Efficacy of *Equisetum Arvense* Extract Against Carbon Tetrachloride Induced Liver and Kidney Injury in Rats

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ABSTRACT

Medicinal plants are considered among the most important sources of antioxidants, which are proven to be highly effective against hepatic and nephrotoxicity of many chemical compounds. *Equisetum arvense* (*E. arvense*) plant family Equisetaceae has many uses in traditional medicine and possesses several pharmacological effects, most notably antioxidant effects. This study aimed first to assess the active constituents and antioxidants activities of *E. arvense* extract. Second to evaluate the protective action of *E. arvense* ethanolic extract against carbon tetrachloride (CCl_4) induced hepatic and renal toxicity in rats. This study was carried on 50 rats. Ten rats were served as a control group. Hepato and nephrotoxicity were induced in 10 rats by injection of CCl_4 (3ml/kg 2 times weekly for 2 weeks) and served as CCl_4group. Thirty rats were sorted into 3 groups (n =10) and orally administered with *E. arvense* ethanolic extract (25, 50, and 75 mg/kg) for 2 weeks and then injected with $CCl_4$for another 2 weeks. Results showed that *E. arvense*contains3 main active constituentsbergenin, nilotinib, and glafenin. It also has high total antioxidants and polyphenol contents. Administration of *E. arvense*at all dosage regimen significantly improved rats body weight gain percentage, liver functions (ALT, AST, ALP, total protein, and albumin), kidney functions (creatinine, urea, and uric acid), and lipid profiles (TC, TG, LDL-C, and HDL-C) matched to CCl_4group. Oral feeding with *E. arvense*at all dosagesregimensignificantly ameliorates liver histopathology in favor of the highest *E. arvense*dose (75 mg/kg). Also, *E. arvense*at all dosagesregimensignificantly decreased lipid peroxidation products (MDA) matched to CCl_4group. In conclusion, *E. arvense* exerted hepato and nephroprotective action as well as hypolipidemic effects against CCl_4-induced toxicity in rats. The mechanism may involve antioxidant effect and mitigation of lipid peroxidation.

Key Words: *Equisetum arvense, hepatotoxicity, nephrotoxicity, dyslipidemia, antioxidant.*

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INTRODUCTION

The liver is the largest and one of the most vital organs that functions to regulate detoxification and metabolism of exogenous and endogenous compounds [1, 2]. Hepatotoxicity is a prevalent health problem that represents 38% of all hepatic problems worldwide [3]. Toxic damage happened in the liver frequently compared to the other organs as all the absorbed substances first reach the liver to be metabolized and eliminated [4]. Carbon tetrachloride (CCl_4) has long been known as a toxicant in animal models for made of acute and chronic liver and renal injuries. CCl_4 model has been utilized in numerous *in vivo* and *in vitro* toxicological researches [5, 6]. Itinduceslipid peroxidation and lowering antioxidant enzyme activities [7]. Although, when the balance between the oxidative stress and the antioxidant was impaired, the liver is the utmost organ to be in danger for tissue injury associated with the reactive oxygen species [8, 9].

Many *in vitro* and *vivo* researches assess natural therapeutic medicine for curing and protecting several debilitating diseases [10, 11]. Nowadays, there is a growing interest in medicinal plant usage. *Equisetum arvense* (*E. arvense*) belongs to the Equisetaceae family famous as field horsetail. It is a plant with a wide prospectus. *E. arvense* contains numerous flavonoids, alkaloids, phenol, phenolic, petrosins, triterpenoids, sterols, saponins, phytosterols, tannin, volatile

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oils, minerals, ascorbic acid, silicic acid, and many other biologically active constituents [12-14].

In folk medicine, *E. arvense* is used to treat tuberculosis, pulmonary, gastric, hemorrhages, rheumatic diseases, gout, wound healing, ulcers, and fractures. It possessed numerous pharmacological properties including antimicrobial, antioxidant, anticancer, and anti-inflammatory actions [13, 15-20]. Numerous researches have documented the hypoglycemic action of *E. arvense* extract in diabetic models [21, 22]. The hepatoprotective action of the *E. arvense* extract versus the hepatitis model made by tetrachloromethane has been confirmed [23]. Moreover, a hepatoprotective effect of the phenolic petrosins and flavonoids separated from *E. arvense* has been documented [24]. Also, *E. arvense* extract showed a renoprotective and pressure-lowering impact in an experimental model of chronic kidney diseases [25].

As far as we know, no previous researches reported the liver and kidney protective efficacy of *E. arvense* ethanolic extract versus CCl_4. Subsequently, the current research was performed to estimate the preventive effect of ethanolic extract of *E. arvense*againstCCl_4producedhepatic and renal damage in rats.

MATERIAL AND METHODS

Plants, chemicals, and animals

E. arvense plant was obtained fromHarazfor herbs and medicinal plants Company, Cairo, Egypt. Carbon tetrachloride (CCl_4) was bought from Sigma-Aldrich (St. Louis, USA). All chemicals were bought from EL-Gomhoria, andBiodiagnostic, Egypt. Fifty male Sprague Dawley rats (n=50) of 200 g ± 10 average body weights, were obtained from Helwan Experimental Animals Farm, Giza, Egypt.

Gas chromatography-mass spectroscopy (GC-MS) analysis of E. arvenseactive constituents

The assay was performed utilizing a GC-MS (Agilent Technologies 7890A) connected to a mass-specific detector (MSD, Agilent 7000). Helium was the carrier gas. The recognition of constituents was carried out by comparing their mass spectra and retention time with the library of authentic compounds (NIST and WILEY) [26].

Preparation of E.arvenseethanolicextract and estimation of its antioxidant content

The ethanolic extract of *E.arvense* was prepared according to Safiyeh et al. [21]. The phosphomolybdenum assay was adopted to estimate the antioxidant content [27], and Folin–Ciocalteuassay was adopted to estimate the total phenols content [28].

Induction of hepato-nephrotoxicity

Rats were subcutaneous (s.c.) injected3ml/kg of 50% v/v CCl_4/oilve oil (2 times weekly for 2 weeks) to induce liver and renal toxicity according to Hismiogullari et al. [6]; Jayasekhar et al. [29].

Experimental design

Rats were housed in suitable cages under optimal laboratory situation and fed a standard ratschowone week for adaptation [30]. They were sorted into five groups (10 rats). Group 1(Control): rats s.c. injected with olive oil. Group 2 (CCl_4): ratss.c.injected with CCl_4. Groups 3-5 (*E.arvense* + CCl_4); rats received orally 25, 50, and 75 mg/kg *E.arvense* ethanolic extract, respectively, for two weeks before CCl_4s.c.injection [21].

Feed intake was recorded over the four weeks, initial body weight (IBW), and final body weight (FBW) estimated to calculate body weight gain percent (BWG%). At the end of the experiment, rats in all groups were anesthetized, and blood was collected from the aorta and centrifuged 10 minutes at 3000 rpm. The serum was then collected and preserved at -80 °C for the measurement of the biochemical parameters.The liver and kidney were detached and washed with normal saline, then weighted.

Measurement of liver enzymes

In serum, the activities of aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), and alkaline phosphatase (ALP) were measured using colorimetric assay kits as claimed by the manufacturer.

Measurement of total proteins and albumin

Serum total proteins and albumin were estimated using colorimetric assay kits as claimed by the manufacturer.

Measurement of renal functions

Creatinine, urea, and uric acid were measured in serum using colorimetric assay kits as claimed by the manufacturer.

Measurement of lipids

Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein-cholesterols (LDL-C) were estimated by colorimetric assay kits as claimed by the manufacturer.

Measurement of malondialdehyde

Malondialdehyde (MDA) was measured using ELISA kits, as claimed by the manufacturer.

Histopathological examination

The formalin-fixed liver tissues were dehydrated in graded alcohol, clarified in xylene, and paraffin-embedded. Then 3-5 µm thick sections were cut, stained with hematoxylin and eosin (H & E), and scanned *via* a light microscope.

Statistical calculations

Values are displayed as mean ± standard deviation (SD) and statistically tested for significance by ANOVAtest preceded by LSD multiple comparison test utilizing SPSS software program, version 24 (p-value ≤ 0.05 was statistically significant).

RESULTS

Active constituents of E. arvense

The GC-MS analysis of *E. Arvense* is presented in Figure 1 and Table 1. The results showedthat *E. arvense*contains several active constituents. There were 3 main compounds present in *E. arvense,* includingbergenin (12.73%), nilotinib (11.55%), and glafenin (9.71%). Followed by gardenin (6.42%), dimethylfraxetin (5.86%), sepiapterin (5%), 3-(3,4-Dimethoxyphenyl)-4-methylcoumarin (4.97 %), and (S)-(-)-Citronellic acid (4.66%).

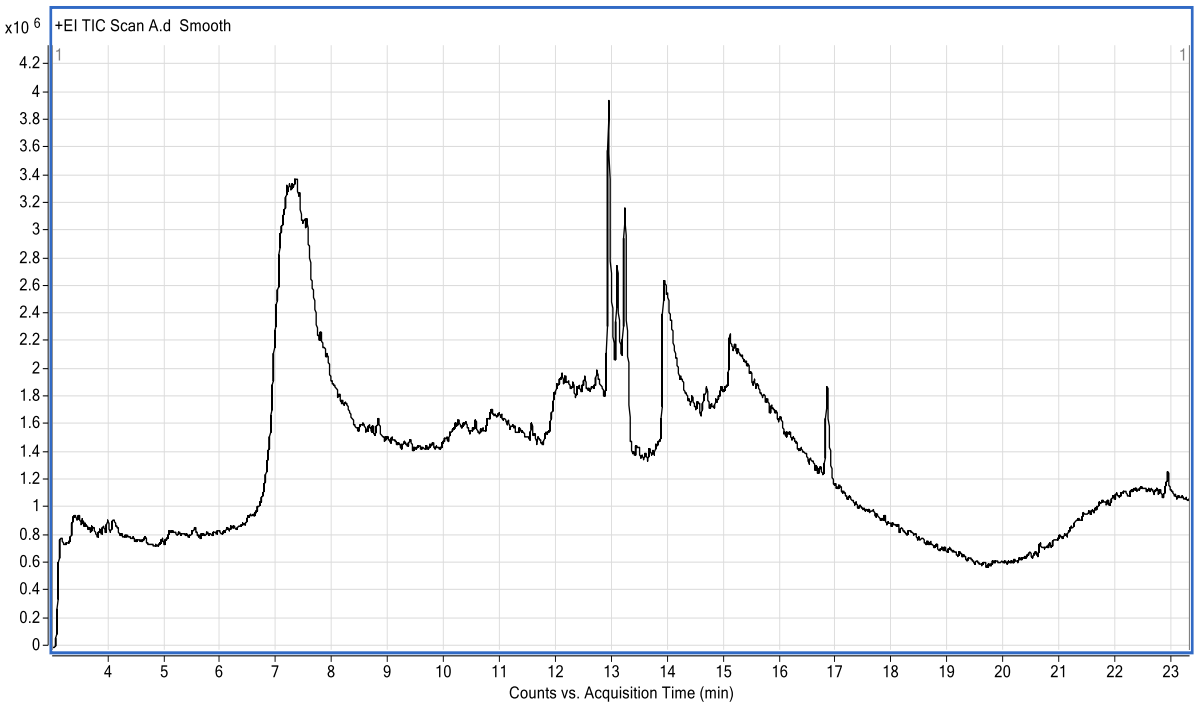

Figure 1: Gas chromatography-mass spectroscopy spectra of *E. arvense* active contents

Table 1: Active constituents of *E. arvense* (gas chromatography-mass spectroscopy)

Constituents	RT (min)	Concentrations (%)
(S)-(-)-Citronellic acid	11.407	4.66
6,7-Dimethoxy-4-ethylcoumarin	12.515	1.77
Sepiapterin	12.997	5
Tetrahydro-L-biopterin	13.136	2.62
Retinyl propionate	13.271	2.45
3,5,3',5'-Tetra-tert-butyldiphenoquinone	13.393	1.29
2'-Hydroxy-3,4,4',5-tetramethoxychalcone	13.798	1.09
Nilotinib	14.001	11.55
Dimethylfraxetin	14.622	5.86
3-(3,4-Dimethoxyphenyl)-4-methylcoumarin	15.095	4.97
7,3',4',5'-Tetramethoxyflavanone	15.244	1.93
Bergenin	15.428	12.73
3,2',4',5'-Tetramethoxyflavone	15.671	1.92
all trans-Retinal	15.816	1.51
Astilbin	16.014	4.79
Cholic acid	16.351	2.73
Glafenin	16.986	9.71
Gardenin	17.689	6.42
Linoleic acid	18.247	2.07

3-Hydroxy-7,8,2'-trimethoxyflavone	18.715	2.05
Isovitexin	19.04	0.96
Dodecanedioic acid	19.431	1.51
Phytanic acid	20.634	4.39
9-cis-Retinoic acid	21.872	1.58
Quercetin 3,5,7,3',4'-pentamethyl ether	22.926	3.51
Irbesartan	23.106	0.92
Non-identified compounds	> 23.2	0.01

Antioxidant contents ofE.arvense

The total antioxidants content of *E. arvense* amounted to2020.6 ± 20.0 mg/ 100 g ascorbic acid, where the total phenolic constituentsamounted to218.0 ± 18.0 mg/ 100 g gallic acid (Table 2).

Table 2: Total antioxidants and total phenols of *E. arvense*

Antioxidant constituents	Mean ± SD
Total antioxidants (mg/ 100 g ascorbic acid)	2020.6 ± 20.00
Total phenols (mg/ 100 g gallic acid)	218.0 ± 18.00

Values were presented as the mean of three replicates ± SD.

Impact of E. arvense on biological evaluation and organs (liver and renal) weighton CCl_4-induced toxicity in rats

Administration of CCl_4 to rats significantly decreased FBW and BWG% ($p \leq 0.001$), with significantly raised ($p \leq 0.001$) liver and renal weight-matched to the control group. *E. arvense* oral ingestion significantly increased the FBW and BWG% in a dose-dependent way ($p \leq 0.001$) matched to CCl_4 intoxicated rats. Besides, the ingestion of *E. arvense* 75 mg/kg significantly decreased the liver and renal weight matched to CCl_4 group, *E. arvense* 25 mg/kg+CCl_4, and *E. arvense* 50 mg/kg+CCl_4 ($p \leq 0.001$). Concerning FI, there was a non-significant decline between CCl_4 intoxicated rats and control rats. Moreover, the ingestion of *E. arvense* in all doses non-significantly increased FI matched to CCl_4 group Tables 3 and 4.

Table 3: Impact of *E. arvense* on some biological parameters on CCl4-induced toxicity in rats

Experimental groups	IBW (g)	FBW (g)	BWG%	FI (g/day/rat)
Control	202.4 ± 4.27	265.8 ± 10.43	31.32 ± 7.49	24.70 ± 2.96
CCl_4	202.7 ± 4.90	187.2 ± 4.32 [a]	-7.65 ± 1.94 [a]	23.74 ± 4.14
***E. arvense*25 mg kg+ CCl_4**	201.2 ± 5.27	220.3 ± 19.97[b]	9.49 ± 3.62 [b]	24.52 ± 2.41
***E. arvense*50 mg kg+ CCl_4**	200.1 ± 5.80	240.5 ± 5.99[b,c]	20.19 ± 4.78 [b,c]	24.75 ± 3.24
***E. arvense*75 mg kg+ CCl_4**	200.6 ± 5.76	254.3 ± 9.78[b,c,d]	26.77 ± 5.09 [b,c,d]	25.36 ± 5.45

Values were offered as mean ± SD (n=10). Values were offered as mean ± SD (n=10). Results were significantly varied ($p \leq 0.05$) from [a]control, [b]CCl_4 group, [c]*E. arvense* 25 mg/kg+CCl_4 group, [d]*E. arvense* 50 mg/kg+CCl_4 group.

Table 4: Impact of *E. arvense* on liver and renal weight on CCl4-induced toxicity in rats

Experimental groups	Liver (g)	Renal (g)
Control	3.92 ± 0.43	0.61 ± 0.04
CCl_4	5.07 ± 0.74 [a]	0.73 ± 0.08 [a]
***E. arvense*25 mg kg+ CCl_4**	4.94 ± 0.64	0.70 ± 0.05
***E. arvense*50 mg kg+ CCl_4**	4.84± 0.55	0.71 ± 0.06
***E. arvense*75 mg kg+ CCl_4**	4.02 ± 0.45[b,c,d]	0.70 ± 0.05

Values were offered as mean ± SD (n=10). Results were significantly varied ($p \leq 0.05$) from [a] control, [b]CCl_4 group, [c]*E. arvense*25 mg kg+CCl_4 group, [d]*E. arvense*50 mg kg+CCl_4 group.

Impact of E. arvense on liver enzymes function on CCl4-induced toxicity in rats

The rats injected with CCl_4 displayed a significant rise ($p \leq 0.001$) in hepatic enzymes (ALT, AST, and ALP) matched to the control. *E. arvense*oral ingestion significantly decreased hepatic enzymes (ALT, AST, and ALP) in a dose-dependent way ($p \leq 0.001$) matched to the CCl_4 intoxicated rats. Besides, there was a significant variation between the *E. arvense* 25 mg/kg and the other two doses. Also, there was a significant variation between the *E. arvense*50 mg/kg+CCl_4 group and the *E. arvense* 75mg/kg+CCl_4 group Figure 2.

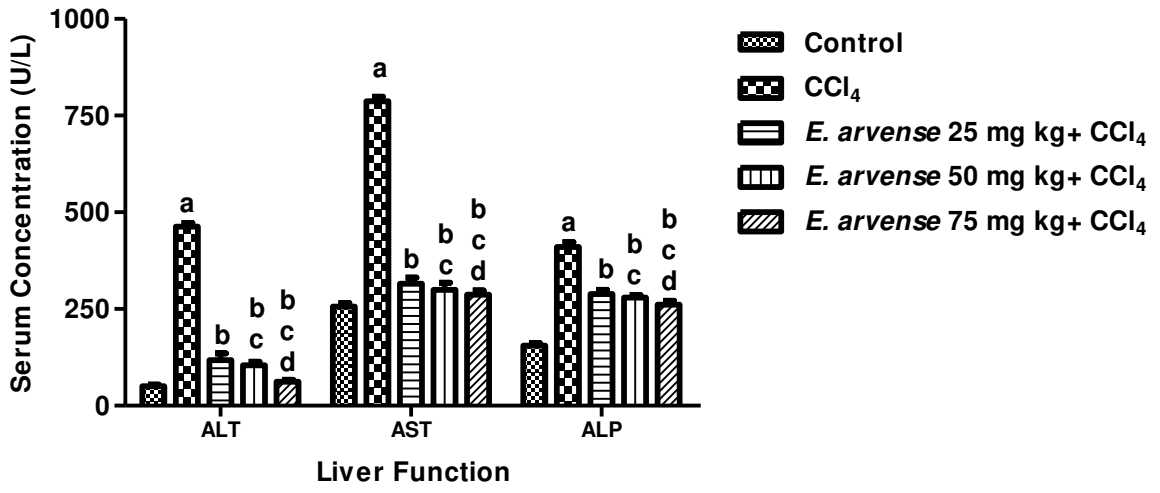

Figure 2: Impact of *E. arvense* on liver enzymes function on CCl4-induced toxicity in rats. Values were offered as mean±SD (n=10). Results were significantly varied ($p \leq 0.05$) from [a]control, [b]CCl_4 group, [c]*E. arvense* 25 mg/kg+CCl_4 group, [d]*E. arvense* 50 mg/kg+CCl_4 group.

Impact of E. arvense on total protein and albumin on CCl_4-induced toxicity in rats

The rats injected with CCl_4 displayed significant decline ($p \leq 0.001$) in total protein and albumin values matched to the control group. *E. arvense*oral ingestion significantly increased total protein and albumin values in a dose-dependent ($p \leq 0.001$) matched to the CCl_4 intoxicated rats. Besides, there was a significant variation between the *E. arvense*25 mg/kg and the other two doses. Also, there was a significant variation between the *E. arvense*50 mg/kg+CCl_4 group and the *E. arvense*75mg/kg+CCl_4 group Figure 3.

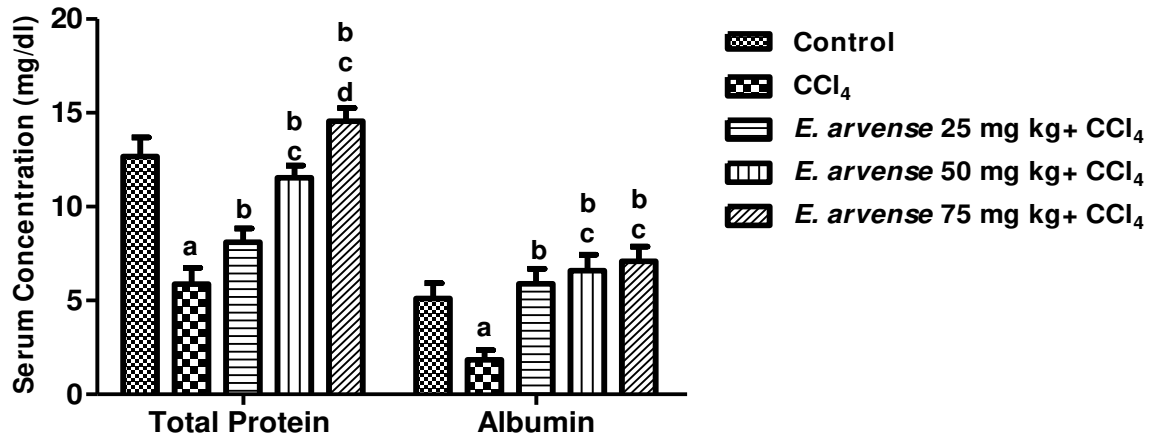

Figure 3: Impact of *E. arvense*ontotal protein and albumin on CCl4-induced toxicity in rats. Values were offered as mean ± SD (n=10). Results were significantly varied ($p \leq 0.05$) from [a] control, [b]CCl_4 group, [c]*E. arvense*25 mg/kg+CCl_4 group, [d]*E. arvense*50 mg/kg+CCl_4 group.

Impact of E. arvense on kidney function on CCl4-induced toxicity in rats

The rats injected with CCl_4 displayed a significant rise ($p \leq 0.001$) in serum creatinine, uric acid, and urea levels matched to the control group. *E. arvense* oral ingestion significantly decreased serum creatinine, uric acid, and urea values in a dose-dependent way ($p \leq 0.001$) matched to the CCl_4 intoxicated rats. Concerning uric acid, there was a significant variation between the *E. arvense* 25 mg/kg and the other two doses. Also, there was a significant variation between the *E. arvense* 50 mg/kg+CCl_4 group and the *E. arvense*75mg/kg+CCl_4 group Figure 4.

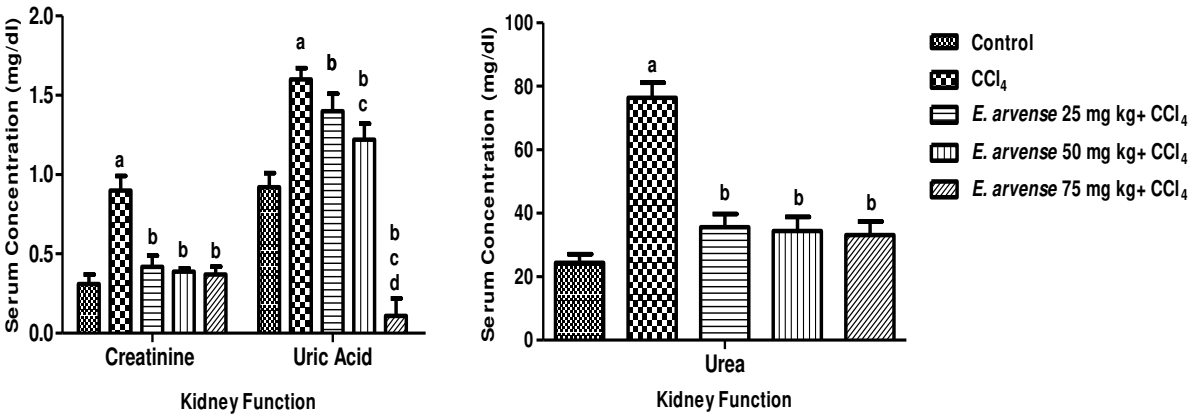

Figure 4: Impact of *E. arvense* on kidney function on CCl4-induced toxicity in rats. Values were offered as mean ± SD (n=10). Results were significantly varied ($p \leq 0.05$) from [a] control, [b]CCl_4 group, [c]*E. arvense* 25 mg/kg+CCl_4 group, [d]*E. arvense* 50 mg/kg+CCl_4 group.

Impact of E. arvense on lipid profile parameters on CCl4-induced toxicity in rats

The rats injected with CCl_4 displayed a significant elevation ($p \leq 0.001$) in serum values of TC, TG, and LDL-C, concurrent with a significant elevation ($p \leq 0.001$) in serum HDL-C concentration matched to control. *E. arvense* oral ingestion significantly improvesalllipid profile parameters in a dose-dependent way ($p \leq 0.001$) matched to CCl_4 intoxicated rats. Besides, there was significant variation between the *E. arvense*25 mg/kg+CCl_4 group and the *E. arvense*75 mg/kg+CCl_4 group in all tested lipid parameters Table 5.

Table 5: Impact of *E. arvense* on lipid profile parameters on CCl4-induced toxicity in rats

Experimental groups	**TC**(mg/dl)	**TG** (mg/dl)	**LDL-C** (mg/dl)	**HDL-C** (mg/dl)
Control	81.52 ± 3.89	73.84 ± 5.58	30.06 ± 5.87	54.11 ± 8.26
CCl_4	115.31 ± 7.22 [a]	147.04 ± 19.06 [a]	49.39 ± 10.79 [a]	33.85 ± 9.04 [a]
***E. arvense*25 mg kg+ CCl_4**	89.30 ± 5.15 [b]	90.97 ± 9.88[b]	35.06 ± 4.02[b]	40.16 ± 9.15[b]
***E. arvense*50 mg kg+ CCl_4**	86.68 ± 5.88 [b]	82.72 ± 5.15[b]	30.07 ± 9.41[b]	42.13 ± 6.14 [b]
***E. arvense*75 mg kg+ CCl_4**	82.09± 6.24[b,c]	78.01± 5.45[b,c]	23.28 ± 7.38[b,c]	50.02 ± 12.62 [b,c]

Values were offered as mean ± SD (n=10). Results were significantly varied ($p \leq 0.05$) from [a] control, [b]CCl_4 group, [c]*E. arvense*25 mg kg+CCl_4 group.

Impact of E. arvenseon serum malondialdehyde on CCl4-induced toxicity in rats

The rats injected with CCl_4 displayed a significant rise ($p \leq 0.001$) in MDA matched to control. *E. arvense*oral ingestion significantly decreasesMDAin a dose-dependent way ($p \leq 0.001$) matched to the CCl_4 intoxicated rats. Besides, there was a significant decrease between the *E. arvense*75 mg/kg+CCl_4 group and the *E. arvense*25 mg/kg+CCl_4 group Figure 5.

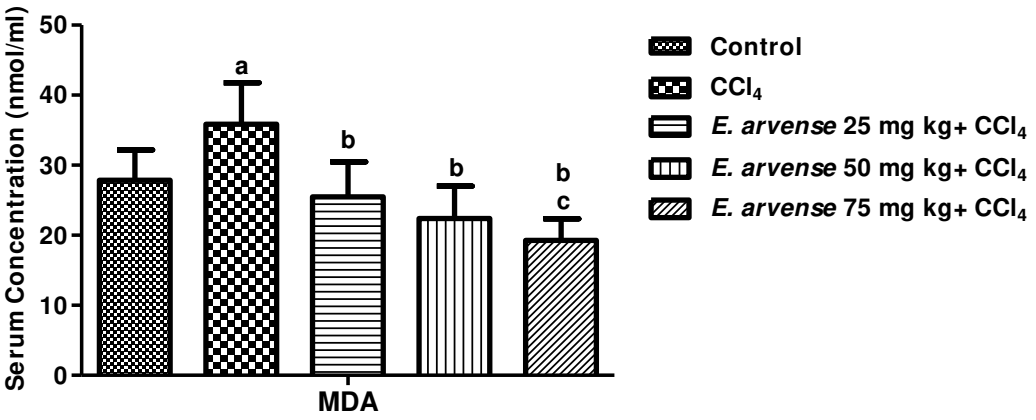

Figure 5: Impact of *E. arvense* on serum malondialdehyde (MDA) on CCl4-induced toxicity in rats. Values were offered as mean ± SD (n=10). Results were significantly varied ($p \leq 0.05$) from [a] control, [b]CCl_4 group, [c]*E. arvense*25 mg kg + CCl_4 group.

Impact of E. arvense on liver tissue histopathology

The histological results of hepatic sections of the control rats displayed a normal histological structure of hepatic lobules (Figure 6A). Hepaticsectionsof rats from the CCl_4 group displayed hepatic sinusoids congestion, Kupffer cell activation, presence of karyomegaly, binucleated cells, sporadic hepatocytes necrosis, and inflammatory leukocytes infiltration (Figure 6B&C). Liver sections of CCl_4 pretreated with *E.arvense*25 mg/kg displayed regeneration nodules separated by fibrous septa with slight loss of normal architecture (Figure 6D). Liver sections of CCl_4 pretreated with *E.arvense*50 mg/kg displayed proliferated hepatocytes replacing the degenerated ones (Figure 6E). Liver sections of CCl_4 pretreated with *E.arvense*75 mg/kg displayed apparent normal hepatic lobule structure where the portal tract was surrounded by proliferated hepatocytes replacing the degenerated ones (Figure 6F).

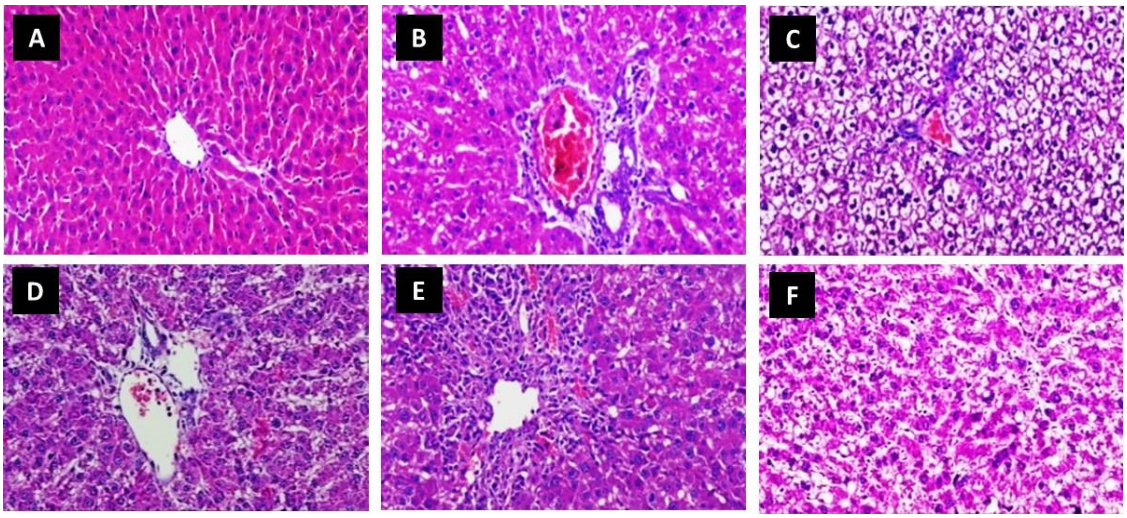

Figure 6. Effect of *E. arvense* on hepatic tissue histopathology displayed in hepatotoxic rats. The histological examination of liver sections of control displayed a healthy histological appearance of hepatic lobules (A). The liver of rats from the CCl_4 group displayed hepatic sinusoid congestion, kupffer cell activation, presence of karyomegaly, binucleated cells, sporadic cell necrosis (B), along with sporadic hepatocytes necrosis and inflammatory leukocytes infiltration (C). Liver sections of *E.arvense*25 mg/kg +CCl_4group displayed regeneration nodules separated by fibrous septa with loss of normal architecture of the liver (D). Liver sections of *E.arvense*50 mg/kg+CCl_4group displayedproliferated hepatocytes replacing the degenerated ones (E). Liver sections of *E.arvense*75 mg/kg +CCl_4group displayed a portal tract surrounded by proliferated hepatocytes replacing the degenerated ones (F)(H&E X 200).

DISCUSSION

The liver regulates numerous functions of the body [31]. CCl_4 is well documented to induce hepatic and nephrotoxicity *via* oxidative stress mechanisms [1]. The extract *E. arvense* has many bioactive components that quench free-radicals and possess antioxidant properties [15, 17, 18]. Since the damaging effects of CCl_4 involve oxidative stress, this study postulated that *E. arvense* extract

might protect the liver and renal versus CCl_4 induced toxicity.

In this study, the CCl_4 induced significant decreases in FBW, BWG%, with significant increases in liver and kidney weight. The obtained results agree with with Ezejindu et al. [32], who revealed that injection with CCl_4 caused a significant loss of body weight of rats concomitant with a relative increase in liver weight. This increase in liver weight was not growth, but inflammation occurs *via* CCl_4. Besides, recently Ullah et al. [33] attributed the reduction in body weight to the decreased feed intake, which was observed in the present study.

Liver enzymes are utilized to estimate the liver cell damage, while total protein is usually applied to estimate the hepatic activity [1, 10]. This study showed that CCl_4-induced severe hepatic and renal destruction as evidence by significant increases in hepatic enzyme levels (ALT, AST, and ALP) coupled with significant increases in renal function levels (creatinine, urea, and uric acid), as well as disturbing in serum total protein and albumin values. The biochemical findings confirmed with the histopathological results of CCl_4 intoxicated rat's liver sections, which showed the occurrence of focal hepatocytes necrosis together with the infiltration of inflammatory cells. These findings pointed to kidney and liver impairment, cellular infiltration, injury, and disturbed cell membrane integrity in the kidney and liver.

The obtained results agree with several studies [3, 33-36] who showed that CCl_4 treated mice had extended necrosis around the central vein and vacuole formation, thus indicated increased hepatic injury. The hepatotoxicity following CCl_4 application explained *via* membrane damage and significant disturbance in renal and liver tissues induced by CCl_4, which is metabolized by cytochrome p450-2E1 to trichloromethyl radicals that begin free radical-induced lipid peroxidation, thus cause hepatic and renal damage [33, 37].

The nephrotoxic effect of CCl_4 was confirmed in several previous studies [38, 39]. Adewole et al. [40] revealed that CCl_4 caused severe kidney damage as estimated by elevated serum creatinine, blood urea nitrogen, and urea concentration, which was explained through CCl_4-induced oxidative stress that boosts the production of diverse vasoactive substances which directly disturbed the kidney function by prompting renal vasoconstriction and impaired the glomerular filtration [41].

Furthermore, CCl_4- induced overt oxidative stress was marked by significant elevation of serum MDA level, thus confirmed in several types of researches [10, 34, 40]. Aziz et al. [7] revealed that many xenobiotics, including CCl_4 toxicity induced *via* the free radicals' production, which are toxic and implicated in the pathophysiology of ailments. Recently, Ullah et al. [33] reported that the CCl_4 brought a high level of MDA and noticeable exhaustion of endogenous antioxidant molecules.The CCl_4 intoxication formed free radicals that induced a cascade of actions inducing in its toxicity [38, 39].

Besides, in this study CCl_4 injection caused a significant increase in lipid profile parameters. Recently Ullah et al. [33] and Elsawy et al. [35] revealed that CCl_4 injection induced a statically significant increase in liver and serum lipids (free fatty acids, TC, total lipids, and TG), while decrease serum HDL-C.CCl_4-induced oxygen-free radicals generation, which catalyzes the oxidation of LDL that caused cell injury [42]. Besides, alteration of lipid profile considered a causal factor for oxidative stress and excessive MDA as found in this study.

The pretreatment effect of *E. arvense* extract (25, 50, and 75 mg/kg) is remarkably protected against both liver and renal injury caused by CCl_4. There were significant decreases in serum liver and kidney markers, as well as improve serum protein and albumin levels. The biochemical results confirmed by the histopathological investigation, which showed that pretreatment of intoxicated rats with *E. arvense* extract significantly protects the liver as minimal changes were seen. The high dose of *E. arvense* (75 mg/kg) was the most effective.*E. arvense* extract administration maintains liver and renal function homeostasis *via* acting as a membrane stabilizing agent through its active antioxidant constituents effects, which was confirmed by the analysis of the active constituents of *E. arvense* by GC-MS done in the current study.*E. arvense* comprises antioxidant activity through its numerous biological active constituents including flavonoids, phenolic, and phytosterols [13, 14, 43].

The hepatoprotection activity of *E. Arvense* herbs extract was investigated in a model of acute hepatitis produced by tetrachloromethane. The results offered that the extract protected the membrane through antioxidant action. This was displayed through lowered liver enzymes, total bilirubin, and lipid peroxidation products, besides the absence of reduced endogenous alpha-tocopherol and glutathione-based enzymes [23]. Oh, et al. [24] showed that the methanolic extract of *E. arvense* produced a marked protective action against tacrine-prompted cytotoxicity in the Hep G2 cell line. *E. arvense* extract decline serum level of MDA induced by CCl_4 injection. This effect was further explained by *E. arvense* phytochemical antioxidant constituents, which possesses a potent radical scavenging ability [44-47]. To the best of our knowledge, no previous study was reported concerning the protective role of *E. arvense*extract against the toxic effects induced by CCl_4 in rats, and our study is the first in this line.

CONCLUSION

The biochemical and the histopathological findings of this study concluded that *E. arvense extract* dose-dependently protects against hepatotoxicity, nephrotoxicity, and hyperlipidemia induced by CCl_4 in rats. The mechanism

behind *E. arvense* action could be explained by its antioxidant and free radicals scavenging efficacy.

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