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Original Research Article

Effects of *Thesium viride* Extract and Fractions on Some Liver Biochemical Parameters in CCl_4-Induced Damage in Wistar Rats

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ABSTRACT

Thesium viride is a hemi-parasite used in the treatment of jaundice, liver enlargement, and splenomegaly. The study investigated the effects of 70% aqueous ethanol extract (AETV) and fractions of *T. viride* on some liver biochemical parameters in carbon tetrachloride (CCl_4) induced liver damage in rats. Rats were grouped into 9 groups; normal, liver damage, silymarin treated, AETV 200 mg/kg treated, AETV 400 mg/kg treated, ethyl acetate fraction (ETV) 200 mg/kg treated, ETV 400 mg/kg treated, butanol fraction (BTV) 200 mg/kg treated and BTV 400 mg/kg treated rats. Treatment was for 7 days while on the 8th day CCl_4 was administered and after 24 hours the rats were sacrificed. Blood samples were collected and the serum was separated for biochemical parameters evaluation. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed and oxidative stress parameters in liver specimen were analysed using spectrophotometric method. Results showed that extract and fractions significantly reduced ALP level. AST and ALT level were significantly reduced in the fractions treated group in a dose-dependent manner. No significant difference was observed between different extract treated group in AST and ALT level. There was a significant reduction in lipid peroxidation by the amount of malondialdehyde (MDA) in all treated groups in a dose-dependent manner. The antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were significantly increased in all the treated groups. The study indicates that the extract and fractions of *T. viride* protect and improves the antioxidant enzymes in liver against CCl_4-induced liver damage.

Keywords: Liver, Serum, Biochemical, Oxidants, Thesium

Introduction

Thesium viride Hill called "Huntu' in Hausa belongs to the family Santalaceae[1]. It is a hemi-parasite subshrub with tufts of greyish green stems arising from a woody rootstock. The leaves are alternate, simple and entire, sessile, narrowly ovate with acuminate apex.[2] *Thesium viride* is found in wooded savannah areas of dry bush, rocky places, along streams and in fallow fields.[3] The aerial part of the plant is used in the treatment of jaundice, liver enlargement and splenomegaly and as well prescribed to cure ulcers.[4,5] The aqueous ethanol extract of the plant was reported to contain alkaloids, flavonoids, cardiac glycosides, anthraquinones and other phenolic compounds was found to be active against *Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Helicobacter pylori* and *Shigella dysenteriae*.[1] The aqueous ethanol extract of the plant was reported to exhibited significant protection against ulcer in rat on ethanol and Aspirin ulcer-induced models.[6]

Hepatotoxicity refers to liver dysfunction that is associated with an overload of drugs such as acetaminophen, cadmium chloride, ethanol, carbon tetrachloride (CCl_4) and allyl.[7]

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These hepatotoxins are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals such as CCl4, and natural chemical.[8] Protecting the liver from the harmful ingested hepatotoxins or counteracting the alterations in the antirradical defense mechanisms is very important.[9] Previous researches reported that plant extract protective effect against $CC1_4$ may be related to polyphenolic compounds, terpenoids, alkaloids, coumarines and phytosterols.[10]

The study investigated the effects 70% aqueous ethanol extract and fractions of *T. viride* on some liver biochemical parameters in carbon tetrachloride (CCl_4) induced liver damage in rats.

Materials and Methods

Preparation of plant materials

The plant was collected at Karaukarau, Giwa Local Government Area of Kaduna State in the September 2019 and it was identified and authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria with voucher number 90000415. The aerial part of the plant collected was air dried under the shade and powdered.

The dried pulverised plant 500 g was extracted with 1 L of aqueous ethanol (70%v/v) in a glass jar for 7 days at room temperature with gradual agitation and change of solvent after 48 hours. The combined extract was filtered through whatman filter paper no. 2, and concentrated on a Büchi rotary evaporator (Büchi Rota vapor R-124) at 50ºC and reduced pressure. The aqueous ethanol extract (20 g) was suspended in 100 ml distilled water and filtered through filter paper size. The filterate was transferred into a separating funnel and partitioned twice with equal volume of ethyl acetate. The aqueous portion was then partitioned twice with equal volume of saturated n-

butanol on equilibration. The partitions ethyl acetate (ETV), n-butanol (BTV) and residual aqueous were concentrated and dried.

Animals used
A total of 63 healthy Wistar rats of both sexes 100-150 g were obtained from the Animal House, Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State after ethical approval was granted by the Committee of Animal Use and Care of Ahmadu Bello University with approval number ABUCAUC/2021/065. The animals were separated into male and female in well aerated laboratory cages in the Animal House, Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State and were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the study. They were fed daily with grower mash from Vital Feeds Company and water *ad libitum* during the stabilization period.

Acute toxicity study
Acute toxicity of the fractions of *T. viride* was carried out using OECD Guideline 423.[11] The studies were carried out using oral route of administration. Three (3) female rats weighing 140-160 g were housed individually in a clean plastic cage in the laboratory. The first rat was starved of food (and not water) for 3 hours before dosing with the 2000 mg/kg aqueous ethanol extract (AETV) fraction. After the fraction was administered, food was withheld for another 1-2 hours and was observed for any signs of toxicity (tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma) and mortality within 24 hours. Two other rats were dosed with the extract 2000 mg/kg orally after the first rat did not die after 24 hours. The dosed rats were observed for signs of toxicity and mortality. After 24 hours, the rats survived and the limit test was determine and the rats were observed for 14 days for any sign of toxicity. The same procedure was carried out using ethyl acetate (ETV) and n-butanol (BTV) fractions.

I*nduction of liver damage*
The model used by Abdel-Kader *et al*[12] was adopted with slight modification. Male Wistar rats were grouped into nine groups of six animals each. The animals were pretreated as follows: Group I receive normal saline (1 ml, p.o.) and served as the control group. The negative control group II received CCl_4 only. Groups III-V received a single dose of CCl_4 (1.2 ml/kg body weight in 50% olive oil). The positive control group III was administered with silymarin (20 mg/kg p.o). Groups IV and V were treated with 200 and 400 mg/kg of the 70% aqueous ethanol extract extract of *T. viride* aerial parts. Groups VI-XI were treated with fractions of ethyl acetate, n-butanol and aqueous as doses of 200 and 400 mg/kg. Treatment was for seven days. On the 8th day CCl_4 was administered and after 24 hours the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture and the serum was separated for evaluation of the biochemical parameters.

Analysis of serum biochemical parameters of the liver
Biochemical parameters were analyzed by standard biochemistry automated analyzer. Blood samples were collected from the rats and centrifuged at 3000 rpm for 10 minutes. Then the separated serum samples were used to analyze liver biochemical parameters including alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST).

Assays of the oxidative stress parameters in liver specimens
The levels of malondialdehyde (MDA) was determined by the method described by Akanji *et al*.,[13] catalase (CAT) by Aebi [14] and superoxide dismutase (SOD) by Fridovich[15] in liver homogenate.

Histopathological examination
A portion of the lobe of the liver was fixed in 10% neutral formalin solution for at least 24 h, processed and paraffin embedded as per the standard protocol. Sections of 5 mm in thickness were cut, deparaffinised, dehydrated, and stained with haematoxylin and eosin (H&E). Histopathological examination of the liver was carried out by means of light microscope at magnification of X 100.[16]

Statistical analysis
All data were expressed as mean ± SEM and percentage (%) where appropriate. One-way analysis of variance (ANOVA) with multiple comparison method by Dunnett was used to determine the level of significance obtained. Results were regarded significant at $p < 0.05$, $p < 0.01$ and $p < 0.0001$.

Results and Discussion

Acute toxicity study
The World Health Organisation encourages appropriate ethnomedicinal use and signifies safety evaluation of herbal medicines thereby preliminary toxicological evaluation is necessary for authentication of safety of herbal medications.[17] In order to determine the safety margin of substances for human consumption, toxicological evaluation was carried out in experimental animals to predict toxicity and to provide guidelines for selecting a "safe'' dose in animals and also used to estimate the therapeutic index of substance.[18] The median lethal oral dose (LD_{50}) of the fractions of *T. viride* was carried out orally in rats. The LD_{50} of the extract and fractions (ETV and BTV) was found to be greater than 2000 mg/kg when administered orally in rats. According to globally harmonized classification system, chemicals are divided into five groups on their LD_{50} basis[19]. The fractions can be put in group 5 (LD_{50} > 2000 mg/kg), falling in lower toxicity class. Initial report on the 70% aqueous ethanol extract by Shehu et al[6] shows the extract was practically non-toxic in rat at an oral dose of 5000 mg/kg body weight.

Serum biochemical parameters of the liver
There were significant (p<0.001) increase in serum ALP, AST and ALT in CCl_4 only treated rats, which were 2-3 times higher compared to normal control. Pretreatment with silymarin, extract and fractions of *T. viride* result in significant (p<0.001) decrease in the levels of serum hepatic enzymes except AST in all doses of extract as shown in Table 1. Liver is the critical organ of the body for maintaining overall health and detoxification of foreign substances. Therefore, diseases of the liver can be devastating, because no other organ in the body can compensate all these important functions.[21] Carbon tetrachloride is metabolized by the cytochrome P450 dependent of monooxygenases, mainly through the CYP2E1 isoform in the endoplasmic reticulum and mitochondria.[22] CCl_4 toxicity is produced by the formation of toxic reactive metabolites such as trichloromethyl (CCl_3^-), trichloromethylperoxy (Cl_3COO^-) and phosgene ($COCl_2$) radicals.[23] These radicals saturate the antioxidant defense system, react with proteins, attack unsaturated fatty acids, generating lipid peroxidation, reduce the amount of cytochrome P450, which leads to a functional failure with the consequent lowering of protein and accumulation of triglycerides (fatty liver), and alter water and electrolyte equilibrium with an increase of hepatic enzymes in plasma.[9] Treatment of the animals with the carbon tetrachloride resulted in significant increase of transaminases (AST and ALT) and alkaline phosphatase (ALP) levels due to hepatocytes damage.[12]
Liver function biomarkers such as aspartate amino transferase (AST) and alanine aminotransferae (ALT) are cytosolic enzymes present in liver cells. Carbon tetrachloride treatment causes the hepatocellular membrane damages through lipid peroxidation which leads to the alteration of the membrane permeability and leakage of liver enzymes in to the circulation system thereby causing cellular leakage, loss of functional integrity of hepatic cell membrane and thus hepatotoxicity.[24] Hence, in the present study experimental animals treated with CCl_4 exhibited significant increase in the serum levels of AST and ALT (table 1), which ensure hepatotoxic effect of CCl_4. Treatment with 70% aqueous ethanol extract of *T. viride* at doses of 200 and 400 mg/kg, do not significantly attenuated the elevated AST and ALT toward the level of normal control group and these results were in comparable with the standard drug silymarin which shows a reduction of 50 and 35% of AST and ALT respectively. These results suggest that the crude extracts of *T. viride* might not protect the hepatocellular membrane structural integrity or enhance the regeneration of damaged hepatocytes at the tested doses.

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Table 1: Effect of *T. viride* extract and fractions on serum biochemical parameter of control and experimental rats

Groups	Treatment	ALP (µ/l)	%	AST (µ/l)	%	ALT (µ/l)	%
I	Normal Saline	99.6 ± 0.93		19.2 ± 1.20		61.2 ± 2.71	
II	CCl_4 (3 ml/kg) only	318.4 ± 1.69		42.0 ± 0.95		107.6 ± 1.03	
III	Silymarin (20 mg) + CCl_4	150.8 ± 1.66 *	51	20.8 ± 0.73 *	50	69.6 ± 0.51 *	35
IV	AETV (200mg) + CCl_4	291.6 ± 2.25 *	9	40.0 ± 0.38	4.5	105.6 ± 0.87	2
V	AETV (400mg) + CCl_4	245.6 ± 1.86 *	22	44.0 ± 0.71	0	102.6 ± 1.50	5
VI	ETV (200mg) + CCl_4	238.6 ± 6.07 *	25	35.6 ± 1.63 *	15	83.8 ± 1.93 *	22
VII	ETV (400mg) + CCl_4	218.2 ± 4.16 *	31	29.60 ± 0.40 *	30	77.0 ± 0.89 *	28
VIII	BTV (200mg) + CCl_4	186.6 ± 1.21 *	41	23.6 ± 1.03 *	44	61.2 ± 3.02*	43
IX	BTV (400mg) + CCl_4	173.2 ± 0.86 *	45	24.8 ± 1.39 *	40	61.4 ± 0.81*	43

All values represent mean ± SEM of n = 6. *p < 0.001, ANOVA, followed by Dunnett's multiple comparison test as compares with CCl_4 only group. % Represent % of reduction with respect to CCl_4 group.
(AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, ALT: Alanine Aminotransferase, AETV: 70% Aqueous Ethanol Extract of *T. viride*, ETV: Ethyl acetate Fraction of *T. viride*, BTV: Butanol Fraction of *T. viride*. CCl_4: Carbon tetrachloride)

Table 2: Effect of *T. viride* extract and fractions on Lipid peroxidation and antioxidant enzymes in control and experimental rats

Groups	Treatment	MDA (nmol/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
I	Normal Saline	32.4 ± 0.75	38.98 ± 0.88	22.74 ± 0.25
II	CCl_4 (3 ml/kg) only	47.76 ± 0.19	15.58 ± 0.90	13.08 ± 0.29
III	Silymarin (20 mg) + CCl_4	36.40 ± 0.51*	35.60 ± 0.89 *	20.14 ± 0.62 *
IV	AETV (200mg) + CCl_4	42.96 ± 0.26*	24.96 ± 1.11*	17.12 ± 0.16 *
V	AETV (400mg) + CCl_4	41.42 ± 0.13*	28.30 ± 0.30*	17.70 ± 0.14 *
VI	ETV (200mg) + CCl_4	42.24 ± 0.20*	28.24 ± 0.44*	14.36 ± 0.28 *
VII	ETV (400mg) + CCl_4	40.94 ± 0.29*	29.92 ± 1.01*	16.04 ± 0.75 *
VIII	BTV (200mg) + CCl_4	40.24 ± 0.24*	32.44 ± 0.57*	16.96 ± 0.35 *
IX	BTV (400mg) + CCl_4	36.48 ± 0.53*	34.88 ± 0.54 *	18.34 ± 0.14 [a]

All values represent mean ± SEM of n = 6. *p<0.001, ANOVA, followed by Dunnett's multiple comparison test as compares with CCl_4 only group.
(CAT: Catalase, MDA: Malondialdehyde, SOD: Superoxide Dismutase, AETV: 70% Aqueous Ethanol Extract of *T. viride*, ETV: Ethyl acetate Fraction of *T. viride*, BTV: Butanol Fraction of *T. viride*. CCl_4: Carbon tetrachloride)

There was a significant attenuation of the elevated AST and ALT onward the normal control group in groups treated with *T. viride* fractions. Increased biliary pressure during the liver damage increases the alkaline phosphatase (ALP) synthesis and thus the increased serum level of them.[25] CCl_4 induced the elevation of serum level of ALP which were reduced toward normal control level with the treatment of *T. viride* extract at 200 and 400mg/kg by 9% and 22% respectively as against 51% of reference silymarin standard. The fractions where found to have a higher reduction in a dose depended manner with butanol fraction having the highest percentage reduction. These results suggest the ability of the plant extract for the stability of biliary dysfunction in rats at a higher dose.

Oxidative stress parameters in liver specimens

Lipid peroxidation can be described generally as a process under which oxidants such as free radicals attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids. One of the main primary product of lipid peroxidation is malondialdehyde (MDA) which is the most mutagenic product widely used as a convenient biomarker for lipid peroxidation and free radical activity because of its ease reaction with thiobarbituric acid.[26] Lipid peroxidation in cellular membranes can cause severe membrane damage and potential cell death.[27] Endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and reduced glutathione scavenges the reactive free radicals to maintain a balance.[28] These enzymes serve as biomarkers of liver injury as they are easily inactivated by lipid peroxides or reactive oxygen specie formed by the use of alcohol and drug.[29,30] SOD is clinically important endogenous antioxidative enzymes as the first line of antioxidant defense by virtue of the ability to convert highly reactive superoxide radicals into hydrogen peroxide and molecular oxygen.[31] Decrease in the level of these antioxidant is considered as a sensitive index of liver cell damage.[25]

Results of the present study revealed that CCl_4 intoxication caused the imbalance between free radical production and the antioxidant defence which resulted in oxidative stress in the liver with significant reduction in the SOD and CAT and marked increase in the MDA as shown in Table 2. This suggests that CCl_4 induce the hepatocellular damage through the oxidative stress via CCl_3O^- radicals which interact with oxygen to produce highly reactive peroxy radicals that promote lipid peroxidation through removing hydrogen groups from unsaturated fatty acids.[32] However, pretreatment with silymarin, *T. viride* extract and fractions significantly accelerated the return of altered activities of SOD and CAT to the values of normal control and markedly reduced the amount of MDA. These evidences suggest that both *T. viride* extract and fractions particularly at higher dose (400 mg/kg) exhibit hepatoprotective activity, at least in part, by improving endogenous antioxidant enzymes status and therefore inhibiting lipid peroxidation.

Histopathological examination

The liver section representing normal control group (Plate I) appeared regular with normal cellular architecture and totally free of any kind of histological changes. The polygonal hepatic cells display intact

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cytoplasm, sinusoidal spaces, visible nucleus, nucleolus and central vein. The hepatoprotective effect of *T. viride* extract and fractions was confirmed by the histopathological findings. CCl_4 administration (Plate II) leads to severe damage, multiple areas of necrosis, severe vacuolization of hepatocytes, fatty changes, parenchymal architecture disruption, and dilatation of sinusoids, central vein congestion and inflammatory infiltration. Treatment with silymarin (Plate III) to some extent prevented the liver from these effects and preserved the liver architecture near to normal. Multiple areas of necrosis, vacuolization of hepatocytes, fatty changes and of a partial central vein congestion ware observed in the crude extract groups (Plate IV). This indicate a poor protection at tested doses of the extract as shown in the level of serum hepatic enzymes. Treatment with ethyl acetate (Plate V) and n-butanol (Plate VI) fractions shows few areas of necrosis, vacuolization of hepatocytes, fatty changes and a partial to absence of central vein congestion with normal liver cells. Based on the serum hepatic enzymes and histology the butanol fraction confers more protection to the liver than the reference standard drug.

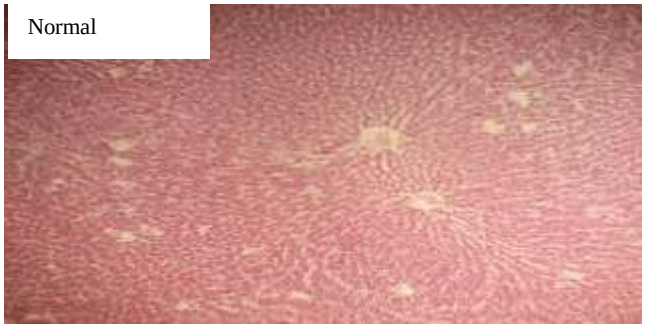


Plate I: Light micrographs of the rat liver sections representing normal control group (Mag. X100).

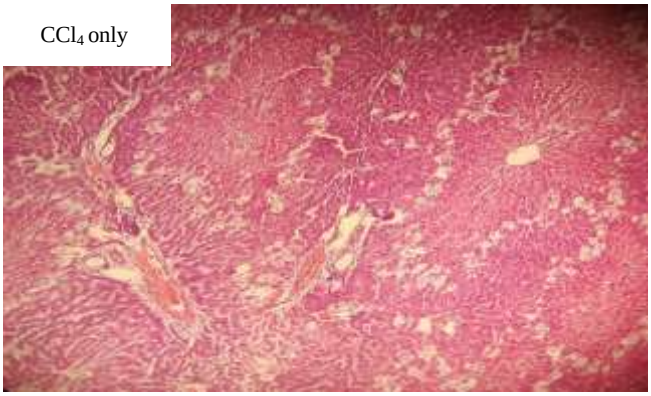


Plate I: Light micrographs of the rat liver sections representing unprotected group (Mag. X100).

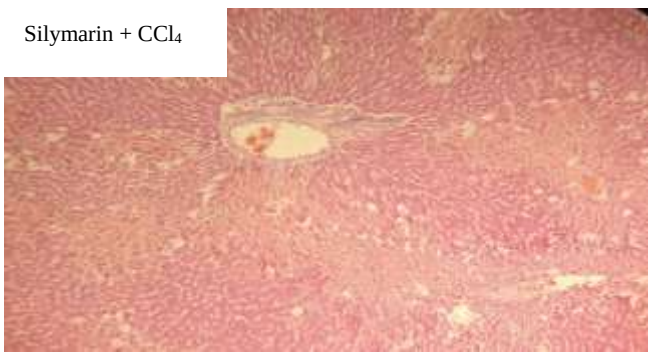
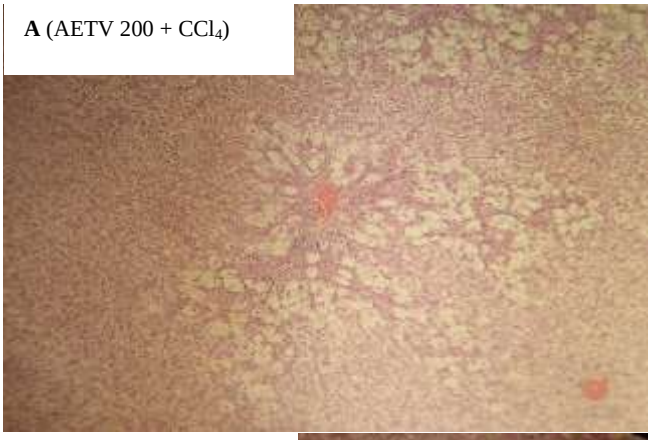


Plate II: The liver tissue section of silymarin control group (Mag. X100).



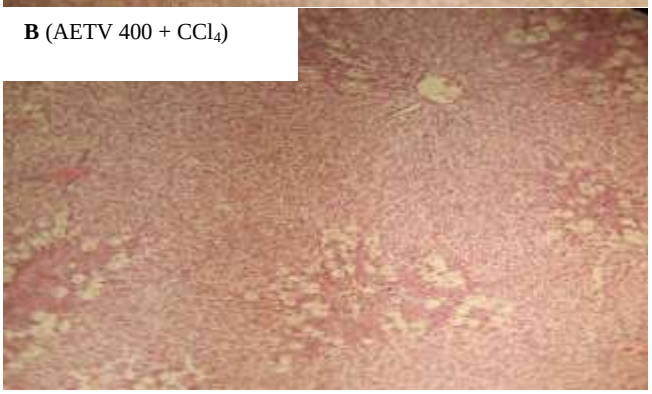
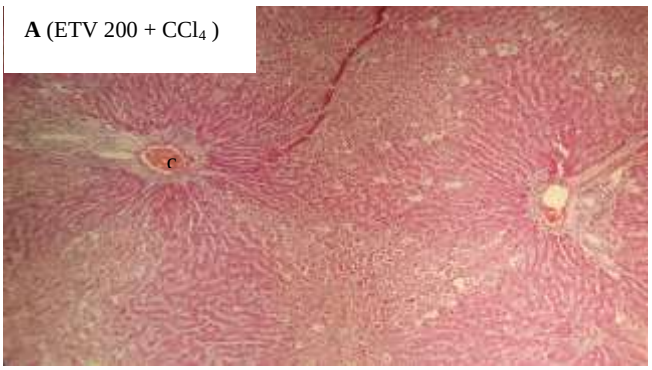


Plate III: Light micrographs of the rat liver sections representing different groups of pretreated with 70% ethanol extract of *T. viride* (Mag. X100).



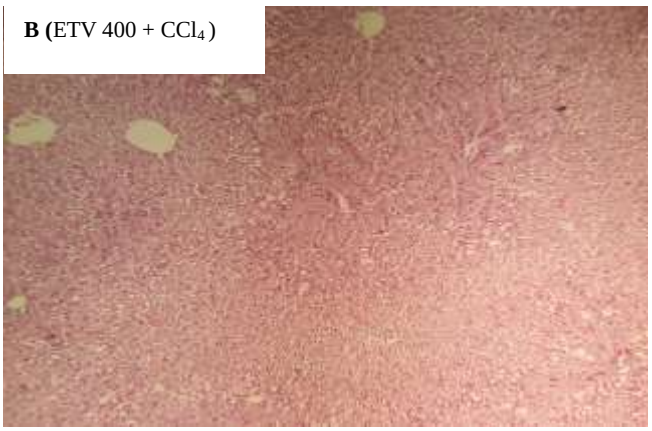


Plate IV: Light micrographs of the rat liver sections representing different groups of pretreated with ethyl acetate of *T. viride* (Mag. X100).

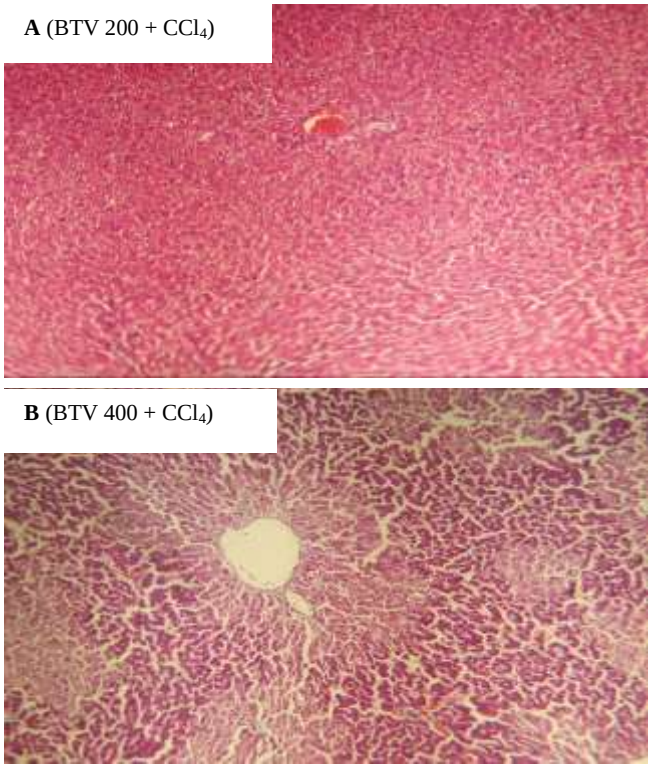


Plate V: Light micrographs of the rat liver sections representing different groups of pretreated with ethyl acetate of *T. viride* (Mag. X100).

Conclusion

The results of the study demonstrated that 70% aqueous ethanol extract and fractions of *T. viride* possess hepatoprotective activity *in vivo* against carbon tetrachloride-induced liver toxicity in rats and significantly improved the overall oxidant status by increasing antioxidant enzymes and reducing lipid peroxidation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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