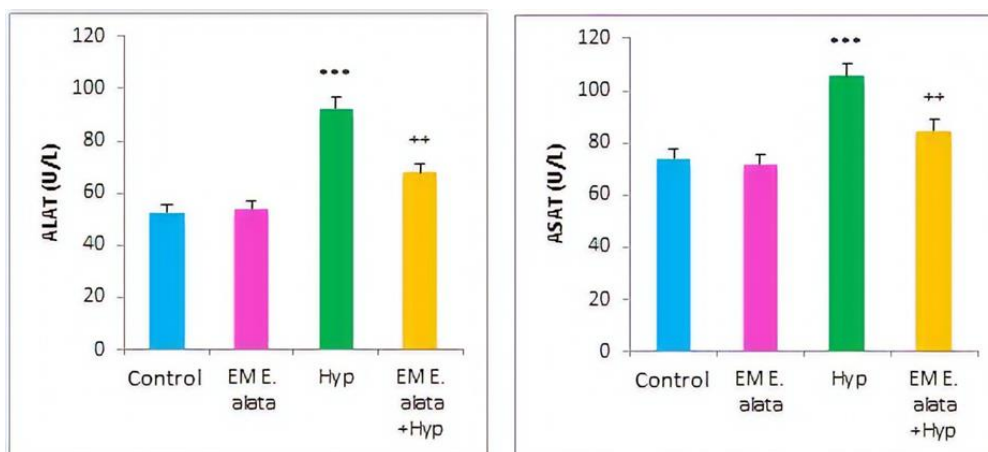

Figure 3.
Variations in ALAT and ASAT enzymes in the control group and the different experimental groups
Values represented as mean ± SEM (n = 6); ***: $p \leq 0.001$ compared to the normal control group; ++: $p \leq 0.01$ compared to the treated group (Hyp)

The analysis of the results revealed that Triton X-100 induced a significant increase in the activity of ALAT and ASAT compared to control rats.

Evaluation of lipid parameters at the plasma level
The effects of Triton X-100 and the extract of *Ephedra alata* on the lipid balance in the different lots studied groups are shown in Figure 4.
Our results showed that Triton X-100 induced a significant increase in the concentrations of the lipid parameters at the plasma level in the group of control rats. These changes in the lipid profile could be attributed either to increased hydrolysis of the stored triglyceride *via* hormone-sensitive lipase activity stimulated by a cyclic adenosine monophosphate dependent protein kinase, or to lipid biogenesis *via* the cardiac cyclic adenosine monophosphate. On the contrary, in the group Hyp + EM *E. alata* the co-treatment with the extract of *E. alata* caused the restoration of these parameters in the plasma. However, there were no significant differences observed for total cholesterol in rats treated with *E. alata* extract alone compared to the control group (p > 0.05).
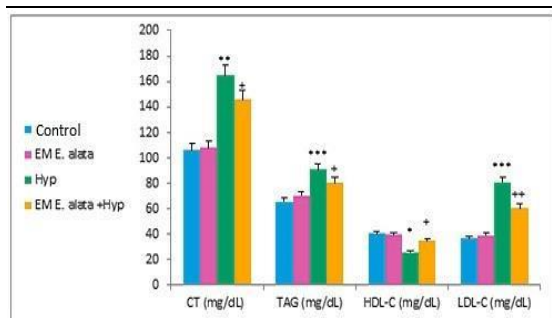
Figure 4.
Variation in serum concentration in CT, TAG,
HDL-C and LDL-C in the control group and the
experimental groups
Values represented as mean ± SEM (n = 6); *: p ≤ 0.05;
**: p ≤ 0.01 and ***: p ≤ 0.001 compared to the normal
control group; + p ≤ 0.05; ++: p ≤ 0.01 compared to the
treated group (Hyp)

*Evaluation of the pro-oxidant/antioxidant profile in
the liver*
Determination of Malondialdehyde (MDA) tissue
The effects of Triton X-100 and the extract of *E.
alata* on the tissue parameters of oxidative stress in
the different studied groups are shown in Figure 5.
Analysis of these results showed that in rats treated
with Triton X-100, hepatic MDA levels increased
significantly (p < 0.001) compared to those in the

control group, which suggests that there is an increased
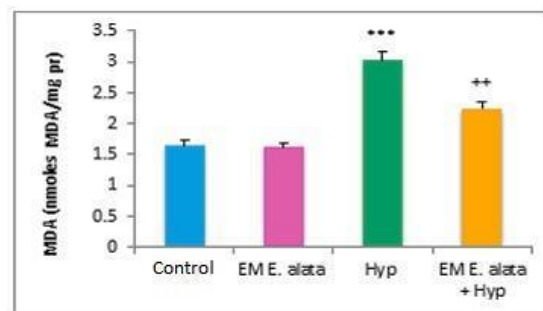production of free radicals.

Figure 5.
Variation of malondialdehyde (MDA) of the liver
in the control and experimental groups
Values represented as mean ± SEM (n = 6);
***: p ≤ 0.001 compared to the normal control group;
++: p ≤ 0.01 compared to the treated group (Hyp)

However, the daily co-administration of the extract
of *E. alata* with Triton X-100 significantly lowered
the MDA level (p < 0.01). This result areas for the
antioxidant potential of *E. alata* extract.
Variations in the activity of tissue antioxidant enzymes
The variations in SOD, CAT and GPx activities of
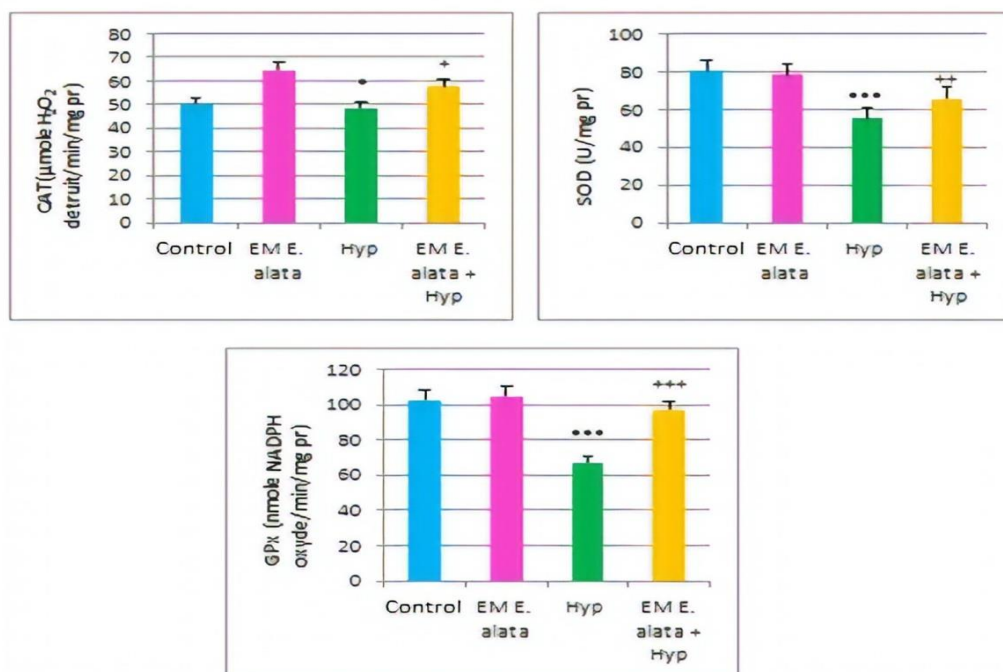the liver in the control and experimental groups are
shown in Figure 6.

Figure 6.
Variations of SOD, CAT and GPx activities of the liver in the control and experimental groups
Values represented as mean ± SEM (n = 6); *: p ≤ 0.05; ***: p ≤ 0.001 compared to the normal control group; +: p ≤ 0.05;
++: p ≤ 0.01 and +++: p ≤ 0.001 compared to the treated group (Hyp)

The results in Figure 6 showed a significant decrease
in the activity of antioxidant enzymes SOD, CAT and

GPx in rats treated with Triton X-100 compared to
the control cases. The results also showed that the

acute treatment associated with pre-treatment with *E alata* extract resulted in a clear restoration of the activity of these enzymes.

The increased activity of ALAT and ASAT in plasma is usually indicative of hepatic cytolysis [20]. The increase in ALAT and ASAT activity is an indicator of cell leakage and failure of the functional integrity of the membrane resulting from liver damage [32, 44]. However, the group treated in parallel with Triton X-100 and EM *E. alata* (Hyp + EM *E. alata*) partially restored the enzymatic activity without reaching the values for the controls or those treated only with the extract of *E. alata*. This implies a potential hepato-protective effect of the extract of *E. alata* probably due to a possible stabilization of the plasma membrane with replenishment of the liver tissue damage caused by the toxic agent, as has been suggested by other authors [41].

This anti-hyperlipidaemic effect can be explained by the ability of this extract to promote the inhibition of HMG-CoA reductase, to promote reactivity, causing a lipid-lowering effect [22].

The current data are in agreement with results previously reported [1, 8]. The highly reactive oxygen metabolites, in particular hydroxyl radicals, act on membrane fatty acids, leading to the production of unstable lipid peroxides, which tend to decompose into products such as MDA [14].

The reduction decrease of SOD, CAT and GPx activities can only be due to the accumulation of reactive oxygen species which leads to harmful results, such as a decrease in the integrity of the cell membrane and loss of function [21]. Our results are in accordance with the work of Abdou RH *et al*. and Uchendu C *et al*. [2, 53] who described a decrease in the activity of antioxidant enzymes following exposure by Triton X-100.

This protective effect could be caused by the indirect effect of the bioactive molecules present in *E. alata* which reduced the levels of reactive oxygen species. Indeed, antioxidant molecules can reduce lipid peroxidation by improving the activity of endogenous antioxidant enzymes [5, 52].

Histological study

Figure 7 shows histological sections stained with haematoxylin-eosin for the studied liver samples.

Our results show that in the control rats, the liver tissue is in the form of hexagonal hepatic lobules and each lobule is formed by well-anastomosed hepatocytes which are arranged in flattened spans around a central-lobular vein, and at the periphery. From each lobule are born sinusoids running between the hepatocyte lamina, each led by an endothelial cell reminiscent of an ordinary hepatic structure. However, the sections of the liver in the rats treated with the Triton X-100 showed remarkable changes, in comparison with those of the control, reflecting the presence of certain hepatocytes arranged irregularly and present a cyto-plasmic vacuolization, a haemorrhage with congestion of the vein central, a large area of necrosis, dilated sinusoidal spaces, a high number of Kupffer cells, cellular infiltrations in the central vein of the lobules, as well as lipid foci.
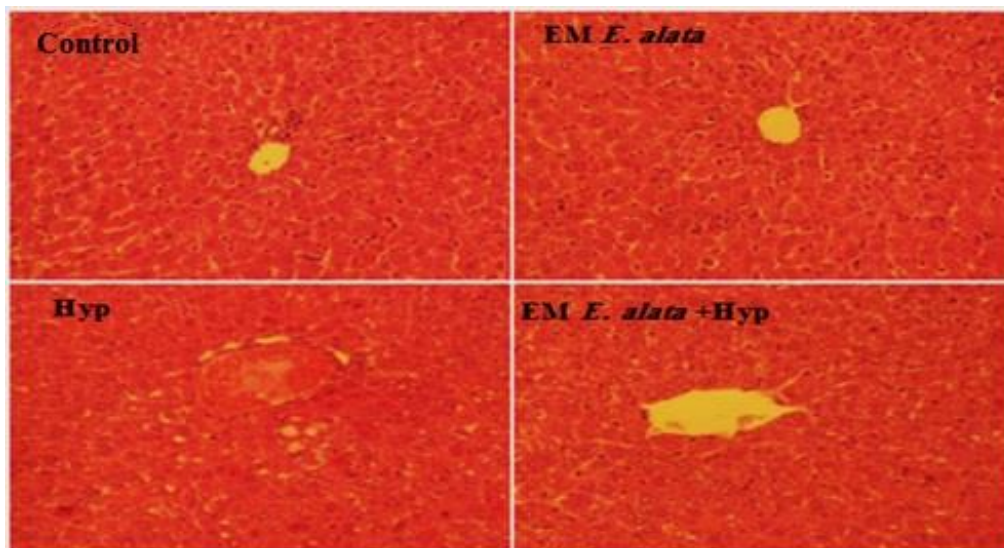

Figure 7.
Microscopic observation of a histological section of the liver (structural exploration) in the rats of different groups (x 400)

However, the combination of the methanol extract of *E.alata* (EM *E. alata* + Hyp) showed protection against the toxicity of Triton X-100, which resulted in improved structural damage demonstrated in animals treated with Triton X-100 alone (Hyp), thus, we noted restoration of the architecture of hepatocytes with

attenuation of the number of sinusoidal dilations and regression of the areas of necrosis and restoration of the number of Kupffer cells.

The histological sections stained with haematoxylin-eosin made in the liver, similar observations had been proved by other authors [13, 47, 51] when treating rats with carbon tetrachloride.

In Figure 8, we used frozen sections using a cryostat to preserve the fatty deposits in the liver.
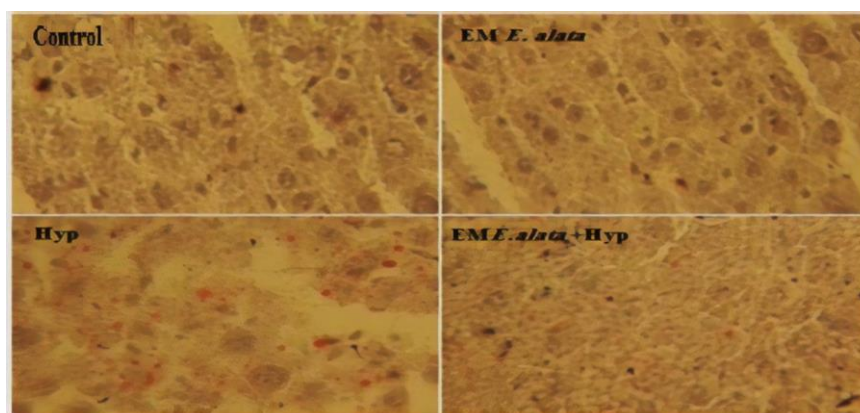

Figure 8.

Microscopic observation of a histological section of the liver (Histological exploration of the lipid deposition) in rats of different groups (x 400)

The histological sections made in the liver of the control rats and the rats pre-treated with the methanolic extract of *Ephedra alata* shows a normal structure of the hepatic parenchyma exhibiting small quantities of lipids distributed in the hepatocytes with a weak red coloration. However, a notable increase in lipid deposition was observed in the group treated with Triton X-100 compared to control rats, which is exerted by the increase in the intensity of the red coloration indicating the installation of a state of hyperlipidaemia in the liver.

This study revealed that the extract of *E.alata* protected the lipid disturbances induced by the Triton X-100, which are expressed by a decrease in the intensity of the red colouring in histological sections stained with red-oil O. Similar effects have been reported by other authors [17, 31].

Analgesic activity (writing test)

Table III shows the dose - effect of the methanolic extract of female cones of *Ephedra alata* as well as the standard anti-inflammatory drug (indomethacin) administered in groups of rats that received acetic acid (0.1%). These effects are expressed as a percentage of inhibition of writhings.

Table III

Effect of the methanol extract of *Ephedra alata* and Indomethacin on abdominal writhings induced by acetic acid in rats

Treatment	Dose	Number of abdominal cramps	Inhibition (%)
Control (acetic acid 0.1%)	10 mL/kg	26 ± 1.32	/
Normal control (distilled water)	5 mL/kg	26 ± 1.32	/
Indomethacin	10 mg/kg	$13 \pm 1.06**$	50
EM *E. alata*	50 mg/kg	$18 \pm 0.52*$	30.7

Values represented as mean $\pm$ SEM (n = 3); *: $p \leq 0.05$; **: $p \leq 0.01$ compared to the normal control group

From these results, we can note the beneficial effect of the methanolic extract of female cones of *Ephedra alata* which induces a significant reduction in abdominal writhings compared to the control group. These values are similar to those obtained for indomethacin.

The appearance of abdominal torsions under the effect of acetic acid engages the effect of local peritoneal receptors [36] as well as the release of mediators such as prostaglandins and cytokines [35]. The analgesic effect of the extract, leaves us to suppose that the biomolecules contained in the female cones of *Ephedra* *alata* could inhibit the release of chemical mediators, as was also reported by Perz-Guerro C *et al*. [38].

Conclusions

The antioxidant activity of the extract from *E. alata* showed a higher inhibition of DPPH• free radical and the highest reductive activity. Also, the haemolysis test showed that the crude extract offered the best protection of erythrocytes. The HPLC analysis has identified eight phenolic compounds in the extract of *E. alata*: gallic acid, chlorogenic acid, vanillic acid, vanillin, p-coumaric acid, rutin, naringenin

and quercetin. The results of the protective effect of hyperlipidaemia caused by Triton X-100 showed that there are noticeable changes in the biochemical and oxidative stress parameters. The treatment with the methanol extract of *Ephedra alata* normalized the biochemical parameters and the stress state. The histological study confirmed the biochemical results. The values obtained for the methanolic extract of *Ephedra alata* regarding the analgesic activity are similar to those obtained for indomethacin.

Conflict of interest

The authors declare no conflict of interest.

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