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EFFECT OF ETHANOL STEM EXTRACT OF *HOMALIUM LETESTUI* ON HISTOLOGICAL FEATURES ON KIDNEY OF PARACETAMOL-INDUCED ALBINO RAT INJURY, USING VARIOUS STAINING TECHNIQUES

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ABSTRACT

Introduction/Aim: Medicinal plant is normally used by most people in under developed and developing countries. In this study the effect of ethanol stem extract of *Homalium letestui* on histological features of paracetamol-induced albino rat injury, using various staining techniques.

Method: Thirty six (36) rats where used for this work. Group one served as the positive control receiving normal saline, group two served as organotoxic group receiving paracetamol 2000 mg/kg body weight, group 3 received silymarin 100 mg/mg bw, while group 4, 5 and 6 received 250, 500 and 750 mg/kg of the extract respectively. General staining technique Hematoxylin and Eosin and specific observation technique, Bence Jones which focuses on glomeruli and Bowman's capsule, were used. Haematological and chempathological investigation were also carried out.

Result: In Bence Jonces technique, there was severe basement membrane degeneration of the Bowman's capsule in the organotoxic group. There was intact basement membrane structure in the normal and Silymarin group, while there was dose dependent improvement in the architecture of Bowman's capsule for the pretreated rats. In Hα E, Paracetamol treated rats showed severe damage in the kidney cells, which were revealed as severe area of epithelial lining degeneration, glomerular inflammation, Tubular necrosis and vascular degeneration. Pretreatment with stem extract of *Homalium letestui* (250 -750 mg/kg) and standard drug, Silymarin (100 mg/kg) helped in reducing the cellular damage induced by Paracetamol. **Conclusion:** Histological work agrees with other parameters suggesting that the plant may prevent or protect kidney architecture

KEYWORDS:*Homaliumletestui*, nephroprotective, paracetamol, rat

. Introduction

The kidneys are two bean-shaped organs present in left and right sides of the body in vertebrates[1]. They are located at the back of the abdominal cavity. They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached to a ureter, a tube that carries excreted urine to the bladder.[1]In humans, the kidneys are located high in the abdominal cavity, one on each side of the spine, and lie in a retroperitoneal position at a slightly oblique angle[2]. The asymmetry within the abdominal cavity, caused by the position of the liver, typically results in the right kidney being slightly lower and smaller than the left, and being placed slightly more to the middle than the left kidney.[2,3,4]. The microscopic structural and functional unit of the kidney is the nephron. It serves the purposes of filtration, reabsorbtion, secretion, excretion, hoemostasis, acid base balance, regulation of osmolality, blood pressure regulation and hormone secretion.[5,6]

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Homalium letestui is a plant of various tradomedical importances. It occurs from Senegal east to the Central African Republic and south to western DR Congo and Cabinda (Angola). The fruits are showy and the young leaf-flush is red before turning green. The tree is thus attractive and worthy of cultivation[7]. In Ivory Coast sap from the bark is used in enemas for the treatment of generalized oedemas while lees from the bark are rubbed over the area[8]. Stem bark and root are used in various decoctions by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, malaria and other inflammatory diseases and also as an aphrodisiac by the Yorubas of Western Nigeria[9].Okokon[10] reported the presence of α-terpineol, Vanillin, 4-phenyl isocoumarin, 3,4,5-trimethoxy phenol, 2-Coumaranone, and xanthones in the stembark extract of *H. letestui*. Also, antiplasmodial[11], antidiabetic[12], anti-inflammatory and analgesic[13], cellular antioxidant, anticancer, and antileishmanial[13], depressant and anticonvulsant[11] antibacterial[12], *in vitro* antioxidant activity against DPPH[11], antiulcer13 and antidiarrheal[12] activities of the plant have been established. Various studies have been done

on the pharmacological activities of Homalium letestui with only one staining technique been employed in most cases. The histopathological nephroprotective ability of ethanol stem extract of *Homalium letestui* plant was studied against carbon paracetamol induced kidney injury using Bence Jonces and Hematoxylin and Eosin staining 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

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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°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, recieved 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

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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.

Kidney function test

The following biochemical parameters were assayed as markers of kidney function using diagnostic kits; Level of electrolytes (Na^+, K^+, Cl^-, and HCO_3^-), creatinine and blood urea.The above parameters were determined at the Chemical Pathology Department of University of Uyo Teaching Hospital.

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Histopathological examinations

The kidneys of each animal used in the study were surgically harvested and fixed in buffered formalin. Kidneys were processed and stained with haematotoxylin and eosin and by Bence Jones Methylamine silver impregnation technique according to the standard procedures. Prepared slides of the organs were mounted on high-defination microscope. The result wereinterpreted 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 study 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 nephrototoxicity.

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.

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Effect of stem extract on the levels of Kidney antioxidant enzymes and compounds

Paracetamol treatment caused significant ($p<0.001$) decreases in the activities of SOD, catalase and GSH levels and significant ($p<0.001$) increase in the concentration of MDA in kidney tissue when compared with control group (Table 3). Pre-treatment with stem extract of *Homalium letestui* (250 – 750 mg/kg) resulted in significant ($p<0.05 – 0.001$) increase in the activities of SOD, catalase and GSH levels as well as significant ($p<0.001$) decrease in the concentration of MDA. Silymarin treated animals also showed a significant ($p<0.001$) increase in antioxidant enzymes activities; SOD, catalase and GSH levels with significant ($p<0.001$) decrease in the level of MDA compared to paracetamol treated rats (Table 2).

Effect of stem extract on kidney weight

The kidney weights of rats 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 (500 - 750 mg/kg) and silymarin (100 mg/kg) had their kidney weights significantly ($p<0.01 – 0.001$) reduced (Table 3).

Evaluation of effect of *Homalium letestui*stem on kidney function parameters of paracetamol-induced kidney injury in rats.

Treatment of rats with paracetamol (2g/kg) caused significant ($p<0.01-0.001$) elevation of serum urea and creatinine levels when compared to normal control. These increased levels of serum urea and creatinine were significantly ($p<0.05 - 0.001$) reduced with the pretreatment of the rats with silymarin and stem extract of *Homalium letestui*(500 – 750 mg/kg) in a dose dependent fashion. However, the administration of paracetamol did not

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affect the electrolytes (K^+, Na^+, Cl^- and HCO^-_3) levels of rats. Pretreatment of the rats did not also affect the levels of the electrolytes as there was no significant ($p>0.05$) different with that of the control rats. (Table 4).

Histopathological studies of rat kidney in paracetamol-induced nephrotoxicity

Histopathological study revealed the normal renal architecture in control group showing normal tubular brush borders and intact glomeruli and Bowman's capsule. Paracetamol treated rats showed severe damage in the kidney cells observed as severe area of epithelial lining degeneration, glomerular inflammation, Tubular necrosis and vascular degeneration (Figure 2 and 3). Pretreatment with stem extract of *Homalium letestui* (250 -750 mg/kg) and standard drug, Silymarin (100 mg/kg) helped in decreasing the cellular damage induced by Paracetamol (Figure 2 and 3). In the Bence Jonces technique, there was basement membrane degeneration of the Bowman's capsule. There was intact basement membrane structure in the normal and Silymarin group, while there was dose dependent improvement in the architecture of the pretreated rats (Figure 4 – 5).

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

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	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	$16.59\pm7.88^{b,f}$	21.87 ± 10.07^{e}	0.50 ± 0.02^{c}	$1.51\pm0.70^{c,e}$	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}	$2.50\pm1.05^{c,e}$	0.00 ± 0.00^{c}	$817.3\pm8.50^{a,d}$
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}	$4.62\pm0.50^{c,f}$	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}	$3.67\pm2.00^{c,e}$	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

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Table 2: Effect of *Homalium letestui*stem extract on kidney antioxidant enzymes in Paracetamol-induced kidney injury in rats.

PARAMETERS/ TREATMENT	SOD (U/mg of protein)	CAT (U/mg of protein)	MDA (U/mg of protein)	GSH (μg/mg of protein)
Normal control	22.30 ± 0.22	50.38±0.86	48.22±1.55	0.36±0.01
PCM +Dist. Water	5.26 ± 0.30^{c}	20.14±1.13^{c}	81.38±3.55^{c}	0.11±0.01^{c}
Silymarin 100 mg/kg + PCM	19.20 ±0.18^{cf}	50.71± 1.44^{f}	50.24±1.24^{f}	0.28±0.01^{f}
HL. 250mg/kg + PCM	9.32 ± 0.28^{cd}	30.05±0.98^{cd}	70.68±2.14^{c}	0.14±0.01^{ce}
HL. 500mg/kg+ PCM	14.45 ± 0. 32^{bf}	34.34±1.33^{f}	58.22±2.16^{cf}	0.19±0.01^{bf}
HL. 750mg/kg+ PCM	18.56± 0.15^{cf}	42.11±1.25^{f}	54.14±1.25^{bf}	0.23±0.01^{af}

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.

Table 3: **Effect of ethanol stem extract of *Homalium letestui* on kidney weight**

Parameters/ Treatment	Kidney (g)
Normal control	1.40±0.02
PCM +Dist. Water	2.61±0.03^{c}
Silymarin 100 mg/kg + PCM	1.46±0.02^{f}
HL. 250 mg/kg + PCM	1.54±0.04^{cd}
HL. 500 mg/kg+ PCM	1.50±0.03^{f}
HL. 750 mg/kg+ PCM	1.45±0.04^{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.

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Table 4: **Effect of ethanol stem extract of *Homalium letestui* on kidney weight and kidney function parameters of rat**

Parameters/ Treatment	Na^+ (mmol/l)	K^+ (mmol/l)	CL^- (mmol/l)	HCO^-_3 (mmol/l)	UREA (mmol/l)	Creatinine (mmol/l)
Normal control	72.68±1.61	7.48±0.38	67.86±1.56	15.6±0.74	5.16±1.02	62.5±3.66
PCM +Dist. Water	78.35±3.57	7.74±0.31	70.15±3.42	18.77±0.89	9.98±1.05^c	87.33 ±4.84^b
Silymarin 100 mg/kg + PCM	65.40±1.42	6.34±0.12	65.4±1.19	18.28± 0.15	6.83±0.80^d	56.50±5.70^f
HL. 250 mg/kg + PCM	67.22±1.47	6.26±0.21	62.63±0.89	17.46±0.60	5.78±0.85^e	48.16±1.95^f
HL. 500 mg/kg+ PCM	76.32 ± 1.41	6.34±0.07	55.83±1.61	17.30± 0.18	5.48±0.84^e	64.83±6.33^d
HL. 750 mg/kg+ PCM	73.59 ± 1.58	5.46±0.17	54.53±1.49	16.82± 0.36	5.15±0.27^e	51.16±1.51^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.

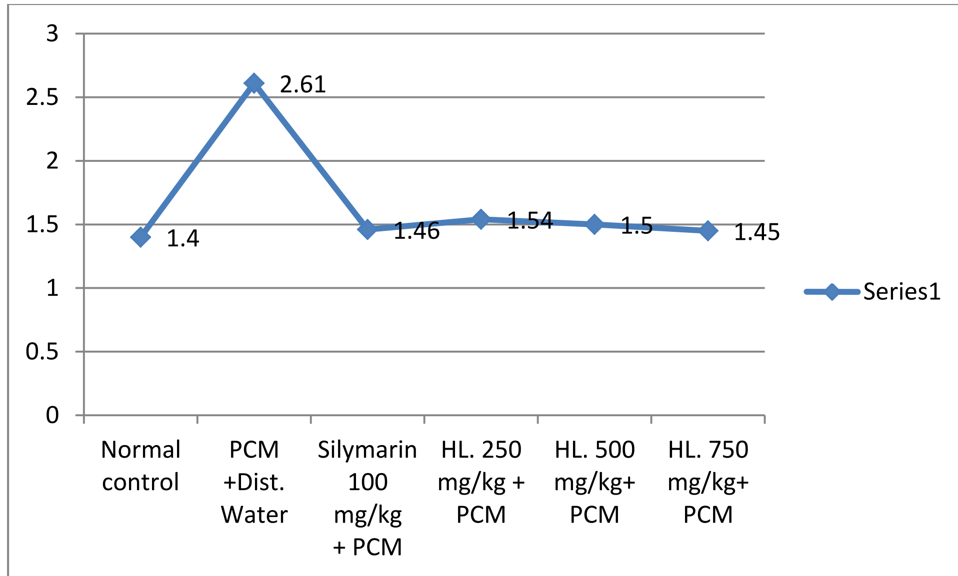

Figure 1: Graph showing effect of paracetamol and extract on weight (on the y-axis) of rat kidney

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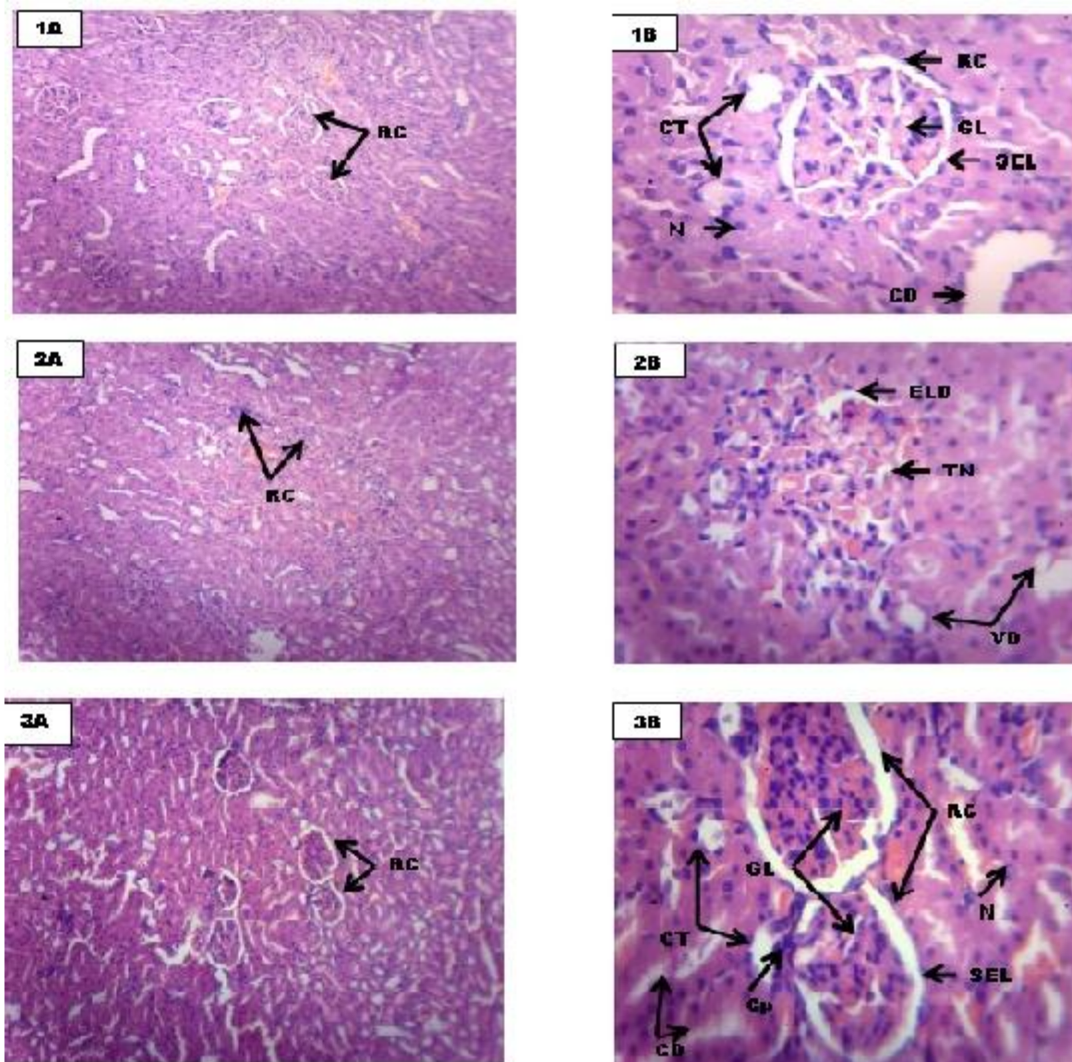

Figure 2: Histological sections of kidneys of rats treated with Normal saline 10 ml/kg bw (1), Paracetamol 2000 mg/kg bw (2) and Silymarin 100 mg/kg mg/kg bw and paracetamol 2000 mg/kg bw (3) at magnification A (x100) and B(x400) stained with H&E technique.

Keys: Renal corpuscle (**RC**), Vascular degeneration (Vd), Convoluted tubules (**CT**), Squamous epithelial lining (**SEL**), Glomerulus (**GL**), Epithelial lining degeneration (**ELD**),Tubular necrosis (TN),Collecting ducts (**CD**) and Nucleus **(N)** and vascular degeneration **(VD)**

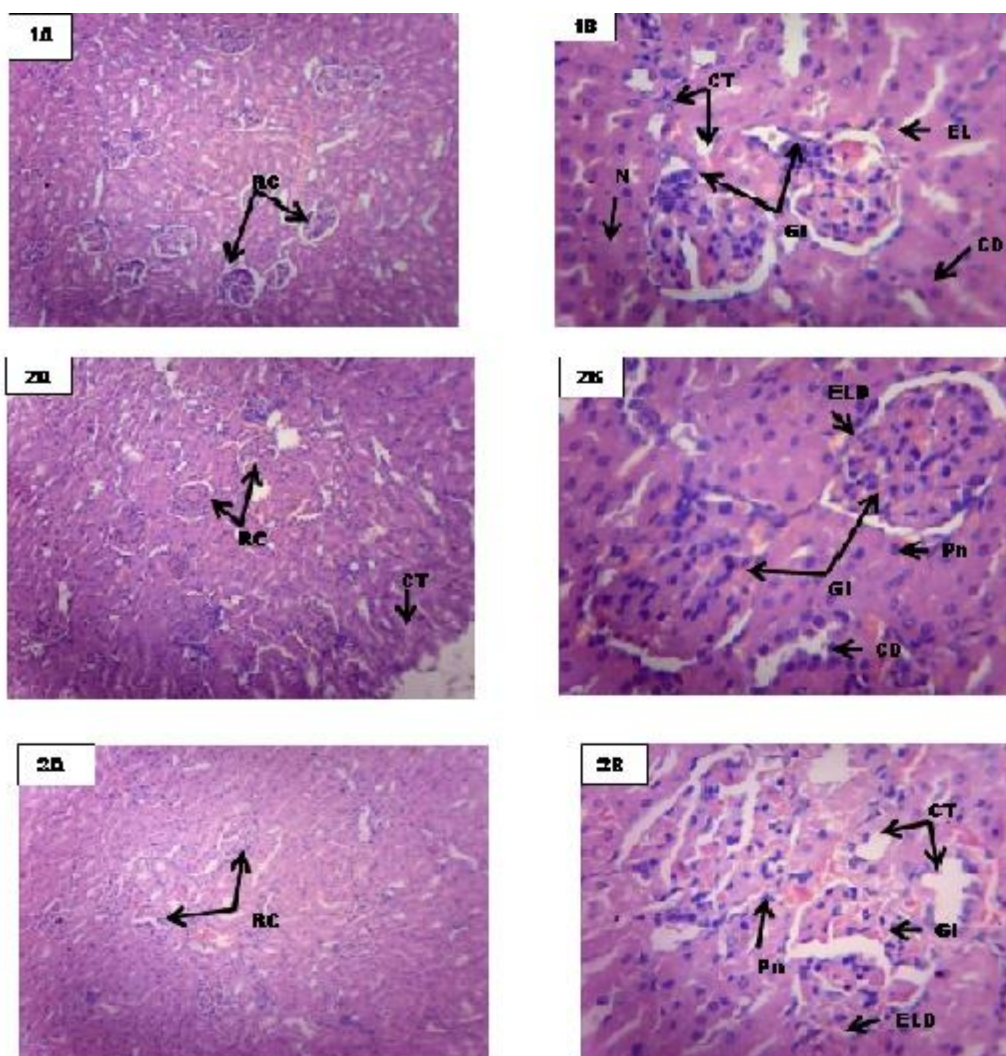

Figure 3: Histological sections of Kidneys of rats treated with *Homalium letestui*250 mg/kg bw and Paracetamol 2000 mg/kg bw (1), *Homalium letestui*500 mg/kg bw and Paracetamol 2000 mg/kg bw (2) and *Homalium letestui*750 mg/kg bw and paracetamol 2000 mg/kg bw (3) at magnification A (x100) and B(x400) Stained with H&E technique.

Keys: Renal corpuscle (**RC**) Convoluted tubules (**CT**), Collecting ducts (**CD**), Epithelial lining

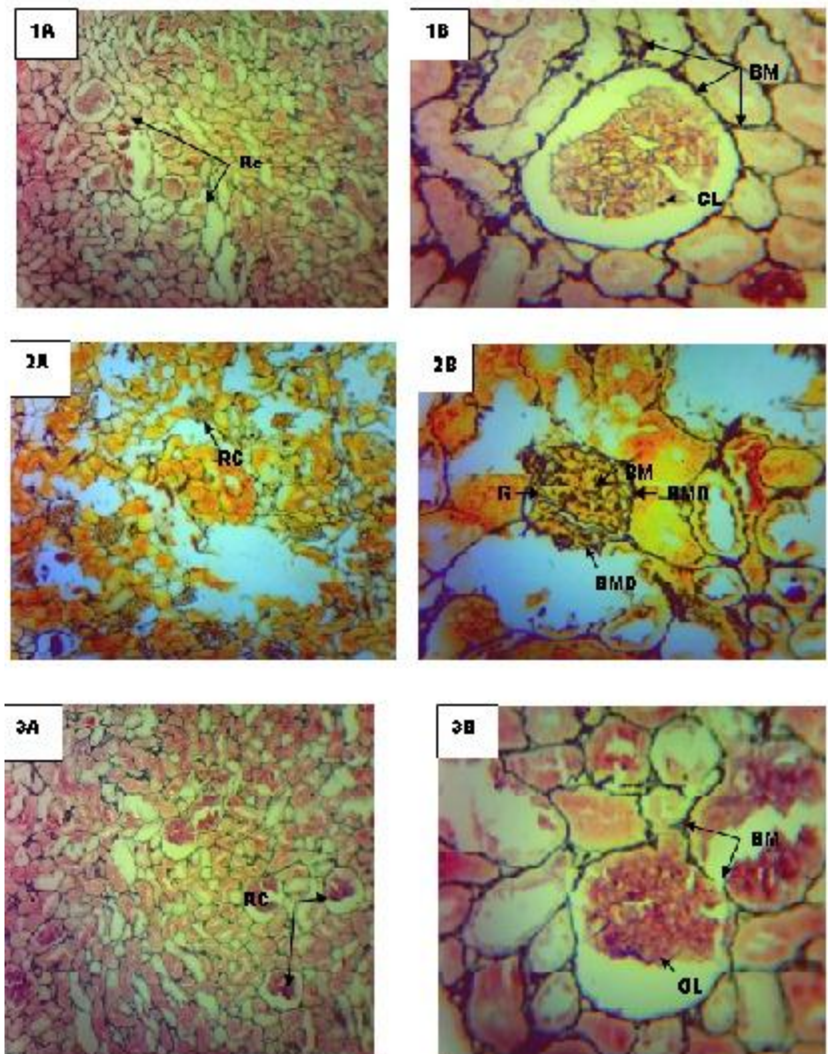

Figure 4: Histological sections of kidneys of rats treated with Normal saline 10 ml/kg bw (1), Paracetamol 2000 mg/kg bw (2) and Silymarin 100 mg/kgbw and paracetamol 2000 mg/kg bw (3) at magnification A (x100) and B(x400) stained with Bence Jones Methylamine silver impregnation Technique.

Keys: Renal corpuscle (**RC**) Basement membrane **(BM), Base** membrane degeneration (BMD),and Glomerulus **(GL)**

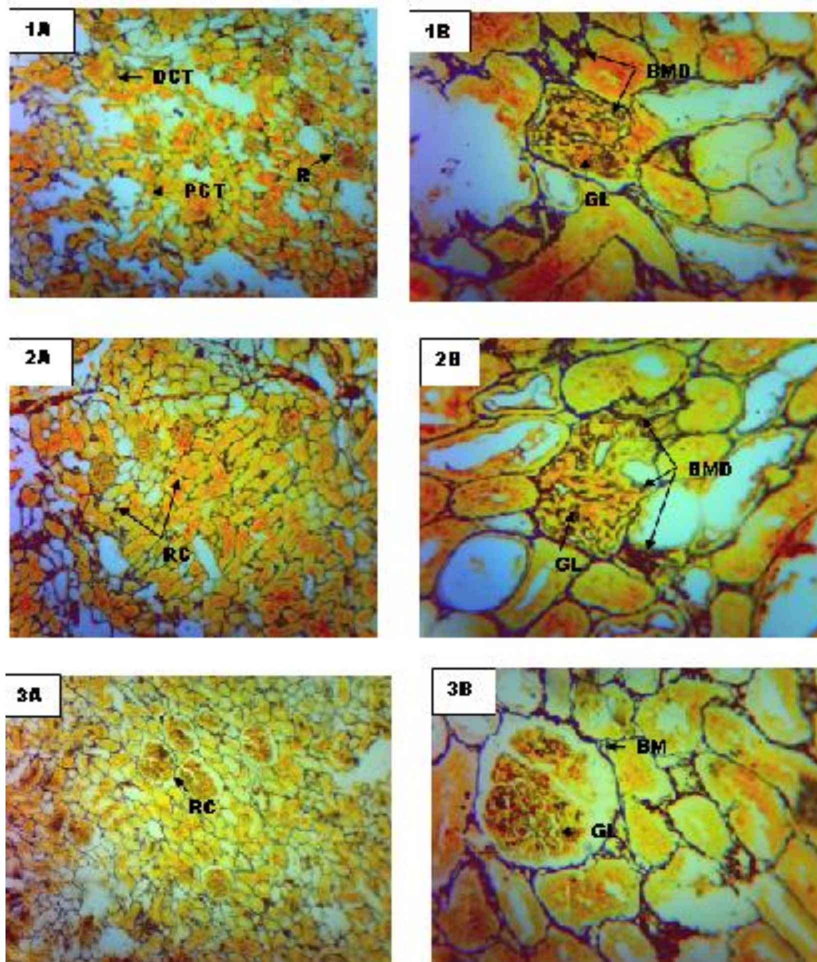

Figure 5: Histological sections of Kidneys of rats treated with *Homalium letestui* 250 mg/kg bw and Paracetamol 2000 mg/kg bw (1), *Homalium letestui* 500 mg/kg bw and Paracetamol 2000 mg/kg bw (2) and *Homalium letestui* 750 mg/kg bw and paracetamol 2000 mg/kg bw (3) at magnification A (x100) and B(x400) stained with Bence Jones Methylamine silver impregnation Technique

Keys: Renal corpuscle (**RC**) Basement membrane **(BM)** and Glomerulus **(GL)**

DISCUSSION

The kidney is a vital organ in the body that is responsible for numerous physiological responsibilities. Any damage or injury to the integrity of the kidney can precipitate severe consequences. Paracetamol, produces toxic effect at high doses which leads to tissue damage. The drug is bioactivated to a toxic electrophile, *N*-acetyl *p*-benzoquinone imine (NAPQI), which binds covalently to tissue macromolecules, and probably oxidizes lipids, or the critical sulphydryl groups (protein thiols) as well as alters the homeostasis of calcium[13]. In the kidney, p-amino phenol is normally formed from paracetamol by deacetylation and excreted in urine. This exposes the kidney to damage by p-amino phenol as it plays a major role in the pathogenesis of paracetamol induced renal damage[14]. Hepatically derived glutathione conjugates are also involved in paracetamol - induced renal injury[15] as well as nitric oxide[16]. Nephrotoxic doses of paracetamol to rats resulted in development of oxidative stress damage in renal tissues. Consequently, the group treated with paracetamol showed significant ($p<0.01$) increase in the serum urea and creatinine concentrations when compared to the normal group. However, pretreatment of the rats with *H. letestui* stem extract, significantly ($p<0.01$) decreased these parameters in a dose dependent manner when compared to the paracetamol group, thus, offering explanation for nephroprotective activity of the plant stem extract. These results are in agreement with those observed by Isik[17]. This elevation in the levels of urea and creatinine was explained by the presence of strong correlation between nephrotoxicity and oxidative stress. Also, there were reductions in the levels of antioxidant enzymes SOD and CAT and antioxidant molecule, reduced gluthatione whereas there was elevated level of MDA. This suggests that the toxic paracetamol metabolite deplete or inhibit the function of physiological molecule against the activities of free reactive radical. These parameters were significantly improved in the extract

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pretreated groups providing information that the extract has the potential to prevent lipid peroxidation and to improve physiological moiety that improves the integrity of the kidney.

It has been proposed that oxidative stress may be responsible for tubular damage. It is well known that the production of ROS causes cell damage due to cytotoxic action of oxygen and nitrogen derived free radical species[19,20,21]. Natural antioxidant systems are inactivated by lipid peroxidation and reactive oxygen species (ROS) [21]. Antioxidants act as cell's defense against free radicals. Enzymatic antioxidants such as SOD, CAT and GPx are the first line of defense against oxidative injury. In the present study, significant decreases in SOD and CAT activities in the paracetamol group could have resulted from ineffective scavenging of ROS and possibly, oxidative inactivation of enzymes[22]. CAT acts as a preventive antioxidant and plays an important role in protecting against the deleterious effects of lipid peroxidation. A significant decrease in catalase activity during paracetamol ingestion indicates inefficient scavenging of H_2O_2[23]. GPx catalyses the oxidation of GSH to GSSG at the expense of H_2O_2[23]. Decreased GPx activity was also observed in paracetamol treatment. This also agrees with Cerutti[22] reports. Thus paracetamol administration suppresses the activities of these enzymatic antioxidants and renders the cells more susceptible to free radical - induced injury. In this study, the concentration of GSH was significantly reduced in paracetamol treated rats, which corroborates other reports[24]. The reduced form of GSH therefore becomes readily oxidized to GSSG on interacting with free radicals[25]. This reduction was significantly reversed by extract pretreatment of the rats suggesting nephroprotective activity.

The nephron- protective property of the extract is further confirmed by significant improvement of the kidney architecture by reversing the paracetamol – induced glomerular congestion, inflammatory cells, tubular necrosis, peritubular necrosis and basement degeneration

in the pretreated group as reveled in H and E stain. Bence Jones stain revealed that paracetamol caused severe degeneration of basement membrane of the rat's kidney. Bence Jones staining technique also revealed that the extract significantly and dose - dependently protected the basement membrane with intact membrane architecture at the high dose administered. This effect may be due to the antioxidant activity of the plant extract. Literature has shown that medicinal plants with nephroprotective properties mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids, phenol and other active compounds[26]. This is in agreement with the findings of this study. The phenolic and flavonoid component of the leave extract may be responsible for this effect. Flavonoids, tannins, and phenols have been reported to exert profound *in vitro* and *in vivo* stabilizing effect on the lysosomes of experimental animals[27,28]. Plant flavonoids which show an antioxidant activity in vitro also function as antioxidants *in vivo*[29,30].

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Conclusion

Result of the histological analysis correlates with biochemical parameters showing that the plants has kidney protecting ability against paracetamol induced kidney injury in rat, which is of immense potential in preventing and managing kidney disease.

Conflict of Interest

There is no potential conflict of interest in this research work

Aknowledgement

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