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Research Article

Histological Study of Effect of Ethanol Stem Extracts of *Homalium Letestui* in Paracetamol Induced Injury in Albino Rat, Using Various Staining Techniques

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Abstract

Introduction/Aim: The use of medicinal plants has attained a commanding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions. Many countries in the world, that is, two-third of the world's population depends on herbal medicine for primary health care. *Homalium letestui* is an evergreen tree. The plant has been of immense benefit to traditional users. A bark-decoction, combined with other medicinal plants, is taken by draught for orchitis, and bark-scrapings enter a prescription given to a newly delivered woman. In this study the histological effect of the ethanol stem extract of *Homalium lestetui* on rat paracetamol induce liver injury was carried out using H&E and Gordon and Sweet silver impregnation Technique.

Method: Thirty-six rats where used for this work. Group one served as the positive control receiving normal saline, group two served as organotoxic group received paracetamol 2000 mg/kg body weight, group 3 received silymarin 100 mg/mg, while group 4, 5 and 6 received 250, 500 and 750 mg/kg of the extract respectively. General staining procedure Hematoxylin and Eosin and the specific staining technique, Gordon and Sweet silver impregnation Technique were carried out on the liver. Haematological and chempathological investigation were also done.

Result: In the groups pretreated with the extract there were slight areas of vacuolation, cellular degeneration, hepatocytic hyperplasia, cellular proliferation and Pyknotic nucleus compared to organotoxic group which revealed severe cellular degeneration, vascular congestion, hepatocytic hyperplasia and pyknotic nucleus in H&E stain. In Gordon and Sweet silver impregnation Technique, there were well structured reticular fibers with no obvious abnormality seen in the *H. letestui* administered group, while there were distortion and degeneration of the reticular fibres in the group that received paracetamol only.

Conclusion: Histological work suggests that the plant may prevent or protect the liver architecture.

Keywords: *Homalium letestui;* Liver; Paracetamol

Introduction

The liver is a reddish brown organ with four lobes of unequal size and shape [1]. Human liver normally weighs 1.44–1.66 kg, and is a soft, pinkish-brown, triangular organ. It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm. The liver lies to the right of the stomach and overlies the gallbladder. It is connected to two large blood vessels, one called the hepatic artery and one called the portal vein. The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels

subdivide into capillaries, which then lead to a lobule [2]. Each lobule is made up of millions of hepatic cells which are the basic metabolic cells. Lobules are the functional units of the liver [3].

The liver is a roughly triangular organ that extends across the entire abdominal cavity just inferior to the diaphragm. Most of the liver's mass is located on the right side of the body where it descends inferiorly toward the right kidney. The peritoneum connects the liver in 4 locations: the coronary ligament, the left and right triangular ligaments, and the falciform ligament. These connections are not true ligaments in the anatomical sense; rather, they are condensed regions of peritoneal membrane that support the liver[1]. The wide coronary ligament connects the central superior portion of the liver to the diaphragm. Located on the lateral borders of the left and right lobes, respectively, the left and right triangular ligaments connect the superior ends of the liver to the diaphragm [1]. *Homalium letestui* Pellegr (Flacourtiaceae) is a tree with a long straight slender bole attaining about 27 m height, occasionally up to 33 m, and to 1 m girth, of dense rain-forest, transition, semi-deciduous, galleried and secondary forests of lowlands and foothills in Senegal to Nigeria and Fernando Po, and also into central Africa to the Congo basin.

It prefers proximity to running water. Bark sap is applied as enema and bark pulp rubbed in to treat edema. Bark decoctions are taken in mixtures to treat orchitis and as tonic for women after childbirth. Root extracts are administered to treat malaria. The tree is decorative with its showy flowers, fruits and reddish young leaves, and is sometimes planted as ornamental [4]. The Yoruba of Nigeria call on the plant in an incantation against small-pox, while the bark, finely ground to a powder, is blown by Liberian witchdoctors into a dragon's lair to stupefy it before slaying it [5]. The aim of this work is to study the hepatoprotective potential of *Homalium letestui* using H&E and Gordon and sweet silver impregnation techniques.

Materials and Methods

Plants collection

Homalium letestui (stem) was collected in a forest in Uruan area, AkwaIbom State, Nigeria. It was identified and authenticated by Dr. Margaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen (FPUU 382) was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

Extraction

The stem was washed and dried under shed for two weeks. The dried plant material was then cut into smaller pieces and grounded to powder. The powdered material was macerated in 70% ethanol. The liquid filtrate was evaporated to dryness *in vacuum* 40°C using rotary evaporator. The ethanol extract was stored at -4^{0}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 water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

Animal treatment

36 rats were weighed and divided into six groups with 6 animals per group. Treatment was 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 and rats in this group were administered 100 mg/kg body weight of silymarin orally 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 the animals in group 2-6 were administered paracetamol 2000mg/kg body weight orally. Twenty hours later all animals were weighed again and sacrificed under light diethyl ether vapour.

Hematological study

Blood samples were collected from each rat by cardiac puncture immediately after the animals were sacrificed under diethyl ether anesthesia, using 21-gauge (21 G) needles mounted on a 5 ml syringe into ethylene diamine tetra-acetic acid (EDTA) - coated sample bottles for analyzed. 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). These parameters were analyzed using automatic hematological system.

Liver function test

Serum was separated from the blood of each animal sacrificed and the sera were stored at -20^{o}C in a freezer until used for biochemical determinations such as total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, total and direct bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols [6].

Histopathological examinations

The livers were processed and stained with haematotoxylin and eosin (H&E) and by Gordon and Sweet [7] silver impregnation technique. Prepared slides of the organs were mounted on high-defination microscope. The result was interpreted by a Pathologist in the Department of Chemical Pathology, University of Uyo, Uyo. Morphological changes in the excised organs of the sacrificed animals were observed and recorded. Histologic micrographs were taken.

Statistical Analysis and Data Evaluation

Data obtained from these studies were analyzed statistically using Students' t-test and ANOVA (One - way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5%, 1% and 0.1% level of significance i.e. $P \leq 0.05$, 0.01 and 0.001.

Result

Effect of treatment with ethanol stem extract of *Homalium letestui* on the hematological parameters of rats with paracetamol-induced liver injury

The administration of paracetamol (2 g/kgbw) to rats did not significantly affect ($p<0.05$) RBC and WBC counts as well as Hemoglobin concentration, PCV and neutrophils percentages of rats (Table 1). However, there were significant ($p<0.001$) reductions in the percentages of lymphocytes, monocytes and eosinophils of paracetamol-treated rats, while pretreatment with *H. letestui* stem extract caused significant ($p<0.05$ -0.001) increases against reductions induced by paracetamol though in non-dose dependent fashion.

Table 1: Effect of treatment with ethanol stem extract of *Homalium letestui* on the hematological parameters of rats with paracetamol –induced hepato-nephrotoxicity.

Parameters Treatment Dose (mg/kg)	RBC (X 1012/l)	PCV(%)	Hb (g/dl)	WBC (X 109/l)	Neutrophils. (%)	Lympho-cytes (%)	Monocytes (%)	Eosino-phils (%)	Basophils (%)	Platelet
Normal control	7.92±0.19	44.0±9.90	13.46±0.16	17.08±2.12	29.36±1.71	48.13±8.67	3.13±1.19	2.16±0.79	2.20±0.40	700.3±38.93
PCM +Dist. Water	7.75±0.55	43.0±2.00	13.21±0.31	16.34±2.18	24.57±8.71^{c}	21.24±13.32^{c}	2.50±1.30^{c}	1.17±0.97^{a}	0.2±0.20^{c}	665.3±15.31
Silymarin 100 mg/kg + PCM	7.57±0.20	44.2±1.16	13.50±0.35	11.66±1.54	16.59±7.88^{b,f}	21.87±10.07^{e}	0.50±0.02^{c}	1.51±0.70^{c,e}	0.00±0.00^{c}	547.5±26.62^{cf}
HL. 250 mg/kg +PCM	8.05±0.41	48.0±2.10	13.90±0.48	14.40±1.85	23.95±5.48^{c}	54.70±11.24^{b}	2.38±1.30^{c}	2.50±1.05^{c,e}	0.00±0.00^{c}	817.3±8.50^{a,d}
HL. 500 mg/kg+ PCM	7.41±0.46	44.0±1.00	12.51±0.25	11.43±1.17	32.37±9.82^{c}	23.50±12.05^{c}	4.62±0.50^{c,f}	2.17±1.10^{e}	0.00±0.00^{c}	908.5±98.94^{af}
HL. 750 mg/kg+ PCM	8.02±0.28	46.0±1.17	13.38±0.40	10.32±1.88	20.36±9.05^{c}	24.11±10.86^{b}	3.67±2.00^{c,e}	2.34±1.17^{a}	0.00±0.00^{c}	640.3±46.10

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control. dp< 0.05, ep< 0.01, fp< 0.001 when compared to paracetamol. n = 6

Effect of stem extract on liver weight of rats with PCM- induced hepatotoxicity

Table 2: Effect of *Homalium letestui* liver function of PCM –induced liver injury in rats.

Parameters/Treatment	Total Protein (g/dl)	Albumin (g/dl)	Total Bili-rubin (mg/dl)	Direct Bilirubin (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Cholestrol Mmol/L
Normal control	6.75± 0.14	4.12±0.68	3.61±0.28	1.21±0.71	109.33±3.04	35.33±3.59	202.15±12.75	3.98± 0.41
PCM +Dist. Water	3.43±0.27^{c}	1.66±0.22^{c}	5.01±0.61^{c}	1.31±0.09^{c}	168.33±3.46^{c}	96.50±4.07^{c}	303.0±22.85^{c}	7.67±0.25^{c}
Silymarin 100 mg/kg + PCM	6.72±0.11^{f}	4.27±0.99^{f}	4.16±0.44^{f}	1.20±0.22^{f}	112.60±7.63^{f}	44.16±3.48^{f}	248.16±26.92^{f}	5.05±0.29^{f}
Ext. 250 mg/kg + PCM	6.33±0.72^{a}	4.40±0.29^{d}	4.85±0.89^{e}	0.60±0.10^{b}	147.83±8.73^{f}	43.50±3.97^{cf}	283.0±24.60	6.70±0.19^{cf}
Ext. 500 mg/kg+ PCM	6.53±0.97^{e}	4.42±0.93^{e}	4.95±0.29^{f}	0.80±0.06^{f}	129.16±7.09^{f}	59.33±5.33^{cf}	278.5±31.14	6.54±0.09^{bf}
Ext. 750 mg/kg+ PCM	6.39±0.74^{f}	4.81±0.31^{f}	4.11±0.09^{f}	0.73±0.09^{f}	126.24±5.8^{af}	75.83±3.60^{ef}	276.66±25.85^{f}	5.98±0.24^{f}

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control.dp< 0.05, ep< 0.01, f< 0.001 when compared to paracetamol. n = 6

The weights of rat livers treated with paracetamol were significantly ($p<0.001$) increased when compared to that of the control group. However, animals in groups pre-treated with the stem extract and silymarin had their weight significantly ($p<0.01$ – 0.001) reduced when compared to paracetamol group (Table 2).

Effect of Homalium letestui stem on liver function test of paracetamol-induced liver injury in rats

Paracetamol (2 g/kg) caused a significant ($p<0.001$) elevation in the level of AST, ALT , ALP, total cholesterol, total and direct bilirubin and decreases in total protein and albumin levels of rats when compared to control. Pre-treatment with the stem extract of *H. letestui* (500 – 750 mg/kg bw) caused observable significant ($p<0.01$ - 0.001) decreases in enzyme levels and that of total cholesterol, total and direct bilirubin in the extract treated groups when compared with the paracetamol group. However, the decreases were dose-dependent. Total protein and albumin levels were significantly ($p<0.05$ - 0.001) elevated dose-dependently in the groups pre-treated with the stem extract when compared to the paracetamol group. The effects of the highest dose of the extract on all the parameters evaluated were comparable to that of silymarin (Table 3).

Table 3: Effect of *Homalium letestui* liver weight of PCM –induced liver injury in rats.

Parameters/Treatment	Liver Wt (g)
Normal control	6.53±0.23
PCM +Dist. Water	8.46 ± 0.16^{c}
Silymarin 100 mg/kg + PCM	6.56 ± 0.12^{f}
Ext. 250 mg/kg + PCM	7.63 ± 0.20^{bd}
Ext. 500 mg/kg+ PCM	6.76 ± 0.14^{f}
Ext. 750 mg/kg+ PCM	6.62 ± 0.15^{f}

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control. dp< 0.05, ep< 0.01, f< 0.001 when compared to paracetamol. n = 6

Histopathological studies of rat liver in paracetamol-induced hepatotoxicity

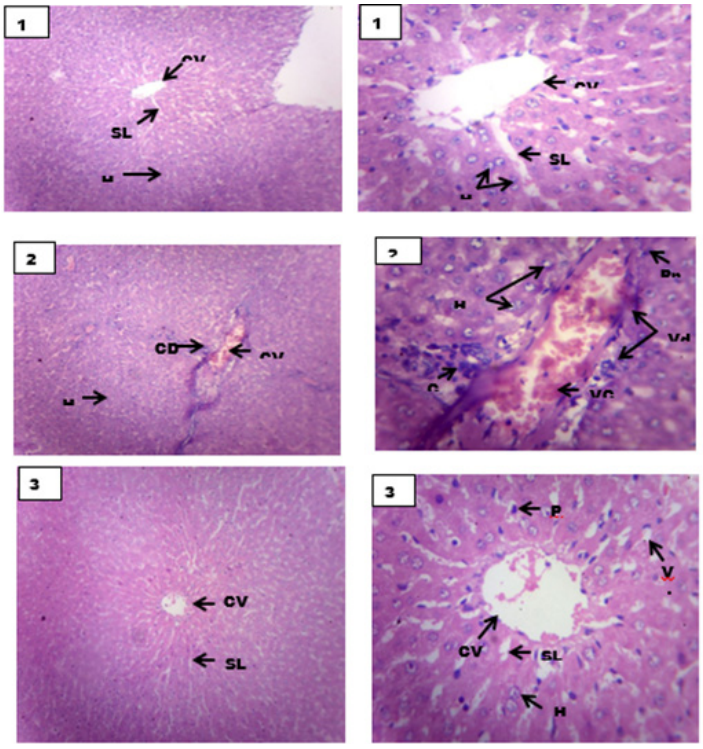

Figure 1: Histological sections of Livers of rats treated with Normal saline 10 ml/kg (1), Paracetamol 2000 mg/kg bw (2) and Silymarin 100 mg/kg bw and paracetamol 2000 mg/kg bw (3) at magnification A (x100) and B(x400) stained with H&E method.

Keys: CV: Central Vein; CD: Cellular Degeneration; V: Vacuolation; I: Inflammation; H: Hepatocyte; Pn: Pyknotic Nucleus; CV: Central Vein; SL: Sinusoidal Lining; HV: Hepatic Vein; Vd: Vascular Degeneration.

Histologic section of the liver treated with normal saline (10 ml/kg) at magnification A (x100) and B(x400) stained with H&E method reveled areas of cellular profile of central vein, sinusoidal lining and numerous hepatocyte all within normal cellular architecture. The organotoxic group revealed severe cellular degeneration, vascular congestion, hepatocytic hyperplasia and pyknotic nucleus. The silymarin group was observed to show similar effect as the control group. In the groups pretreated with the extract (Groups 4 –6) there were slight areas of vacuolation, cellular degeneration, hepatocytic hyperplasia, cellular proliferation and Pyknotic nucle-

us (Figure 1 & 2). Gordon and Sweet silver impregnation technique revealed well structure reticular fibre, no damages or abnormality in normal control and Silymarin treated group while there were distortion and degeneration of the reticular fibres in the group that received paracetamol only while there were well structured reticular fibers with no obvious abnormality seen in the *H. letestui* administered group (250- 750 mg/kg bw) (Figure 3 & 4).

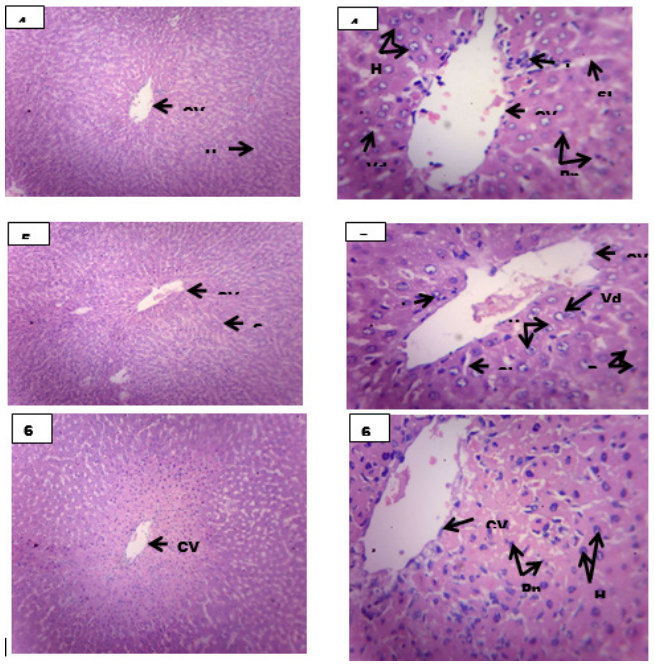

Figure 2: Histological sections of Livers of rats treated with Group *Homalium letestui* 250 mg/kg bw and Paracetamol (4), *Homalium letestui*500 mg/kg bw and Paracetamol 2000 mg/kg bw (5) and *Homalium letestui* 750 mg/kg bw and paracetamol 2000 mg/kg bw (6) at magnification A (x100) and B(x400) stained with H&E method.

Keys: CV: Central Vein; V: Vacuolation; Vd: Vascular Degeneration; SL: Sinusoidal Lining; I: Inflammation, H: Hepatocyte; Pn: Pyknotic Nucleus**.**

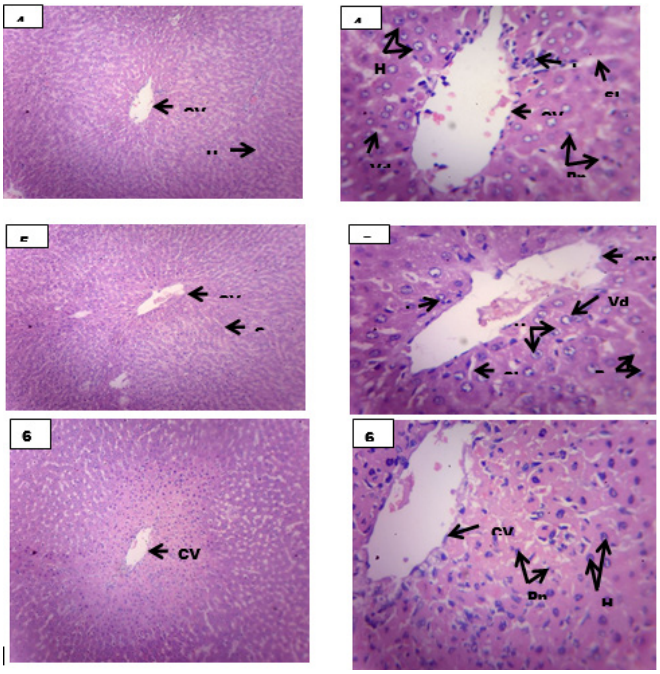

Figure 3: Histological sections of Livers of rats treated with Normal saline 10 ml/kg bw (1), Paracetamol 2000 mg/kg bw (2) and Silymarin 100 mg/kg bw and paracetamol 2000 mg/kg bw (3) at magnification A (x100) and B(x400) stained with Gordon and Sweet silver impregnation technique.

Keys: BD: Bile Duct; PT: Portal Triad; HA: Hepatic Artery; HV: Hepatic Vein; H: Hepatocytes; PT: Portal Triad; PTD: Portal Triad Degeneration, RF: Reticular Fiber; RFD: Reticular Fiber Degeration; RBC: Red Blood Cell.

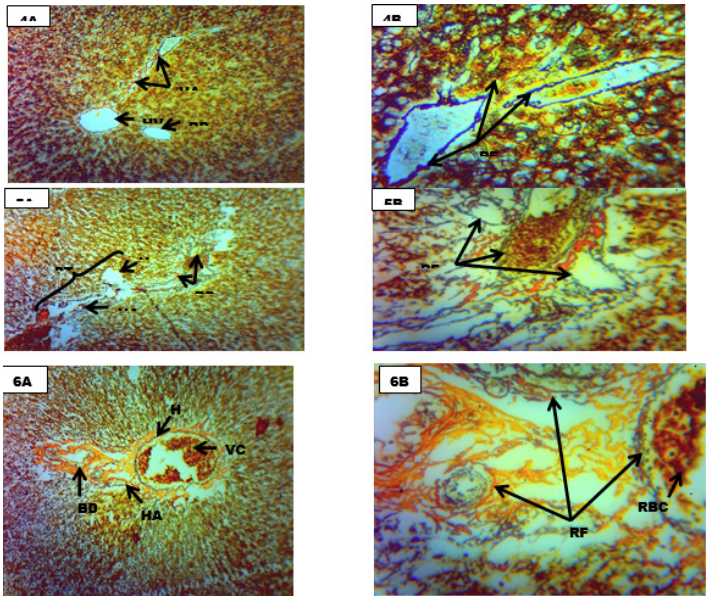

Figure 4: Histological sections of Livers of rats treated with *Homalium Letestui* 250 mg/kg bw and Paracetamol (4), *Homalium letestui* 500 mg/kg bw and Paracetamol 2000 mg/kg bw (5) and *Homalium letestui* 750 mg/kg bw and paracetamol 2000 mg/kg bw (6) at magnification A (x100) and B(x400) Gordon and Sweet silver impregnation technique.

Keys: BD: Bile Duct; HA: Hepatic Artery; HV: Hepatic Vein; H: Hepatocytes; RF: Reticular Fiber; PT: Portal Triad; RBC: Red Blood Cell.

Discussion

Histology is the study of tissues, including their role in the body, their anatomy, their interaction with body systems and the ways they are affected by disease [8]. Tissues are made from large groups of cells that cluster together to complete a shared function. From tissues arise organs, and organs keep the body operating. Histology can help gain a better understanding of cell behavior and reproduction, making cellular biology more understandable [9]. Likewise, because tissues are the building blocks of virtually everything in the body, understanding histology enables students to predict and understand organ behavior and function [10]. Staining is used to highlight important features of the tissue as well as to enhance the tissue contrast. Hematoxylin is a basic dye that is commonly used in this process and stains the nuclei giving it a bluish color while eosin (another stain dye used in histology) stains the cell's nucleus giving it a pinkish stain. However, there are other several staining techniques used for particular cells and components [11].

Result of the study showed that paracetamol did not significantly affect the haematological parameters of rats treated with it when compared to the control except reductions in the percentages of lymphocytes, monocytes and eosinophils of paracetamol-treated rats. This is an indication that there was no destruction of red blood cells and no change in the rate of production of RBC (erythropoiesis). The result also showed that paracetamol does not have the potential to induce erythropoietin release from the kidneys, which is the humoral regulator of RBC production [12]. The non-significant effect of treatment of rats with paracetamol also indicates that there was no change in the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC and haemoglobin (Hb) are very important in transferring respiratory gases [13]. It has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anaemic conditions while higher values are suggestive of polycythemia. Thus, the treatment of rats with paracetamol does not have the potential to induce anaemia or polycythemia.

Also, treatment of rats with paracetamol may not have adverse effects on the bone marrow, kidney and haemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and haemoglobin metabolism [14]. Result from the study inferred that ethanol extract of *Homalium letestui* at normal doses may have no toxic effect on the haematological parameters which include red blood cell count, haemoglobin concentration, packed cell volume and WBC but may stimulate the body immune system against disease or infection due to increased percentages of neutrophils, basophils, lymphocytes and monocyte as were observed in this study. Immunomodulatory activity of the stem extract of *H. letestui* has previously been reported [15]. There was significant improvement in the percentages of basophils and lymphocytes in groups pre-treated with extract which were significantly low in the organotoxic group. The increase maybe an immunological response by the body defence system to heal or repair injury done on the rat organ by paracetamol administration [16].

Decrease in the platelet level correlated with the study done by Shorr, Kao, Pizzo, Rauckman and Rosen [17] that normal platelet function is dependent on the production of proaggregatory thromboxane A_2(TxA_2) through COX-1, and acetaminophen has been shown to inhibit platelet function both *in vitro* and in high intravenous doses *in vivo*, which suggests that the plant may be able to reverse and protect against the thinning effect of paracetamol and may also decrease the risk of surgical bleeding. The effect of the

extract on the platelet level of the hepatotoxic rats appeared to be biphasic, that is, the effect was optimal at median dose used in this work, beyond which the effect was reversed. This may be due to the chemical constituents of the plant and the plant maybe acting as a partial agonist. The reversal of increased serum enzyme levels in paracetamol-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity.

Increase in serum level of ALP in the paracetamol group is due to increased synthesis, in the presence of increasing biliary pressure [18] and reflects the pathological alteration in biliary flow [19]. Therefore, an improvement in level of ALP in the pretreated rats provides a valuable indication that the plant may be useful in management and prevention of conditions such as of gallstone and cholescytitis. Paracetamol-induced toxicity in rats may have altered membrane structure and function as well as lipids metabolism in the liver as suggested by the increased cholesterol levels of rats. Alteration of bio-membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system [20] and this could affect lipid transport in the liver. This effect was reduced by the protective activity of the stem extract which reversed the level of total cholesterol, although it could not reduce it to the range of the control or that of the standard drug used in the experiment. This suggests that the extract help in preventing lipid peroxidation.

Histologically, H&E staining technique is used for general tissue structure observation. It's mainly used for observing nucleus, cytoplasm and any other abnormality base on general tissue property. Gordon and Sweet's silver staining method is a histological index for liver study with major focus on the reticular fiber. It is useful for demonstrating liver architecture; hepatocyte necrosis and hepatocyte regeneration through assessing the degree of fibrosis or damage to the reticular fibers [7]. In H and E staining, paracetamol caused severe cellular degeneration, vascular congestion, hepatocytic hyperplasia and pyknoptic nucleus which were much reduced in the *Homalium letestui* pretreated group. Gordon and Sweet silver impregnation technique revealed distortion and degeneration of the reticular fibres in paracetamol group while all extract - administered group showed well-structured reticular fibers with no obvious abnormality seen. Histology result agrees with other parameters and further confirms that the extract may exert a dose – dependent hepatoprotective effect on paracetamol - induced liver toxicity.

Okokon et al. [15] and Oyepata et al. [21] reported the presence of polyphenolic compounds such as vanillin, 2-Coumaranone, 3, 4, 5-trimethoxy phenol and 4-phenyl isocoumarin, and 4-(3-hydroxy-1-propenyl)-2-methoxy phenol and α-Terpineol as revealed by GC - MS analysis of the dichloromethane fraction. These compounds have been reported to exhibit antioxidant activity [22-25] highlighted the importance of vanillin in combating parasite infections by acting as an antioxidant. Also, Geoffrey, Eliud, Alex, Laura and Mungiria [26] reported that α-Terpineol and furan present in *Ocimum americanum* is responsible for antioxidant and antibacterial activities. The chemical constituent of the plant may reduce free radicals and ROS reaction with biomolecules, thus preventing the initiation of a chain reaction of free radical formation that consequently leads to tissue damage. The plant may be useful in the treatment and management of conditions like hepatitis, hepatotoxicity, liver fibrosis, cirrhosis or liver cell cancinoma.

Conclusion

Result from the study suggests that *Homalium letestui* possesses hepatoprotective activity on vital organs and cells in the body. The plant may be useful in the treatment and management of conditions like hepatitis, hepatotoxicity, liver fibrosis, cirrhosis or liver cell cancinoma.

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