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Phenolic Compounds From *Ephedra Alata veill* Growing in Libyaand The Ameliorative Effect Towards CCl_4-Induced Hepatotoxicity in Rats

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Abstract
Ephedra alata family Ephedraceae is a Libyan medicinal plants that are traditionally used in folk medicine for treating many diseases. This study designed to evaluate the phytochemical investigation of its methanolic extract and to analyze their phenolic and flavonoid constituents by using HPLC chromatography.
The ameliorative effect of the methanolic extract of *Ephedra alata veill* leaves towards the CCl_4 induced hepatotoxicity in male Wister rats was investigated. The CCl_4-treated rats showed a significant decline in the studied serum levels of high-density lipoprotein (HDL), albumin (ALB) as well as the hepatic levels of glutathione (GSH) and activities of catalase (CAT), superoxide dismutase (SOD) , glutathione reductase (GR), elevation in the levels of total lipids (TL), triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), globulin (G), total bilirubin (TBil) , alanine and aspartate aminotransferase and alkaline phosphatase (ALAT and ASAT, ALP) and the hepatic levels of malondialdehyde (MDA).
The HPLC chromatography revealed the presence of Phenolic acids which identified as gallic, chlorogenic, catechin, , caffeic, syringic, ellagic, coumaric, cinnamic, ferulic acid, taxifolin beside kaempferol, methyl gallate , pyrocatechol and vanillin. Biochemical investigation showed that the administration of *Ephedra alata* methanol extract significantly enhanced all the examined biochemical parameters. Moreover, results indicated that Wistar rats exposed to high doses of CCl_4 could experience both certain metabolic changes and liver damage. The investigation on tissue histology was also included. The bioactive phenolic compounds in methanol extract of *Ephedra alata*, which scavenges free radicals, enhances liver functions, and normalizes the histological architecture of the liver, are therefore may be promising candidates for treating CCl_4-induced hepatotoxicity.

keywords **:** *Ephedra alata veill*., phenolic, flavonoids, HPLC, CCl_4-Induced, Hepatotoxicity.

Introduion

Ephedra alata belongs to the genus Ephedra which is one of the largest genera of the Ephedraceae family; which contains about 70 species of nonflowering seed plants distributed throughout continents. It has been commonly used in folk medicine in Libya and most of the Arabian countries for treatment of asthma, and the common cold [1].
Ephedra is a common phytomedicine with a long history and a wide range of pharmacological effects. Recently, many researchers have conducted in-depth studies on the pharmacological effects of extracts or monomeric compounds from *Ephedra* herb. It appears that not only the stems, roots, but chemical components of Ephedra fruits also have the multiple pharmacological functions. Additionally, there were approximately 10 kinds of pharmaceutical dosage forms of Ephedra herb statistically, including ordinary tablets, chewable tablets, capsules, syrups, drops. And more than 500 kinds of drugs are consisted of Ephedra herb [2].

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E. alata species are short evergreen, almost leafless shrubs that grow in height (60-90 cm).Stems are thin, erect or reclining, green, small, ribbed and pointed, about 1.5 mm in diameter and usually ending in a sharp point. The nodes diverge from 4 to 6 cm. Small, triangular leaves appear at the stem nodes. The knots are distinctively reddish-brown in color. The stems usually branch from the base. It bears delicate yellow-green flowers and fruits, emits a strong pine-like aroma and has an astringent taste. [3]
The major active ingredients of *Ephedra* are alkaloids that constitute fewer than 5 percent of the total mass, and are referred to as ephedrine-type (E-type) alkaloids [4]. The main importance of Ephedra species in the medical field is due to the presence of the alkaloids derived from phenyl-alanine, which act on the sympathetic nervous system as a sympathom-imetic. Where, Previous pharmacological studies revealed that *Ephedra* species crude extracts possessed antimicrobial, antioxidant, antidiabetic, hepatoprotective, cardiovascular, anti-inflammatory, anticancer, anti-obesity, diuretic activities, antifungal and antitumor activities [5].
E. alata constitutes a natural source of potent antioxidants that may prevent many diseases and could be potentially used in food, cosmetics, and pharmaceutical products [6]. Chlorogenic acid, rutin, catechin, quercetin, coumaric acid and flavonoid compound included,vicenin II, lucenin III,kaempferol 3-rhamnoside, quercetin 3-rhamnoside, herbacetin 7-glucoside,herbacetin 8-methyl ether 3-*O*-glucoside-7-*O*-rutinoside, herbacetin-7-*O*-6″- quinylglucoside, furanofuran (±)-syringaresinol, digalloyl-glucose and nilocitin, *p*-coumaric acid were isolated [7].
Ephedra herb is a common phytomedicine with a long history and a wide range of pharmacological effects. Recently, many researchers have conducted in-depth studies on the pharmacological effects of extracts or monomeric compounds. It appears that not only the stems, roots, but chemical components of *Ephedra* fruits also have the multiple pharma-cological functions. Additionally, more than 500 kinds of drugs are consisted of Ephedra herb [8,9].
Gold nanoparticles (Au-NPs) of *E. alata* extract, which is a biological activity, was chosen for its superiority over physical and chemical activities for being environmentally friendly and less toxic [10]. The ephedra plant extract showed phytoconstituents as ; flavonoids and phenolic compounds [11]. Additionally, Au-NPs of its extract in *vitro* evaluations of antioxidant efficacy, and biocompatibility study in three different cell lines were performed to establish the applicability of the formulated NPs [12].
In current research, HPLC was estimated for part of the methanol extract of *E. alata* after the phytoscreening showed that, it is rich with phenolics and flavonoids, the other part used for hepatopertctive activities which showed important results. The development of modern plant-based hepatoprotective medicines represents a significant research potential at the moment. In the present study, we demonstrate the hepatoprotective capabilities of the aerial portions of *E. alata* towards rat liver injury caused by carbon tetrachloride (CCl_4). These plant varieties, which are typically produced in tropical and subtropical areas, have the potential to provide the pharmaceutical sector with a fresh source of herbal medicines

Materials and Methods

All chemicals used are analytical grade and purchased from Sigma-Aldrich and Merck (Germany).

Plant Material

Fresh leaves of E. alata were collected on May 2021 from wadi sawfajjin, Beni walid, Libya, The plant is authenticated by Dr. Alaaeldin Sayed Ewase, Ministry of Environment. Voucher specimens were deposited at the herbarium of the National Research Center (NRC), Egypt.

Extraction

The powdered *E. alata* (100 g) were defatted with petroleum ether (60-80ºC), using Soxhlet extractor , the extract is evaporates under reduced pressure. The polar and free sugars were extracted by methanol 95% . The resulted aqueous methanolic and pet ether extracts were concentrated to give (9.64 and 5.5 g) , respectively in refrigerator at -4°C for future research

Paper Chromatography

Two-dimensional paper chromatography (TDPC) was carried out on whatman (IMM) for comparative studies of of *E. alata* leaves extract under

investigation using BAW for the first dimension, followed by 15% AcOH for the second dimension.

High Performance Liquid Chromatography, HPLC

The HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Kromasil C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35°C.

Biology Methods

Evaluation of Biological Activities

Hepatoprotective Activities *in vivo*

Animals

Male Wistar albino rats weighing from 120 to 150 g were supplied from Animal House at the National Research Centre. All animals were maintained in climate-controlled environments with unlimited access to food and water. Egyptian Medical Committee's Ethical Standards were followed in all aspects of anesthesia and animal handling.

Experimental Design

In this study, 18 male rats were employed. The animals were split into three groups: Rats in Group 1 were used as typical, healthy controls. Group 2 rats received 500 microliters of CCl_4 intraperitoneally twice weekly for six weeks in a row, diluted 1:9 (v/v) in olive oil (0.1 ml). Group 3 rats received an intraperitoneal injection of CCl_4 (0.1 ml) before SOD, and glutathione reductase, GR) antioxidants were determined. The 5, 5'-Dithiobis-2-nitrobenzoic acid and GSH reaction constituted the basis for the method used to test GSH content [16]. The approach provided in [17] was used to estimate the CAT activity. Based on SOD's capacity to prevent the nitroblue- tetrazolium dye's reduction process, which is mediated by phenazine methosulphate, the SOD receiving 200 mg/kg of E. alata methanolic extract orally.

Examination of some Biochemical Parameters

Sample Preparations

Each animal's sublingual vein was punctured to obtain its blood. To separate serum, blood samples were put into dry test tubes and centrifuged at 3000 rpm. For additional biochemical investigation, the sera were stored at -20°C. Rats were promptly dissected to obtain the liver tissues. The liver was homogenised with a 10% w/v ratio in a 50 mM Tris HCl buffer at a pH 7.4 temperature and centrifuged for 20 minutes at 10,000 rpm. For additional analysis, the supernatant was gathered and stored in deepfreeze at -20 C.

Determination of Serum Biochemical Parameters

Total lipids, total cholesterol, triglycerides, low density lipoprotein cholesterol, high density lipoprotein cholesterol, total proteins, albumin, globulin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, and direct bilirubin were all colorimetrically measured in the serum of all experimental groups using Biodiagnostics kits (Dokki, Giza, Egypt).

Antioxidant Assay:

Non-enzymatic and Enzymatic

Test for lipid peroxide: Malondialdehyde (MDA) levels in liver homogenate were measured using the method described before [15]. The underlying mechanism of this procedure relies on the interaction of the freed MDA with thiobarbituric acid in an acidic solution following lipid peroxidation (LPO) of the cell membranes.

In the liver homogenates of control and treated rats, the amounts of non-enzymatic (glutathione, GSH) and enzymatic (catalase, CAT, superoxide dismutase, activity was evaluated [18]. The ability of the GR to catalyze glutathione (GSSG) reduction served as the basis for assessing GR activity [19].

Histological Analysis

Animals' livers were removed, individually weighed, and then sliced into slices of 5 m thickness, fixed in 10% paraformaldehyde, and embedded in paraffin

wax blocks. Hematoxylin and eosin was used to stain tissue sections that were 5 m in thickness (H&E).

Data were statistically evaluated with the help of SPSS version 23 (Statistical Package for the Social Sciences) (copyrighted by IBM SPSS software, USA). Information was presented as a mean and standard error of the mean (SEM).

Results and Discussion

Phytochemical Screening

Phytochemical Screening of the methanol extract of *E. alata* plant; showed that: phenolics, flavonoids, alkaloids compounds are the major components in the extract.

HPLC analysis

HPLC analysis of methanol extract indicate the presence of cardiac glycosides, reducing sugars, alkaloids, phenolic and flavonoids compounds. Phenolic compounds included : gallic acid, chlorogenic acid, catechin, methyl gallate,coffeic acid , syringic acid, pyrocatechol, ellagic acid, coumaric acid, vanillin, ferulic acid, taxifolin , cinnamic acid and kaempferol (Fig 1,Table 1).

Compounds	$R_{t\ (min)}$	Area	Conc. (µg/ml = µg / 20mg)	Conc. (µg/g)
Gallic acid	3.33	3669.80	341.71	17085.53
Chlorogenic acid	3.99	633.26	42.60	2129.76
Catechin	4.31	370.83	63.35	3167.34
Methyl gallate	5.62	95.68	1.55	77.69
Caffeic acid	5.91	104.92	3.18	158.95
Syringic acid	6.44	44.28	1.48	74.04
Pyro catechol	7.22	143.77	14.25	712.29
Ellagic acid	7.90	169.79	9.25	462.34
Coumaric acid	9.07	110.43	1.93	96.64
Vanillin	9.76	270.33	6.46	323.17
Ferulic acid	10.03	634.64	20.38	1018.99
Taxifolin	12.56	50.12	6.23	311.44
Cinnamic acid	14.31	27.07	0.31	15.59
Kaempferol	15.30	28.06	2.05	102.43

Table 1 : Compounds determined from HPLC analysis of *E. alata* plant methanol extract

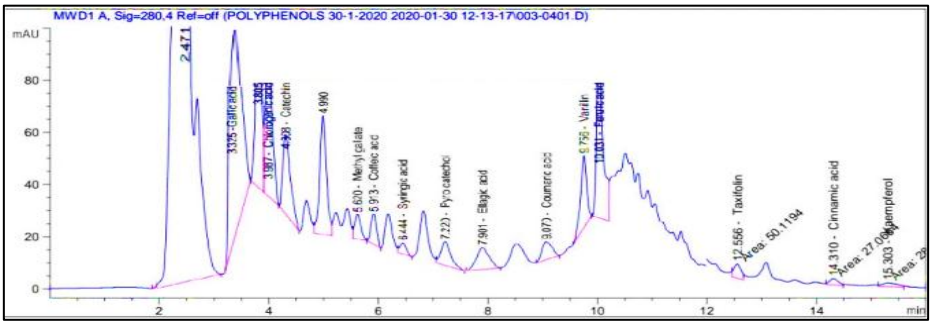

Figure 1 : HPLC , Base Peaks of Methanol Extract

Biology results

Impact on serum biochemical variables

The effects of administering CCl_4 alone, *E. alata* methanolic extract in addition to $CCl_4$and CCl_4 on the lipid profile of the exploratory organisms are shown in (Table 2). The type of treatment had a particularly negative impact on the rats' serum levels of TL, TC, TG, LDL-C, and HDL-C. All of the envisioned lipid profile measures in the CCl_4-treated group were higher than those in the control group, with the exception of

the HDL-C levels, which were significantly lower. However, compared to the rats treated with CCl_4, those given *E. alata* methanolic extract also showed a significant decrease in the levels of TL, TC, TG, and LDL-C. Following confirmation from the results of the current experiment that CCl_4 treatment may have affected hepatic lipid digesting (fatty oil and cholesterol levels). This is supported by the most recent findings, which show that CCl_4 significantly increased the levels of lipid parameters ($p < 0.05$). According to this association [20], CCl4 induction is similar to hepatitis if the breakdown of fatty substances occurs. This situation may also be attributed to a loss in lipase activity, which may result in a decrease in fatty substance hydrolysis [21]. The damage to hepatic parenchymal cells that resulted in an aggravation of lipid digestion in the liver, however, is very likely what caused the hypercholesterolemia in the CCl_4-intoxicated rats [22]. However, when compared to CCl_4-intoxicated rats, rats fed with *E.alata* methanolic extract showed a significant ($p<0.05$) drop in triacylglycerol and cholesterol values. The system of lipid-lowering effects of the methanolic extract of *E. alata* can be attributed to an inhibitory effect on in *vitro* microsomal acyl coenzyme A: cholesterol acyltransferase. This substance is responsible for the liver's acylation of cholesterol to cholesterol esters [23].

Table 2 : Effect of oral CCl_4 treatment on several biochemical parameters in male albino rats, either alone or in combination with *E. alata* methanolic extract

Parameters	Experimental groups			
	Control	CCl4	Methanolic extract + CCl4	Significance
TL ($mgdL^{-1}$)	512.04 ± 43.5	686.4 ± 54.3	598.2 ± 35.1	$p < 0.05$
TC ($mgdL^{-1}$)	118.20 ± 12.97	241.5 ± 33.8	179.1 ± 27.2	$p < 0.05$
TG ($mgdL^{-1}$)	104.40 ± 7.34	172.3 ± 19.7	137.1 ± 14.6	$p < 0.05$
LDL-C ($mgdL^{-1}$)	61.20 ± 9.87	151.9 ± 18.6	127.2 ± 11.3	$p < 0.05$
HDL-C ($mgdL^{-1}$)	36.60 ± 6.40	25.6 ± 7.2	31.7 ± 5.1	$p < 0.05$
TP ($g\ d\ L^{-1}$)	6.68 ± 0.22	6.12 ± 0.9	6.42 ± 0.7	$p < 0.05$
A ($g\ d\ L^{-1}$)	4.42 ± 0.13	3.7 ± 0.4	4.21 ± 0.3	$p < 0.05$
G ($g\ d\ L^{-1}$)	2.46 ± 0.24	4.3 ± 0.5	3.4 ± 0.6	$p < 0.05$
A/G ratio	1.72 ± 0.16	1.01 ± 0.04	1.41 ± 0.07	$p < 0.05$
ASAT ($U\ L^{-1}$)	33.02 ± 1.30	1130.6 ± 25.7	89.3 ±17.5	$p < 0.05$
ALAT ($U\ L^{-1}$)	25.60 ± 1.50	78.3 ± 8.3	51.6 ± 6.8	$p < 0.05$
ALP ($U\ L^{-1}$)	55.30 ± 3.84	90.4 ± 11.7	63.7 ± 5.4	$p < 0.05$
TBil ($mg\ d\ L^{-1}$)	0.66±0.02	0.91 ± 0.06	0.79 ± 0.02	$p < 0.05$
DBil ($mg\ d\ L^{-1}$)	0.11 ± 0.005	0.17 ± 0.009	0.13 ± 0.003	$p < 0.05$

Data are represented as mean ± standard error.

The kind of treatment had a discernible impact on the serum protein profile of the various groups of rats in Table 2, as evidenced by the fact that CCl_4-only-treated animals had markedly lower levels of albumin and higher levels of globulin than control rats. In this way, this group's A/G proportion was remarkably decreased. However, in contrast to the CCl_4-treated group, the rats from the E. alata extract and CCl_4-treated groups displayed a controlled increase in the levels of albumin and the A/G percentage but a significant drop in the levels of globulin.

The significant ($p < 0.05$) decrease in serum albumin of CCl_4-treated rats when compared to control rats may indicate weakened liver functions or debilitated union, either necessary due to injury to liver cells or

optional due to decreased protein intake and decreased amino acid assimilation brought on by malabsorption conditions or ill health, or loss of protein in urine as a result of nephritic disorder and persistent glomerulonephritis [24]. However, compared to rats receiving only CCl_4, rats receiving E. alata methanolic extract also showed a significant ($p < 0.05$) increase in grouping of serum egg whites. Egg white fixation increased after treatment with the methanolic extract of C. sinaica, which may be attributed to the treatment's decreased lipid peroxidation cycles and increased plasma protein thiol activities [25].

The treatment of CCl_4-*E. alata* extract alone and in combination had an effect on liver function indicators, which were introduced in (Table 2). The kind of therapy had an overall impact on the activities of ASAT, ALAT, and ALP as well as TBil in rats' serum, but none of the parameters under consideration had an impact on the levels of DBil. In comparison to the controls, the CCl_4-treated rats showed significant increases in the activities of ASAT, ALAT, and ALP as well as TBil levels. Contrary to expectations, the ALP, ASAT, and ALAT activities as well as the levels of TBil and DBil of *E. alata* extract and CCl_4-treated rats were essentially not the same as those of the benchmark group.

In the current study, rats treated with CCl_4 compared to controls had significantly higher serum levels of the liver biomarkers AST and ALT activity (p 0.05). Similar to the current experiment, earlier studies showed that CCl_4 significantly increased serum ALP levels as well as absolute protein and egg white levels [26]. Given that these proteins are present in the cytoplasmic portion of the cell and are released into flow if there should be an incidence of cell harm, the increased serum levels of hepatic markers have been attributed to the liver injury [27]. The expansion of serum AST and ALT activity was, however, shown to be suppressed ($p < 0.05$) by treatment with E. alata extract in addition to CCl_4 . The administration of poly phenolic extricates from chicory (Cichorium intybus), for example, resulted in completely standardised serum AST and ALT levels in mice exposed to thioacetamide, a hepatotoxic organosulfur compound [28]. According to the current results, many other plant extracts were accounted for to have impressive restorative impacts on liver injury induced by substance specialists. Comparative effects of barberry extract on CCl_4 - activated hepatotoxic animals have also been documented [29]. These results suggest that it is difficult to protect liver tissue against CCl_4 harm.

Antioxidant Assay:

Non-enzymatic and Enzymatic

Impact on endogenous cancer prevention mechanisms and hepatic lipid peroxidation: The effects of CCl_4 treatment on hepatic MDA and GSH levels as well as the activities of endogenous cell reinforcement proteins, either alone or in combination with *E. alata* methanolic extract, were reported in (Table 3). The type of therapy had a significant impact on the levels of MDA and GSH in the liver as well as the functions of CAT, SOD, and GR. Rats whose livers were solely controlled by CCl_4 showed significant increases in MDA levels along with considerable decreases in GSH substance, SOD, and GR activity as compared to controls. The mean estimates of hepatic MDA fixation in the *E. alata* methanolic extract + CCl_4 treated groups of rats were fundamentally lower than those in the CCl_4-treated rats and were essentially different from those of the controls. However, the mean estimates of hepatic GSH substance in rats treated with CCl_4 and *E. alata* extract were significantly higher than those in the CCl_4-treated group. The animals administered *E. alata* extract in addition to CCl_4 revealed a modest height in the activities of CAT, SOD, and GR, which did not significantly differ from those of the controls when compared with the CCl_4-treated group.

Table 3 : Effect of oral administration of CCl_4 alone or with *E. alata* methanolic extract, on the levels of some antioxidants of male albino rats

Parameters	Experimental groups		
	Control	CCl_4	Methanol extract+ CCl_4
MDA (nmol g^{-1} liver)	4.48 ± 0.11	12.8 ± 0.7	8.3 ± 0.6
GSH (mg g^{-1} liver)	40.04 ± 5.10	21.5 ± 9.6	32.1 ± 6.9
CAT	104.3 ± 17.1	48.2 ± 7.3	83.8 ± 18.4

(U g^{-1} liver)			
SOD (U g^{-1} liver)	9.56 ± 0.17	4.2 ± 0.3	7.1 ± 0.5
GR (U g^{-1} liver)	73.20 ± 2.71	41.5 ± 3.2	59.3 ± 4.7

Data are represented as mean ± standard error.

The results in the current investigation is based on the findings of various researcher [30],who found that CCl_4's hepatotoxic effects are lipid peroxidation-related and are largely caused by its active metabolite CCl_3 (which can extract hydrogen from unsaturated fats, starting the lipid peroxidation), cause cell damage, and ultimately cause liver damage. Another report [31] mentioned that any hepatoprotective medication's effectiveness depends on its capacity to either lessen the harmful effect or restore the normal hepatic physiology that has been altered by a hepatotoxin. The present examination found that E. alata methanolic extract caused elevated catalyst levels in tested groups to decrease ($p < 0.05$), indicating the primary uprightness of the hepatocytic cell film or recovery of damaged liver cells. As recently stated, our perceptions can be attributed to the cell reinforcement elements C, like the results achieved for other plants [32]. oxidative pressure is likely reduced by E. alata extract that inhibits lipid peroxidation. Thus, the cell films remain spotless and cells are prevented from moving on to the corruption step.

The Histopathological Outcomes.

Small analyses of liver tissue from common control rats reveal the normal design of hepatic lobules. The focal veins are located at the center of the lobules, which are encircled by ropes of hepatocytes. Hepatic sinusoids can be detected in the spaces between the strands of hepatocytes (Figure 2). Rats' liver tissue was disrupted with loss of lobular plan, spanning fibrosis with collagenous septa development extended gateway lot to localised vein with mononuclear cells, vacuolar degeneration, and corruption of hepatocytes, according to a histopathological analysis of the liver (Figure 3). Rat liver sections exposed to CCl_4 and a methanolic extract of *E. alata* showed vacuolar degeneration, putrefaction of hepatocytes, and mild incendiary cell invasions surrounding focal veins. Kupffer cells with binuclei and activity were observed (Fig. 4).

The hepatic lobules' typical design can be seen in the microscopic evaluations of the liver regions of regular control rats. The convergence of the lobules surrounded by hepatocyte ropes is where the central veins are located. Hepatic sinusoids can be detected in the spaces between the strands of hepatocytes (Fig. 3). Rat livers treated with CCl_4 alone underwent histopathological analysis, which revealed interference with the liver tissue, including loss of lobular arrangement, spreading over fibrosis with collagenous septa advancement stretched-out passage parcel to central vein with mononuclear cells, vacuolar degeneration, and debasement of hepatocytes (Fig. 4). Kupffer cells that were binucleated and active were discovered (H&E, 400). Rats exposed to CCl_4 and *E. alata* methanolic extract had liver segments that displayed delicate irritable cell assaults surrounding the central vein, vacuolar

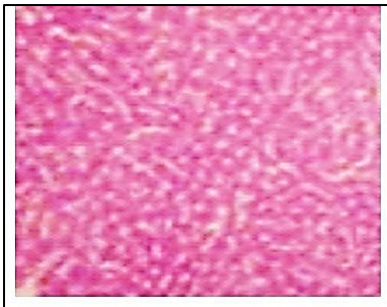

Figure 2: A photomicrograph of a section through the liver of a control rat reveals the typical histological organization of the liver's lobules, central vein, hepatocytes, blood sinusoids, and nuclei (H&E, 400).

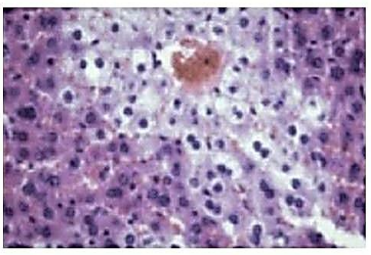

Figure 3: A photomicrograph of a section of the rat liver after administration of Ccl_4 alone reveals disruption of the liver tissue with loss of lobular arrangement, bridging fibrosis with collagenous septa formation, an expanded portal tract to the central vein with mononuclear cells, and necrosis of hepatocytes.
Pyknotic nuclei and a dilated, obstructed central vein were seen (H&E,400).

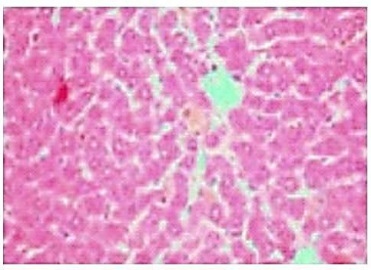

Figure 4: Photomicrograph of section in liver of rat treated with Ccl_4 and E. alata methanol extract demonstrating vacuolar degeneration, necrosis of hepatocytes, and minor inflammatory cell infiltrations surrounding central vein. Kupffer cells that were binucleated and active were discovered (H&E, 400).

degeneration, hepatocyte degeneration, and binucleated and activated Kupffer cells (Fig. 5).

CONCLUSION

Phytochemical screening of the aqueous methanol extract of *E. alata* leaves indicated the presence of phenolic metabolites. The aim of this study was to evaluate the potential hepatoprotective activity of *E. alata* extract. The aqueous methanol extract have shown a remarkable protective effect against CCl_4-induced hepatocyte injury. The histopathological

findings in the current investigation supported the biochemical findings. The changes typically include hepatocellular putrefaction or apoptosis, greasy group, aggressive cell invasion, and other histological manifestations that were also consistent with the conclusions of other creators. Hepatoprotective activity mechanism is attributed to the free radical scavenging and antioxidant activity of the phenolic compounds present in the extract.

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