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Hepatoprotective activity of extract of *Homalium Letestui* stem against carbon tetrachloride-induced liver injury

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ABSTRACT

Background and aims: *Homalium letestui* Pellegr (Flacourtiaceae) is used traditionally by the Yorubas of Western Nigeria as an antidote and by the Ibibios of Southern Nigeria to treat stomach ulcer, malaria and other inflammatory diseases. The aim of this study is to determine the hepatoprotective effects of ethanol extract of *H. letestui* stem (250-750 mg/kg) on carbon tetrachloride (CCl4)-induced liver injury in rats.

Methods: A total of 36 rats were divided into six groups of 6 animals each. Group 1 was administered with normal saline (10 ml/kg) for eight days, group 2 received CCl4, group 3 served as the standard group, while groups 4, 5 and 6 were administered p.o with 250, 500 and 750 mg/kg of *H. letestui* stem extract, respectively, for 8 days. Liver function and histopathological parameters were investigatesd to assess hepatoprotective activity of the extract.

Results: Administration of the stem extract (250-750 mg/kg body weight) caused significant ($P<0.05 – 0.001$) reductions in levels of liver enzymes (ALT, AST and ALP), total cholesterol, direct and total bilirubin and elevation of serum levels of total protein and albumin. Optimal effects on most parameters were observed at 500 mg/kg dose. The effects of the extract/fraction were comparable to that of the standard drug used. Thus, the local use of this plant, at appropriate doses, as an antidote could be supported.

Conclusions: The plant may provide protection against substances that react with membrane lipids to induce peroxidation and subsequent dysfunction of membranes by acting as an effective scavenger of reactive oxygen species. This positive effect may be similar to the established effects of certain substances such as silymarin, vitamin E, vitamin C and other free radical scavengers that reduce the toxic effects of CCl4, especially on the liver.

Keywords: *Homalium letestui*, Hepatoprotective, Rat.

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INTRODUCTION

Homalium letestui Pellegr (Flacourtiaceae) is a forest tree growing up to 80–100 feet and of dense rainforest, transition, semi-deciduous, galleried and secondary forests of lowlands and foothills in Senegal to Nigeria and Fernando Po, and also from central Africa to the Congo basin. It flourishes well around running water.[1] various parts of the plant have been of immense local benefits in many African countries. The tree is decorative with its showy flowers, fruits and reddish young leaves, and is sometimes cultivated as ornamental.[2] In Ivory Coast sap from the bark is used in enemas for the treatment of generalised edemas while lees from the bark are rubbed over the area.[3] In Gabon, a bark-decoction with other medicinal plants is taken for orchitis, and bark-scrapings have been incorporated into a prescription given to a newly-delivered woman. Plant parts, particularly stem, bark and root, are traditionally used in various decoctions by the Ibibio's of the Niger Delta of Nigeria to treat stomach ulcer, malaria and other inflammatory diseases and also are used as aphrodisiac agents by the Yorubas of Western Nigeria.[4]

Several researches have been done to evaluate properties and activities of this plant. Okokon reported the presence of α-terpineol, vanillin, 4-phenyl isocoumarin,[3,4,5] -trimethoxy phenol, 2-coumaranone, and xanthones in the stem bark extract of *H. letestui*.[5] In addition, antiplasmodial[4], antidiabetic[6], anti-inflammatory, analgesic[5], cell antioxidant, anticancer, antileishmanial[7], depressant, anticonvulsant[8] antibacterial[9], in vitro antioxidant activity against DPPH,[9] antiulcer[10], and antidiarrheal[10] activities of the plant have been established. In this study, the hepatoprotective activity of this plant against carbon tetrachloride (CCl4)-induced liver injury was investigated to provide scientific basis for its use in traditional medicine.

METHODS

Sample collection

The stems of *H. letestui* (stem) were collected in a forest in Uruan area, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPUU 382) was issued at the Herbarium of Department of Pharmacognosy and Natural Medicine.

Extraction

The stem was washed and shadow dried for two weeks. The dried plant material was further chopped into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40°C in vacuum using rotary evaporator. The ethanol extract was stored at -4°C until used.

Animals

Adult male albino rats were obtained from the University of Uyo Animal House. They were maintained on standard animal pellets and given water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of University of Uyo.

Animal treatment

A total of 36 rats were weighed and divided into six groups of 6 each and treated as follows: Group 1 consisted of normal animals that were administered with normal saline (10 ml/kg) for eight days; group 2, the organotoxic group, received normal saline (10 ml/kg) for eight days; group 3 served as the standard group (orally administered with 100 mg/kg body weight (BW) of silymarin) for 8 days, while groups 4, 5 and 6 were administered p.o with 250, 500 and 750 mg/kg of *H. letestui* stem extract, respectively, daily for 8 days. On the 8th day, animals in groups 2-6 were given CCl4 dissolved in corn oil mixed at a ratio of 1:3 and at a dose of 1.5 ml/kg BW intraperitoneally. Twenty hours after last treatment, all animals were sacrificed under anesthesia with diethyl ether vapor. Blood samples were collected by cardiac puncture and used immediately.

Hematological investigations

Immediately after the animals were sacrificed under diethyl ether anesthesia, blood samples were collected by cardiac puncture using 21 gauge (21 G) needles mounted on a 5 ml syringe into ethylene diamine tetra-acetic acid (EDTA)-coated sample bottles for analysis. Hematological parameters such as full blood count (FBC), hemoglobin, (Hb), packed cell volume (PCV), platelet concentration (PLC) and total and differential white blood cell count (WBC) were analyzed using automatic hematological system.

Evaluation of the protective effect of the extract against CCl4-induced liver injury on biochemical parameters and histology of liver of rats.

Sera were separated from the blood samples and were stored at -20oC until used for biochemical investigations such as measuring total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, and total and direct bilirubin. The measurements were done spectrophotometrically using Randox analytical kits according to the manufacturer's instructions[11]. The livers of the animals were surgically removed and weighed and a part of each was fixed in 10% formaldehyde for histological processes.

Statistical analysis

Data of this work were analyzed using Student's t-test and one-way ANOVA followed by a post hoc test (Tukey-Kramer multiple comparison test). Differences between mean values were considered significant at 0.1% and 5% level of significance, i.e., $P \leq 0.001$ and 0.05.

RESULTS

Effect of *H. letestui* stem extract on the blood hematological parameters of rats with CCl4-induced hepatotoxicity.

The administration of CCl4 (1.5 ml/kg BW) to rats did not significantly affect ($P \geq 0.05$) RBC and PCV percentage and haemoglobin (Hb) concentration (Table 1). However, there were significant ($P<0.001$) reductions in the percentages of neutrophils in CCl4-treated rats, while pretreatment with *H. letestui* extract did not significantly increase RBC, PCV, Hb and monocytes ($P<0.05$). There were significant reductions in the percentages of eosinophil's following CCl4 administration ($P<0.05$ - 0.001). Pretreatment with the stem extract caused significant, dose dependent elevations of eosinophil's percentages ($P<0.001$). Lymphocyte percentage and platelet count significantly increased after CCl4 administration when compared to normal control ($P<0.001$). Extract and carbon tetrachloride pretreatment significantly caused reduction in the increased lymphocyte percentage and platelet count ($P<0.001$). However, monocytes percentage was not affected by CCl4 and extract pretreatment (Table 1). [4.1.7] Effect of *H. letestui* on liver function after CCl4-induced liver injury in rats.

The results in Table 2 show the effects of the stem extract of *Homalium letestui* on liver function parameters of rats with CCl4-induced liver injury. Administration of CCl4 caused significant increases in the level of ALT, AST, ALP, total cholesterol, total and direct bilirubin and liver weight when compared with the control ($P<0.001$). However, a significant decreases in total protein and albumin were also induced by CCl4 ($P<0.001$). Significant, dose dependent decreases in ALP and AST, total cholesterol and liver

Table 1: Effect of treatment with ethanol stem extract of *Homalium letestui* on the hematological Parameters of rats with carbon tetrachloride-induced hepatotoxicity.

Parameters Treatment Dose (mg/kg)	RBC ($X 10^{12}/l$)	PCV (%)	Hb (g/dl)	WBC ($X 10^{9}/l$)	Neutrophils. (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Platelet (%)
Normal control	3.82±0.32	43.5±1.62	14.53±0.12	15.31±2.15	44.38±8.53	48.50±8.24	3.66±0.70	3.33±0.49	0.10±0.01	261.0±19.15
CCl4 +Dist. Water	3.08±0.34	41.6±1.97	13.83±0.70	18.74±2.21	32.5±4.16^{c}	61.0±4.45^{c}	4.16±0.87	1.83±0.10^{a}	0.12±0.01	308.1±18.30^{a}
Silymarin 100mg/kg + CCl4	4.05±0.12	40.6±1.92	13.50±0.72	12.89±2.34	47.83±4.82^{b,f}	50.6±5.62	3.66±0.95	4.52±0.30^{d}	0.01±0.01^{f}	368.1±17.33^{b}
HL. 250mg/kg + CCl4	3.98±0.52	42.3±1.76	14.18±0.66	12.16±0.75	37.5±6.07^{c}	56.66±6.71	3.54±1.17	3.33±0.91^{d}	0.01±0.01^{f}	244.3±16.64^{d}
HL. 500mg/kg+ CCl4	4.13±0.26	43.0±2.50	14.45±0.24	10.91±1.07	36.8±4.16^{c}	55.36±4.27^{c}	4.16±0.74	3.50±0.92^{d}	0.00±0.00^{f}	287.8±15.01
HL. 750mg/kg+ CCl4	4.18±0.60	41.1±0.70	13.90±0.32	11.12±2.37	46.6±4.82^{f}	42.8±4.42^{cd}	5.66±1.52	3.66±0.84^{d}	0.01±0.01^{f}	244.1±11.88^{d}

Data were expressed as mean ± SEM. significant at $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$ when compared to control. $^{d}p< 0.05$, $^{e}p< 0.01$, $^{f}p< 0.001$ when compared to CCl4. n = 6.

Table 2: Effect of *Homalium letestui* on liver function of CCl4–induced liver injury in rats

PARAMETERS/ TREATMENT	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL CHOLESTEROL Mmol/L
Normal control	5.66± 0.49	4.12±0.96	4.66±0.22	1.06±0.10	89.1±1.70	54.16±9.93	186.0±29.38	4.98± 0.05
CCl4 +Dist. Water	3.66±0.24c	2.16±0.22c	6.31±0.30c	2.05±0.12c	179.33±2.56c	95.33±0.88c	243.0±9.01c	6.48±0.12c
Silymarin 100 mg/kg +CCl4	5.79±0.91f	4.15±0.64f	5.01±0.14f	1.35±0.10d	137.16±7.34cf	92.66±3.63c	227.6±14.50a	5.49±0.16af
Ext. 250 mg/kg + CCl4	5.32±0.74e	5.10±0.33e	5.86±0.53	0.90±0.14f	150.66±9.65cd	92.0±1.36c	180.33±19.47e	5.05±0.24f
Ext. 500 mg/kg+ CCl4	6.00±0.98f	5.04±0.17e	5.30±0.10	1.43±0.15d	134.66±3.09cf	97.83±1.72c	178.83±19.09 e	5.67±0.08bf
Ext. 750 mg/kg+ CCl4	5.52±0.12f	4.52±0.75d	5.38±0.41	0.93±0.08f	139.83±3.91cf	94.33±1.60e	205.83±14.55	5.12±0.18f

Data were expressed as mean ± SEM. significant at $^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$ when compared to control. $^{d}p< 0.05$, $^{e}p< 0.01$, f< 0.001 when compared to CCl4. n=6 6

weight were observed following pretreatment with the stem extract (P<0.05). Treatment with the stem extract did not affect elevated ALT level, but caused significant increases in the level of total protein and albumin (P<0.05 - 0.001).

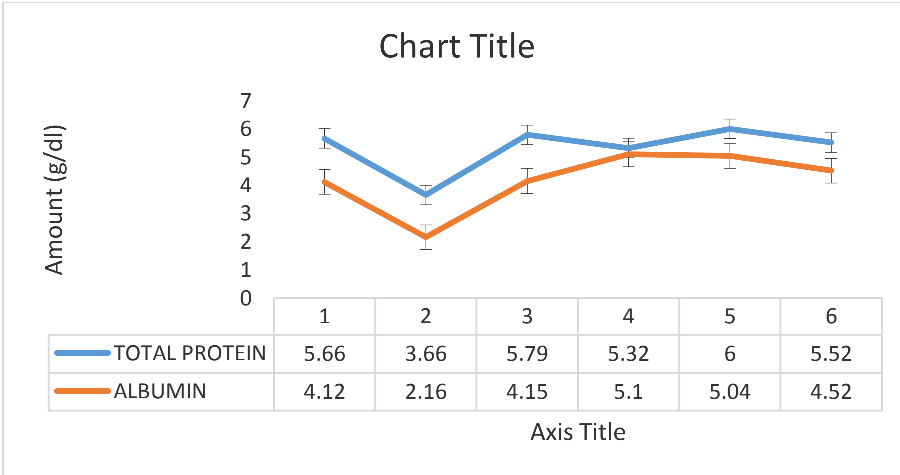

Figure I: Effect of *Homalium letestui* stem extract on level of total protein and albumin in male albino rats.

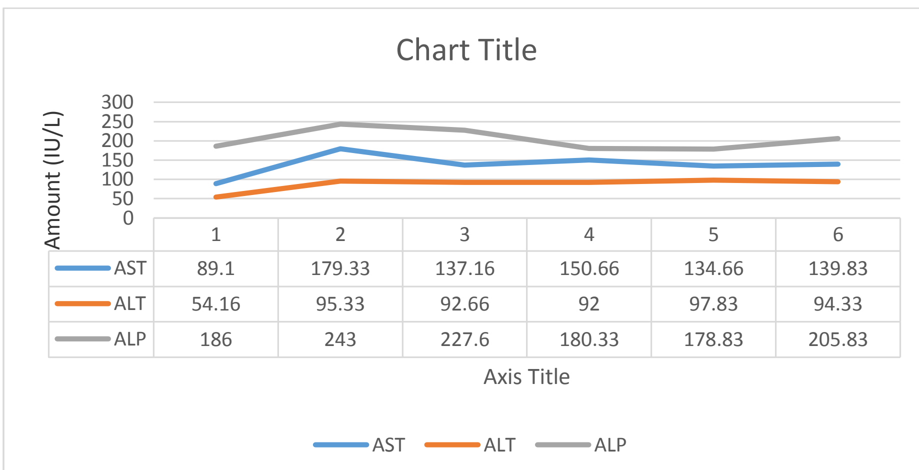

Figure II: Effect of ethanol stem extract of *Homalium letestui* on AST, ALT and ALP in male albino rats

Histopathological Investigations of Rat Liver in CCl4-Induced Hepatotoxicity Histopathological examination of liver sections of normal control group showed normal cellular structure with distinct hepatic cells, sinusoidal spaces and central vein (Figures 3 and 4). Disarrangement of normal hepatic cells with centrilobular necrosis, hyperplasia, vascular and cellular degeneration, inflammation and fatty degeneration were observed in the CCl4-treated rats of group 2. The liver sections of the rats treated with stem extract of *H. letestui* (250-750 mg/kg BW) showed signs of protection, evident by the reduction/ absence of inflammatory cells and vascular and cellular degeneration. Liver sections of the rats treated with silymarin showed significant reduction in fatty degeneration and absence of necrosis and inflammation (Figure 3 and 4).

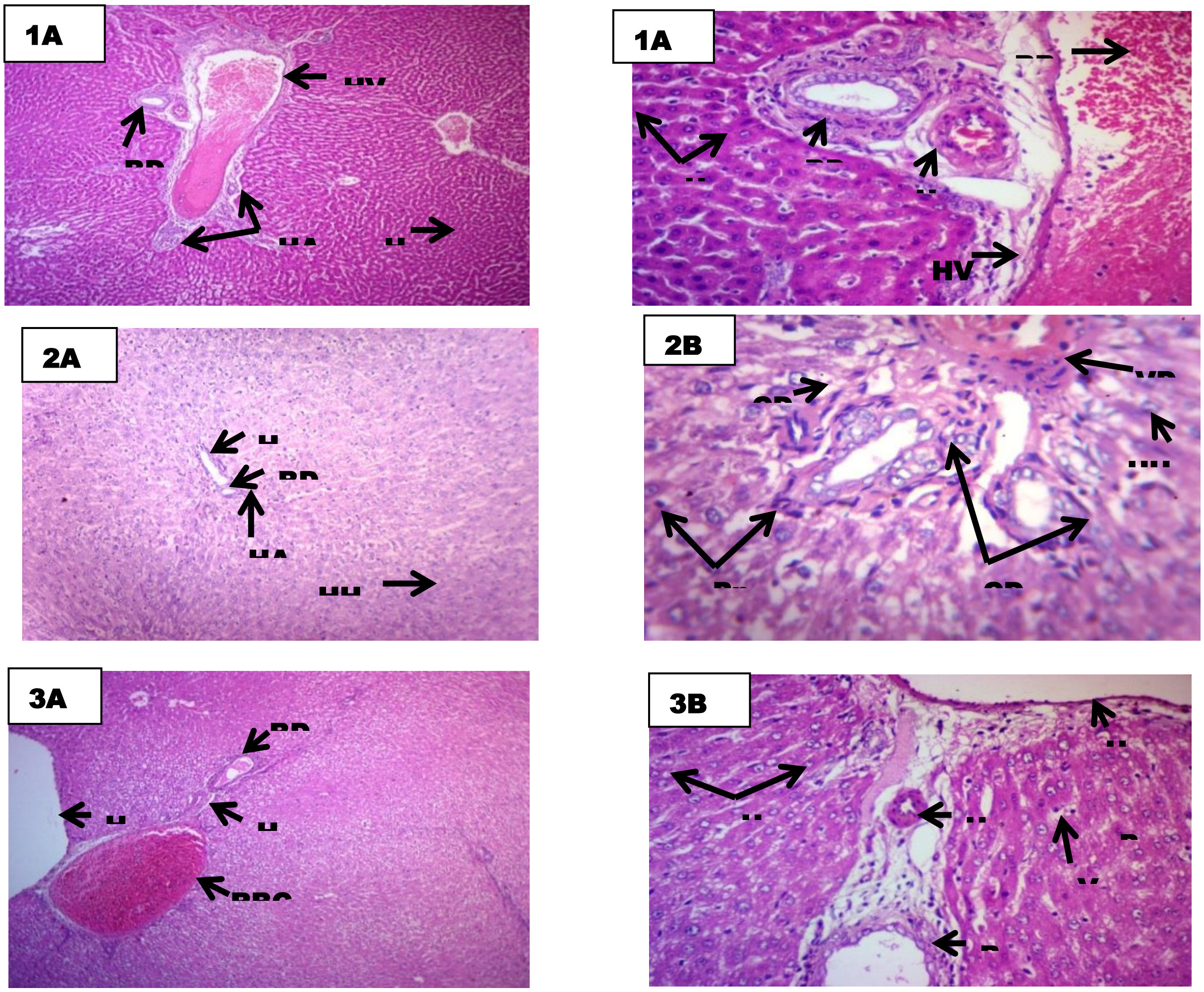

Figure III: Histological sections of Livers of rats treated with Normal saline 10 ml/kg bw (1), CCl_4 mg/kg bw (2) and Silymarin 100 mg/kg bw and CCl_4 mg/kg bw (3) at magnification A (x100) and B(x400) using H&E technique.

Keys: Bile duct (BD), Cellular degeneration (Cd), Inflammation (I), Portal triad (PT), Portal triad degeneration, (PTD), vascular degeneration (Vd), Central vein (CV), Hepatocyte (H) Hepatocytic hyperplasia (HH), Hepatic artery (HA), Hepatic vein (HV), Pyknotic nucleus (Pn) and Vascular congestion (Vc)

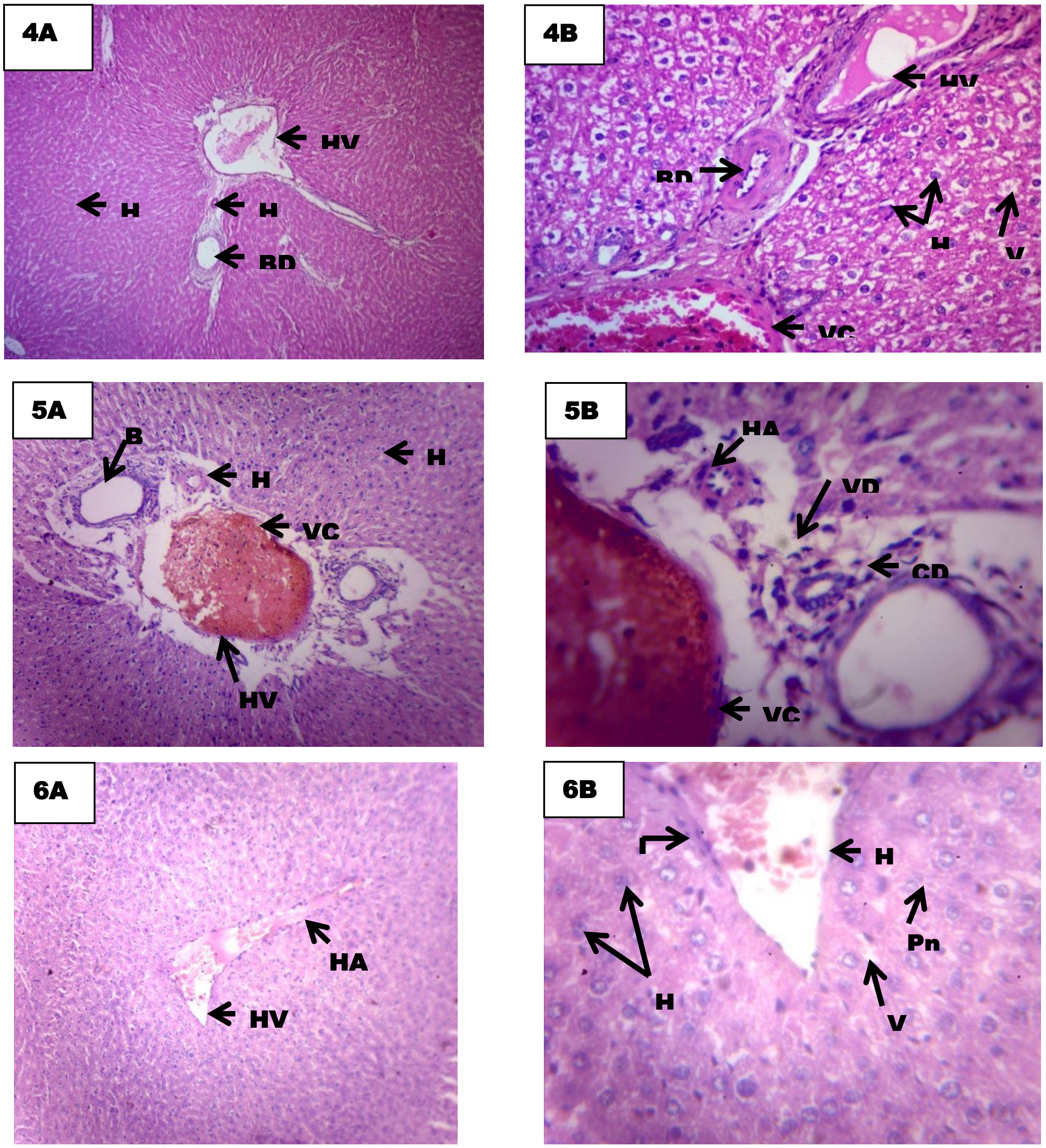

Figure IV: Histological sections of Liver rats treated with *Homalium letestui* 250 mg/kg bw and CCl_4 1.5 ml/kg bw (4), *Homalium letestu* 500 mg/kg bw and CCl_4 1.5 ml/kg bw (5) and *Homalium letestui* 750 mg/kg bw and CCl_4 1.5 ml/kg bw (6) at magnification A (x100) and B(x400) stained with H&E technique.

Keys: Bile duct (BD) Cellular degeneration (Cd), Portal triad (PT), Hepatic artery (HA), Hepatic vein (HV) Inflammation (I), Vascular degeneration (Vd), Hepatocyte (H), Hepatocytic hyperplasia (HH), Central vein (CV), Pyknotic nucleus (Pn) and Vascular congestion (Vc)

DISCUSSION

It has been well established that CCl_4 induces hepatotoxicity by metabolic activation[12]; therefore, it selectively causes toxicity in liver cells, while maintaining semi normal metabolic function.[13] CCl_4 is biotransformed by cytochrome P450 system in the endoplasmic reticulum to produce trichloromethyl (CCl_3-) free radical. Trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen forms trichloromethyl peroxyl radical. Trichloromethyl peroxyl radical may attack lipids on the membrane of endoplasmic reticulum faster than the trichloromethyl free radical.[11] These free radicals are thought to react with membrane lipids to induce peroxidation and subsequent dysfunction of membranes, thereby causing injuries to liver, kidney, heart, testis, and brain. Thus, trichloromethylperoxyl free radical leads to lipid peroxidation. The destruction of Ca^{2+} homeostasis finally results in cell death.[11] Lipid peroxidation and altered levels of some endogenous scavengers are considered indirect in vivo reliable indices for oxidative stress.[13] It has been well documented that CCl_4 is metabolized by mixed-function oxidase system in the endoplasmic reticulum of the liver to the highly reactive trichloromethyl radical. This reactive metabolite leads to autoxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes both functional and morphological disruption of the cell membrane.[11] The hepatocyte membrane distruption is associated with membrane leakage of the hepatocyte cytosolic content which is manifested by a significant elevation of the serum marker enzymes of acute hepatocellular damage, i.e., ALT and AST, and ALP.[14] However, ALT is the most reliable among these marker enzymes. AST is known to be present in abundance in the cardiac muscle, skeletal muscle, kidneys and testes, and ALP is abundant in the growing bone. Thus, any disease state affecting any of these extrahepatic tissues significantly elevates the serum levels of this enzymes.[15]

In the CCl_4 model, CCl_4 slightly increased WBC values compared to the control group. This is in agreement with a previous work in which increased concentration of antigen in the body resulted in elevated values of WBC.[16] In this study, it was observed that the administration of ethanol extract of *H. letestui* caused a significant decrease in lymphocytes, monocytes and eosinophils when compared to the organotoxic group. This trend is consistent with the results obtained by Adisa, Ajayi, Awujo, and Thomas and Ezekiel and Onyeyili.[17-18] This may be due to the ability of the plant extract to counteract deleterious effect of CCl_4. CCl_4 has been known to produce hepatic damage by generating highly reactive trichloromethyl (CCl_3-) and trichloromethyl peroxy radical when metabolized by cytochrome P45019-20. Pretreatment with *H. letestui* extract in this study was observed to slightly improve the PCV, RBC, Hb and eosinophil values when compared to the CCl_4 group. The effect appears to be biphasic, that is, at a median dose the result was optimum while at a much higher dose the effect was reversed.

Extract treatment significantly attenuated the acute elevation of ALT, AST and ALP by CCl_4. The inhibition of protein synthesis and disruption of phospholipids metabolism by CCl_4 might be responsible for the abnormal levels of cholesterol in the serum as observed in the CCl_4-treated rats. Treatment with ethanol stem extract of *H. letestui* significantly reversed these changes. This indicates that the extract preserves hepatic protein synthesis and phospholipids metabolism. CCl_4 induction was also associated with a significant decrease in the serum levels of albumin and total protein.

However, treatment with ethanol stem extract of *H. letestui* protected the liver from the deleterious effect of the toxicant by ameliorating the decrease in the circulatory levels of albumin and total protein and thus stabilizing the endoplasmic reticulum. CCl4 induction causes degeneration of hepatocytes and blockade of the bile ducts which resulted in a significant increase in the serum levels of total and direct bilirubin and ALP.[21] Treatment with *H. letestui* stem extract reduced the elevated serum levels of total and direct bilirubin as well as that of ALP. Therefore, reduction in the levels of ALT and AST to the normal values indicates the process of regeneration from hepatocellular damage. Reduction in the levels of ALP and total and direct bilirubin suggests the stabilization of the function of the biliary system. An increase in the serum levels of total protein and albumin suggests the regeneration of endoplasmic reticulum, leading to protein synthesis. The extract had the most protective effect on liver function at median dose of 500 mg/kg beyond which (750 mg/kg BW) the extract appeared to exacerbate the damaging properties of the toxicant. This may be due to presence of oxidants or degrading chemical substances that become pharmacologically significant as their dose increases.

The CCl4-induced hepatotoxicity in rats that leads to hepatic injury triggers the generation of toxic radicals which can be masked by using a correct antioxidant in sufficient amount.[20] The presence of flavonoids, tannins and terpenoids in the plant explains its role in hepatoprotection by inhibiting the free radical-mediated damage.[20] The hemorrhage caused by CCl4 in the liver was minimized by use of the plant extract as flavonoids are known to be vasculoprotective. Based on our results, it can be suggested that the ethanol extract of *H. letestui* stem may have hepatoprotective activity in rats. Furthermore, histological damage to the rat liver induced by CCl4 administration support other well established finding that intoxication with CCl4 leads to severe necrosis in the liver centrilobular regions around the central veins[22] and fatty infiltration.[23] Interestingly, the microscopic examinations in the extract pre-treated groups also revealed the potential ability of the extract to reduce inflammation, steatosis and necrosis as it was evident by a decrease in histological scoring. This is in agreement with most tabulated parameters that the ethanol stem extract of *H. letestui* have optimum clinical and traditional dose, beyond which the pharmacological effect may be reversed or the toxic properties may become more pronounced.

CONCLUSIONS

The results of the study suggest that the plant may provide protection against substances that react with membrane lipids to induce peroxidation and subsequent dysfunction of membranes by acting as an effective scavenger of reactive oxygen species. This positive effect may be similar to the established effects of certain substances such as silymarin, vitamin E, vitamin C and other free radical scavengers that have been reported to reduce the toxic effects of CCl4, especially on the liver.

CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

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