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Original Research Article

Hepatoprotective and Nephroprotective Activities of Husk Extract of *Zea mays* Against Paracetamol-Induced Liver and Kidney Injuries In Rats

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ABSTRACT

Zea mays L (Poaceae) is used traditionally by the Ibibios of Southern Nigeria to treat stomach ulcer, malaria, inflammatory diseases and as an antidote. The husk extract of *Zea mays* (187-561 mg/kg) was investigated for hepato- and nephroprotective potentials against paracetamol-induced liver and kidney toxicities in rats to ascertain the folkloric claim of its usefulness in the treatment of poisoning. Assays of liver and kidney function parameters as well as histopathological study of the liver and kidney were used to assess hepatoprotective and nephroprotective activities of husk extract. Administration of the husk extract (187-561 mg/kg) caused significant ($p<0.05$-0.001) reductions in the levels of liver biomarker enzymes (ALT, AST, and ALP), direct and total bilirubin and elevation of serum level of total protein. The husk extract also caused significant ($p<0.05$) reduction of high levels of serum creatinine, urea and electrolytes concentrations (K^+, Na^+, Cl^- and HCO^-_3) caused by the toxicant. The effects were dose-dependent in most cases. Histology of the liver and kidney sections of extract and silymarin-treated animals showed reductions in the pathological features compared to the organotoxic-treated animals. The biochemical changes were consistent with histopathological observations suggesting marked hepatoprotective and nephroprotective potentials. The results showed that husk extract of *Zea mays* has hepatoprotective and nephroprotective potentials against injurious agents which may be due to the activities of its phytochemical components.

Keywords: *Zea mays*, husk,liver, hepatoprotective, nephroprotective.

Introduction

Zea mays L.(Poacae) also known as maize or corn, is an annual grass plant cultivated in Nigeria primarily for human consumption and as animal feed. The plant is tall with a fibrous root system and has long narrow leaves on opposite side of the stem and bears ears that are enclosed in modified leaves known as husks.[1] In addition to its nutritive values, various parts of the plant are also used in ethnomedicine for the treatment of several ailments. The corn silk is used as an antidiabetic or diuretic, and decoction of the silk is consumed for the treatment of urinary troubles and gallstones.[2-4]The husks are used for the treatment of pains and arthritis,[5] ulcer,[6] malaria and type 2 diabetes in Ibibio traditional medicine.[7]The husk extract has been reported to possess some pharmacological properties which include; analgesic, anti-inflammatory,[5] antioxidant,[8] antidepressant,[9] antimalarial and antiplasmodial,[7] hepatoprotective,[10] nephroprotective,[11]antidiabetic and hypolipidaemic,[12] and antiulcer[13]activities. The median lethal dose (LD_{50}) of the ethanol husk extract was determined to be 1874.83 mg/kg.[9]Arabinoxylan, which has immunological effects, has been isolated from the husk extract,[14]while eight phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, femlic acid, rutin, resveratrol, and kaempferol) have also been detected in ethanol husk extract of *Zea mays*.[8]Corn husk has also been reported to be rich in anthocyannins.[15]In this study, we report the hepatoprotective and nephroprotective activities of the husk extract against paracetamol-induced liver and kidney injuries in rats to confirm its use in the treatment of liver and kidney diseases in ethnomedicine.

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Materials and Methods

Collection of plant materials

Fresh husks of *Zea mays* were collected in August, 2018 from Farmland in Uyo in Uyo LGA, Akwa Ibom State, Nigeria. The husks were identified and authenticated as *Zea mays* by a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPH, 614) was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

Extraction

The plant parts (husks) were washed, cut into smaller pieces and air-dried on laboratory table for 2 weeks. The dried husks were pulverized using electric grinder.The powdered husk (1.5 kg) was macerated in 50% ethanol for 72 hours. The liquid filtrate obtained was concentrated and evaporated to dryness in vacuo at 40°C using rotary evaporator. The crude extract (yield 2.83%) was stored in a refrigerator at -4°Cuntil they were used for the experiments.

Animals

Wistar male rats (150 – 165 g) used for these experiments were obtained from the Animal house of Department of Pharmacology and Toxicology, University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Permission and approval for animal studies were

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obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CHS/AE/19/034).

Effect of Zea mays husk extract on paracetamol-induced liver and kidney toxicities in rats

In this work, 36 male young adult rats were divided into 6 groups with each group consisting of 6 rats per group. The experimental treatment and design of the group was as follows:

Group 1 (Control group): Rats were orally administered 10 mL/kg body weight distilled water per oral for 8 days.
Group 2 (Organotoxic group): Rats were administered 10 mL/kg body weight distilled water for 8 days.
Group 3 (Standard group): Rats were administered 100 mg/kg body weight Silymarin per oral for 8 days.
Group 4 (Low dose test group): Rats were administered 187 mg/kg body weight of *Zea mays* husk extract orally for 8 days.
Group 5 (Middle dose test group): Rats were administered 374 mg/kg body weight *Zea mays* husk extract orally for 8 days.
Group 6 (High dose test group): The animals in this group were administered 561 mg/kg body weight *Zea mays* husk extract orally for 8 days.
On the 8th day, animals in groups 2-6 received paracetamol at a dose of 2000 mg/kg per body weight orally. Twenty hours later all the animals were weighed again and sacrificed under light anaesthesia using diethyl ether vapour.

Collection of blood samples and organs

After 8 days of treatment (24 hours after the last treatment) the rats were weighed again and sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture and used immediately. Blood were collected into plain centrifuge tubes and EDTA bottles. The blood in the centrifuge tubes were centrifuged immediately at 2500 rpm for 15 minutes to separate the serum at room temperature to avoid haemolysis and used for biochemical assays. The blood samples collected into EDTA bottles were used for haematological analysis. The livers and kidneys were surgically removed, weighed and fixed in 10% formaldehyde for histological process.

Haematological analyses

The following haematological parameters were determined; Haemoglobin concentration (Hb), packed cell volume (PCV), total and differential white blood cell count (WBC), Platelet count, red blood count (RBC). These parameters were determined at Haematology Department of University of Uyo Teaching Hospital using automated Haematologyanalyser.

Biochemical analysis

Liver function test

The following parameters were determined; aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total plasma protein and total and direct bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols [16] at the Chemical Pathology Department of University of Uyo Teaching Hospital.

Kidney function test

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

Histopathological analysis

The kidneys of each animal that were used in the study were surgically harvested and fixed in buffered formalin. They were then processed and stained with haematotoxylin and eosin (H&E) for kidney and liver study according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Morphological changes observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

Statistical analyses

Data obtained from this work were analysed statistically using ANOVA (one –way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance i.e. $p \leq 0.05$.

Results and Discussion

Effect of treatment with ethanol husk extract of Zea mays on the haematological parameters of rats with paracetamol-induced hepatotoxicity.

The administration of paracetamol (2 g/kg) to rats caused significant ($p<0.01$) reduction in RBC counts, haemoglobin concentration, packed cell volume and platelets counts when compared to control. However, pretreatment with *Zea mays* husk extract reversed the reductions significantly ($p<0.001$) in a dose-dependent fashion. Also, WBC counts, lymphocytes, monocytes, neutrophils and basophils percentages of rats were significantly ($p<0.001$) increased by paracetamol. These increases were significantly ($p<0.001$) reduced in a dose-dependent manner by pretreatment with the husk extract (Table 1).

Effect of husk extract on liver weight of rats with Paracetamol-induced hepatotoxicity

The liver 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 husk extract and silymarin had their weight significantly ($p<0.01 – 0.001$) reduced when compared to control (Table 2).

Effect of husk extract of Zea mays on liver function test of paracetamol-induced liver injury in rats

Administration of paracetamol (2 g/kg) to rats caused a significant ($p<0.001$) elevation in the level of AST, ALT, ALP, total and direct bilirubin and decrease in total protein level when compared to control. Pre-treatment with the husk extract of *Zea mays* (187 – 561 mg/kg) caused observable significant ($p<0.001$) decreases of these enzymes levels and that of total and direct bilirubin in the extract-treated groups when compared with the paracetamol group. Also, the decreases were dose-dependent. Total protein level was significantly ($p<0.001$) elevated dose-dependently in the groups pre-treated with the husk extract when compared to the paracetamol group (Table 2).

Histopathological studies of rat liver in paracetamol-induced hepatotoxicity

Histologic sections of livers of rats receiving various treatments at magnification B(x400) stained with H&E method revealed that Group 1 (normal control) treated with distilled water (10 mL/kg) had normal cellular profile of portal triad, bile duct, hepatic artery, hepatic vein, hepatocytes and nucleus, all within normal cellular architecture (Figure 1). The organotoxic group (Group 2) treated with paracetamol (2 g/kg) revealed cellular area of cellular abnormalities including portal and peri portal inflammation, vacuolation, presence of kuffer cells, pyknotic nuclei, vascular degeneration, congestion when compared to control group (Figure 1). The silymarin treated rats (Group 3) revealed moderate area of cellular abnormalities including portal and peri portal inflammation, vacuolation, pyknotic nuclei, vascular degeneration, congestion when compared to control group (Figure 1). Group 4 rats treated with husk extract (187 mg/kg) revealed moderate area of cellular abnormalities including portal and peri portal inflammation, vacuolation, pyknotic nuclei, vascular degeneration, congestion when compared to control group (Figure 1). Group 5 administered with husk extract (374 mg/kg) revealed moderate area of cellular abnormalities including portal and peri portal inflammation, vacuolation, pyknotic nuclei, vascular degeneration, congestion when compared to control group (Figure 1b). Group 6 rats treated with husk extract (561 mg/kg) revealed reversed cellular

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architecture with slight area of abnormal cellular integrities when compared to control group (Figure 1).

Evaluation of effect of Zea mays husk extract on kidney function parameters of paracetamol-induced kidney injury in rats

Treatment of rats with paracetamol (2 g/kg) caused significant ($p<0.01$-0.001) elevation of serum urea, creatinine and electrolytes (K^+, Na^+, Cl^- and HCO^-_3) levels when compared to normal control. These increased levels of serum urea, creatinine and electrolytes were significantly ($p<0.05$ - 0.001) reduced in the groups pretreated with silymarin and husk extract of *Zea mays*(187 – 561 mg/kg) in a dose-dependent fashion when compared to the organotoxic group treated with paracetamol only (Table 3).

Effect of husk extract on kidney weight

The kidney weights of rats treated with paracetamol were significantly ($p<0.05$) increased when compared to that of the control group. However, animals in groups pre-treated with the husk extract (187 - 561 mg/kg) and silymarin (100 mg/kg) had their kidney weights significantly ($p<0.01$ – 0.001) reduced when compared to the organotoxic group (Table 3).

Histopathological studies of rat kidney in paracetamol-induced nephrotoxicity

Histopathological study of the kidney of rats treated with distilled water (10 mL/kg) revealed the normal renal architecture in control group with no pathological observation (Group 1) (Figure 2). Paracetamol treated rats showed severe damage in the kidney cells observed as cellular abnormalities including tubular degeneration and loss of epithelium lining in the renal corpuscle, severe area of epithelial lining degeneration, glomerular inflammation, tubular necrosis and vascular degeneration when compared to control group (Group 2) (Figure 2). Pre-treatment of rats with corn husk extract of *Zea mays* (187 – 561 mg/kg) and standard drug, Silymarin (100 mg/kg) was observed to protect the rat's livers from cellular damage induced by Paracetamol. Histologic section of the kidney of rats treated with silymarin at magnification B(x400) revealed cellular area of cellular abnormalities including tubular degeneration and loss of epithelium lining in the renal corpuscle when compared to control group (Figure 2). Histologic section of rat's kidney treated with 187 mg/kg of cornhusk extract revealed slight recoveries from cellular abnormalities and of loss of epithelium lining in the renal corpuscle, though strong evidence of tubular degeneration were observed when compared to control group (Figure 2). Histologic section of the kidney of rats treated with 374 mg/kg of cornhusk extract at magnification B(x400) revealed noticeable recoveries and reversible effect of cellular architecture with slight area of abnormal cellular integrities when compared to control group (Figure 2). Histologic section of the kidney of rats treated with 561 mg/kg of cornhusk extract at magnification B(x400) revealed recovery effects of the area cellular abnormalities including loss of epithelium lining in the renal corpuscle and tubular degeneration when compared to control group (Figure 2).

The present study was done to investigate the hepatoprotective and nephroprotective activities of *Zea mays* husk extract using adult male Wistar rats. Evaluation of liver and kidney protective properties of *Zea mays* husk extract was carried out using paracetamol-induced liver toxicity model. Effect of the husk extract on liver function test, kidney function test and histology were used as parameters to assess these properties.

Paracetamol (Acetaminophen) is one of the most widely used pharmaceutical analgesic and antipyretic. Paracetamol produces toxic effect at high doses which leads to liver 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.[17]The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), causing damage to the macromolecules in vital biomembranes and liver injury.[18,19]

The values of haematological parameters obtained in this study showed that paracetamol may have caused significant decreases in RBC, Hb, PCV and platelets counts of rats when compared with the control. This is an indication that there was destruction of red blood cells and a change in the rate of production of RBC (erythropoiesis). The result also shows that paracetamol can induce anaemia and does not have the potential to induce erythropoietin release from the kidneys, which is the humoral regulator of RBC production.[20] The significant reduction observed in RBC and Hb concentration of rats treated with paracetamol by implication may likely affect 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.[21] 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.[22] Thus, the treatment of rats with paracetamol demonstrated the potential to induce anaemia. This also shows that paracetamol may 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.[23] Results from the study further showed that ethanol husk extract of *Zea mays* caused reductions in WBC, neutrophils, basophils, lymphocytes and monocytes level which were elevated by paracetamol administration. The increase maybe an immunological response by the body defence system to heal or repair injury done on the rat organ by paracetamol administration.[24] The phytochemical constituents of *Zea mays* husk extract which include flavonoids and phytosterols are possible candidates that increase immunological parameters. Decrease in the platelet level correlated with the study done by Shor*et al*.[25] who reported 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. This may be due to the chemical constituents of the plant.

Liver function was assessed by estimating the activities of serum ALT, AST, ALP, bilirubin (total and direct) and total protein that are originally present in the cytoplasm.[26] When there is hepatopathy, these enzymes and molecules leak into the blood stream which serves as an indicator for the liver damage.[27] The abnormally high levels of serum ALT, AST, ALP, total and direct bilirubin, as well as decrease in total protein level as observed in the negative control in this study are indications of paracetamol-induced liver dysfunction and denote the damage to the hepatic cells. The reduction of increased serum enzymes 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, thereby protecting the animals from paracetamol-induced hepatoxicity. Increase in serum level of ALP in the paracetamol group is due to increased synthesis, in the presence of increasing biliary pressure [28] and reflects the pathological alteration in biliary flow.[29] Therefore, an improvement in the level ALP in the pretreated rats suggests that the extract provides a valuable indication that the plant may be useful in management and prevention of conditions such as gallstone and cholescytitis.

In the present study, reduction in serum total protein level was observed in the paracetamol-treated rats which may be associated with the decrease in the number of hepatocytes which in turn may result in decreased hepatic capacity to synthesize protein. The decreased level of total protein as recorded in paracetamol-treated rats revealed the severity of hepatopathy. This negative effect on total protein was significantly and dose-dependently improved in the extract pre-treated groups, indicating an improvement of the functional status of the liver cells by the husk extract. Bilirubin, a metabolic product of haemoglobin, undergoes conjugation with glucuronic acid in hepatocytes to increase its water solubility. Determination of serum bilirubin represents an index for the assessment of hepatic function, and any abnormal increase in the levels of serum bilirubin indicates hepatobiliary disease and severe disturbance of hepatocellular

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Table 1: Effect of *Zea mays* husk extract on heamatological parameters of rats with PCM-induced liver injuries

Treatment	Dose (mg/kg)	WBC (X 10^9/l)	NEUT. (%)	LYM (%)	MONO (%)	ESINO (%)	BASO (%)	RBC (X 10^{12}/l)	HGB (g/dl)	PCV (%)	PLATELETS. 10^3/µL)
Control	-	4.92 ± 0.33	41.25 ± 2.13	46.5 ± 2.75	34.25 ± 2.68	2.50 ± 0.28	0.00 ± 0.00	8.06 ± 0.22	14.45 ± 0.29	44.0 ± 1.68	760.25 ± 24.75
Paracetamol		8.75 ± 0.75^{c}	74.75 ± 2.68^{c}	94.5 ± 4.05^{c}	11.5 ± 0.64^{c}	11.50 ± 1.19^{c}	1.75 ± 0.25c	4.51 ± 0.74^{c}	7.53 ± 0.53^{c}	18.25 ± 1.54^{c}	155.75 ± 18.50^{c}
Silymarin	100	7.21 ± 0.33^{a}	43.0 ± 5.87	75.5 ± 3.37^{c}	25.25 ± 1.88^{a}	5.50 ± 0.64^{a}	0.25 ± 0.25	7.64 ± 0.34^{f}	12.12 ± 0.49^{a}	38.25 ± 1.79^{f}	504.0 ± 9.70^{c,f}
Crude extract	187	6.66 ± 0.28	46.25 ± 2.49	54.25 ± 2.54	32.75 ± 1.65	2.25 ± 0.25	0.00 ± 0.00	8.46 ± 0.22^{f}	12.80 ± 0.14^{a}	40.75 ± 0.75^{f}	637.75 ± 10.93^{c,f}
	374	6.09 ± 0.44	34.75 ± 2.13	54.25 ± 2.52	38.0 ± 0.91	2.00 ± 0.40	0.00 ± 0.00	8.57 ± 0.35^{f}	12.90 ± 0.61^{f}	45.5 ± 1.89^{f}	830.75 ± 26.71^{c,f}
	561	6.34 ± 0.23	38.0 ± 2.12	55.0 ± 2.01	38.0 ± 1.47	1.75 ± 0.25	0.00 ± 0.00	9.03 ± 0.19^{f}	14.40 ± 0.27	47.75 ± 1.39^{f}	870.25 ± 35.57^{c,f}

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 paracetamol. n = 6.

Table 2: Effect of husk extract of *Zeamays* on Paracetamol -induced liver injury in rats

PARAMETERS/ TREATMENT	Total Protein(g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Alkaline phosphatase (U/L)	ALT (U/L)	AST (U/L)	Liver weights (g)
Normal control	6.62 ± 0.33	0.08 ± 0.02	0.05 ± 0.01	77.5.0 ± 4.66	34.75 ± 1.49	174.0 ± 2.85	6.95 ± 0.51
PCM +Dist. Water	4.52 ± 0.30^{c}	2.29 ± 0.1^{c}	0.14 ± 0.02^{c}	209.0 ± 3.67^{c}	74.5 ± 3.22^{c}	281.0 ± 10.27^{c}	8.27 ± 1.03^{c}
Silymarin(100 mg/kg)	6.52 ± 0.39	0.21 ± 0.01^{f}	0.11 ± 0.01^{cf}	178.5 ± 6.06^{cf}	62.25 ± 2.10^{ce}	238.25 ± 3.96^{cf}	6.51 ± 0.34^{f}
Ext.187 mg/kg	6.35 ± 0.18	0.20 ± 0.01^{f}	0.07 ± 0.01^{f}	161.0 ± 0.91^{cf}	54.25 ± 0.75^{cf}	214.0 ± 1.95^{cf}	6.71 ± 0.26^{f}
Ext. 374 mg/kg	6.20 ± 0.15	0.19 ± 0.01^{f}	0.07 ± 0.01^{f}	158.2 ± 1.10^{cf}	54.0 ± 1.87^{cf}	210.25 ± 3.52^{cf}	6.00 ± 0.29^{f}
Ext. 561 mg/kg	6.32 ± 0.13	0.19 ± 0.01^{f}	0.07 ± 0.01^{f}	155.0 ± 1.08^{cf}	51.0 ± 0.91^{cf}	203.25 ± 2.86^{bf}	5.92 ± 0.08^{f}

Values are 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 paracetamol. n = 6.

Table 3: Effect of husk extract of *Z. mays* on PCM-induced kidney injury in rats

PARAMETERS/ TREATMENT	Creatinine (mg/dl)	Urea (mg/dl)	Bicarbonate (mmol/L)	Sodium ion (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Kidney weight (g)
Normal control	0.41 ± 0.04	27.57 ± 2.40	35.75 ± 2.56	151.0 ± 1.22	5.10 ± 0.30	104.75 ± 3.47	1.02 ± 0.05
PCM +Dist. Water	1.03 ± 0.06^{c}	69.82 ± 5.26^{c}	53.75 ± 3.27^{b}	201.0 ± 5.30^{c}	7.07 ± 0.43^{c}	188.5 ± 5.69^{c}	2.51 ± 0.21^{b}
Silymarin (100 mg/kg)	0.65 ± 0.02^{a,f}	36.72 ± 1.61^{f}	39.0 ± 4.60^{d}	171.0 ± 3.83^{e}	5.26 ± 0.20	130.75 ± 3.70^{b,f}	1.09 ± 0.01^{e}
Ext.187 mg/kg	0.70 ± 0.04^{b,f}	33.90 ± 0.92^{f}	41.50 ± 1.84^{d}	161.0 ± 9.59^{f}	6.18 ± 0.15	114.0 ± 2.67^{f}	1.09 ± 0.06^{e}
Ext. 374 mg/kg	0.65 ± 0.02^{a,f}	29.27 ± 1.07^{f}	38.25 ± 0.44^{e}	162.0 ± 1.35^{f}	5.75 ± 0.25^{d}	114.5 ± 5.90^{f}	1.08 ± 0.07^{e}
Ext. 561 mg/kg	0.57 ± 0.04^{f}	26.47 ± 1.09^{f}	38.25 ± 1.25^{f}	153.0 ± 2.67^{f}	5.80 ± 0.17^{d}	107.5 ± 2.90^{f}	1.01 ± 0.06^{f}

Values are 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 paracetamol. n = 6.

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function.[30]Paracetamol caused elevated amount of bilirubin in the blood of the administered rats which were significantly lowered in the husk extract pre-treated group. Decreased serum bilirubin level following extract treatment indicated the effectiveness of the extract to restore normal functional status of the liver.

Antioxidant enzymes are involved in the scavenging of the free radicals to form hydrogen peroxide and safer molecules, hence reducing the toxic effect caused by these radicals. SOD and CAT are important enzymes in the enzymatic antioxidant defense system.[31] The husk extract has been reported to increase hepatic antioxidant enzymes such as SOD and CAT.[10] This implies that husk extract maybe acting by reducing reactive free radicals due to the presence of antioxidant chemicals, thereby reducing oxidative damage to the tissues besides improving activity of hepatic antioxidant enzymes.

The result suggests that the plant extract may be able to deplete massive production of toxic electrophile metabolite,N-acetyl p-benzoquinone imine(NAPQI), by acting as an essential substitute to biological and physiological antioxidants and thus able to prevent hepatopathy and lipid peroxidation. Active chemical constituent of the plant may be responsible for this activity by acting as an antioxidant against harmful reactive oxygen species. This effect could be due to the antioxidant/free radical scavenging activities of flavonoids (gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, femlic acid, rutin, resveratrol, and kaempferol),[8]p-hydroxycinnamic acid, stigmasterol, sitoterol, anthocyannins and octadecanoic acid.[32-37]These compounds reported to be present in this husk extract [8,12] have been reported to show antioxidant activity.[38-40] The plant may be useful in the treatment and management of conditions like hepatitis, hepatotoxicity, liver fibrosis, cirrhosis or liver cell cancinoma. These results corroborate earlier findings of Okokon *et al*.[10] on the hepatoprotective potentials of the husk extract.

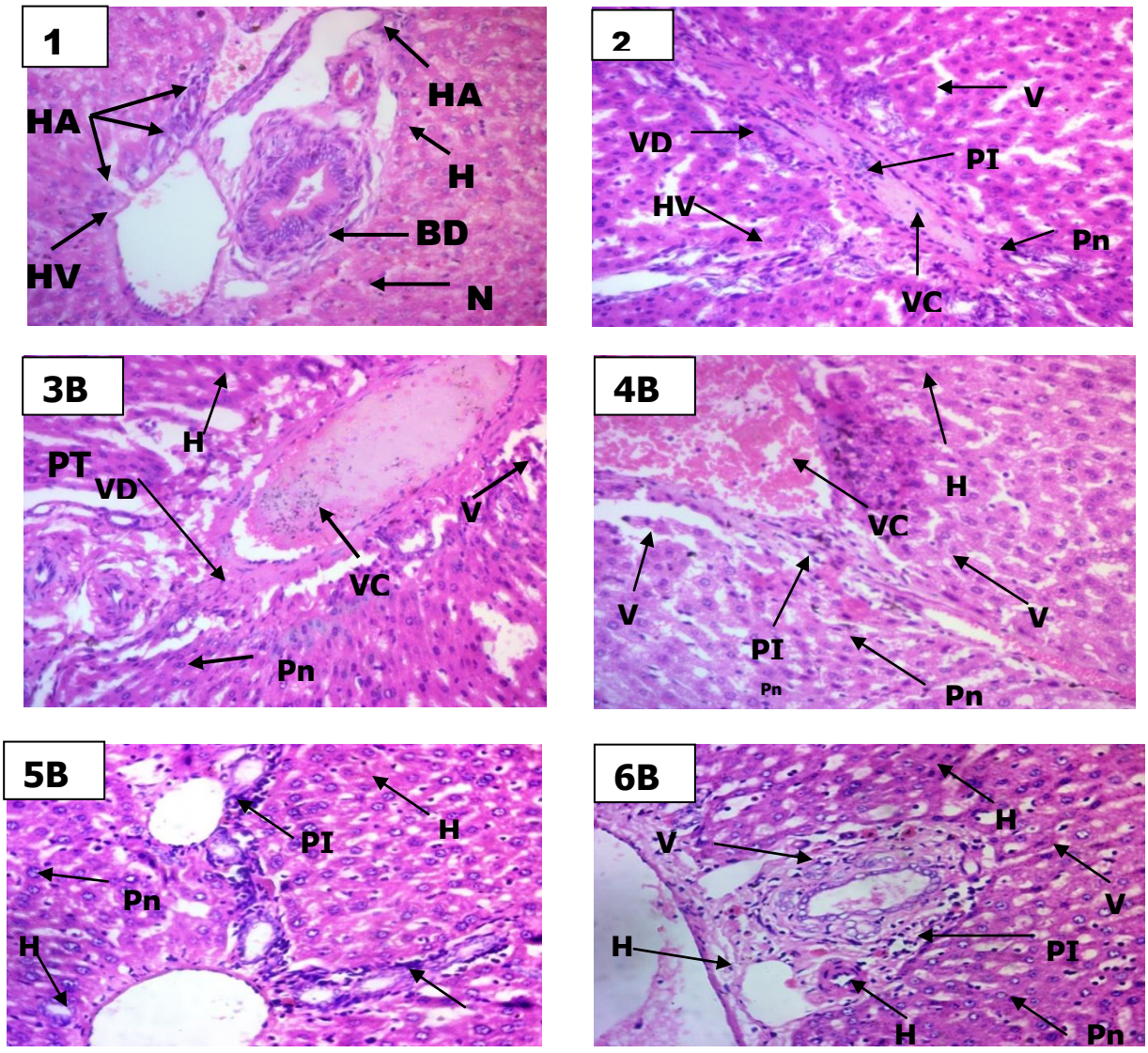

Figure 1: Histological sections of Livers of rats treated with Normal saline 10 mL/kg (1), Paracetamol 2000 mg/kg (2),Silymarin 100 mg/kg mg/kg and paracetamol 2000 mg/kg (3), HE 187 mg/kg and Paracetamol (4), HE 374 mg/kg and Paracetamol 2000 mg/kg (5) and HE 561 mg/kg and paracetamol 2000 mg/kg (6) at magnification A (x100) and B(x400) stained with H&E technique.

Keys: Central vein (**CV**), Cellular degeneration (CD), Vacuolation (**V**),Portal Inflammation (P**I**), Hepatocyte (**H**), Pyknotic nucleus **(Pn)** Central vein (**CV**) Sinusoidal lining (**SL**), Hepatic vein (**HV**), Vascular degeneration (**VD**). Portal triad (**PT**), Bile duct (**BD**), Kuffer cells (**Kc**), Hepatic Artery (**HA**), Hepatic vein (**HV**), Hepatocytes (**H**) and Nucleus (**N**)

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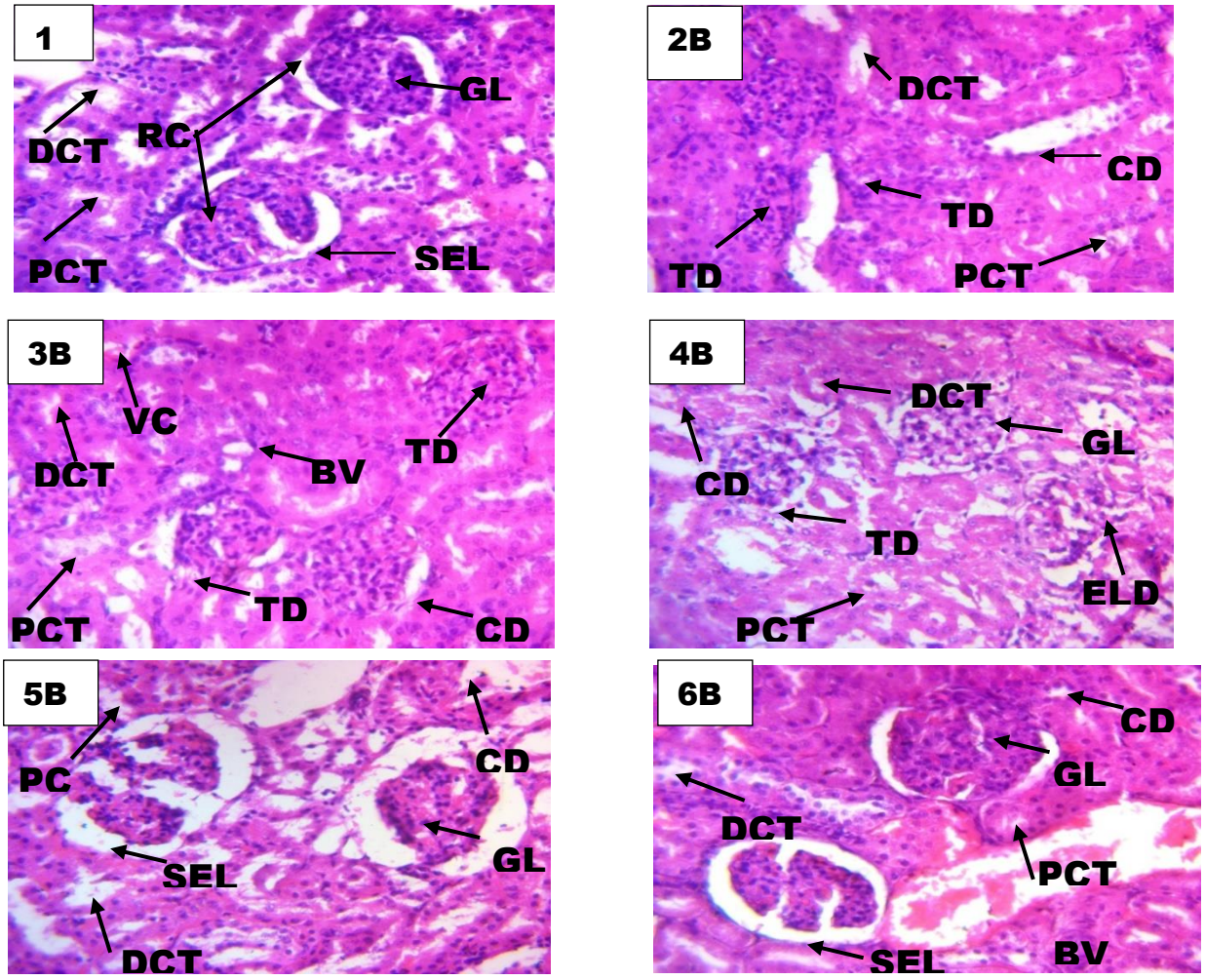

Figure 2: Histological sections of kidneys of rats treated with Normal saline 10 mL/kg (1), Paracetamol 2000 mg/kg (2),Silymarin 100 mg/kg mg/kg and paracetamol 2000 mg/kg (3), HE 187 mg/kg and Paracetamol (4), HE 374 mg/kg and Paracetamol 2000 mg/kg (5) and HE 561 mg/kg and paracetamol 2000 mg/kg (6) 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), Nucleus **(N)**,vascular degeneration **(VD),** Tubular degeneration (**TD**), Medulla (**M**), Distal convoluted tubules (D**CT**), Proximal convoluted tubules (P**CT**), Blood vessel (**BV**), and Collecting ducts (**CD**).

Histologically, H and E staining technique is used for general tissue structure observation. It is mainly used for observing nucleus, cytoplasm and any other abnormality base on general tissue property. In H and E staining, paracetamol caused severe cellular degeneration, vascular congestion,hepatocytic hyperplasia and pyknoptic nucleus which were much reduced in the cornhusk extract pretreated groups. Histology result agrees with values obtained from haematological analysis and liver function parameters that the extract may exert a dose-dependent hepatoprotective effect on paracetamol induced liver toxicity.

In this study, the ethanol husk extract of *Zea mays* was evaluated for nephroprotective activity against paracetamol-induced nephrotoxicity in rats. Paracetamol, produces toxic effect at high doses which leads to tissue damage. The drug is bioactivated to a toxic electrophile, 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.[17]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.[41]Hepatically derived glutathione conjugates are also involved in paracetamol-induced renal injury[42] as well as nitric oxide.[43]In the present study, 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 levels of urea, creatinine and electrolytes concentrations when compared to the normal group. However, pre-treatment of the rats with *Zea mays* husk extract significantly ($p<0.01$) decreased these parameters dose-dependently when compared to the paracetamol group, thus, offering explanation for nephroprotective activity of the husk extract. These results are in agreement with those observed by Isik*et al.*[44] who reported an elevation in serum urea and creatinine in rats after 1 g/kg body weight of paracetamol administration. This elevation in the levels of urea and creatinine was explained by the presence of strong correlation between nephrotoxicity and oxidative stress. These effects on urea and creatinine elevations were significantly and dose-dependently reduced in the pre-treated rats. Husk extract has been reported to cause

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elevation of GSH, GPx, SOD and CAT,[11] demonstrating antioxidant potentials. Besides, corn silk is reported to exert diuretic effect on thekidney by promoting potassium-induced natriuresis.[45]The husk extract may likely possess this effect thereby leading to loss of sodium and chloride, thereby protecting the kidney. These suggest that the kidney protective activity of the husk extract maybe due to the presence of phytochemical compounds in the extract via antioxidative stress action as earlier reported by Okokon *et al*.[10]

The nephro-protective property of the extract is further confirmed by significant improvement of the kidney architecture by preventing the glomerular congestion, interstium with inflammatory cells, tubular necrosis, peritubular necrosis and basement degeneration in the pretreated groups in the H and E stain. The cornhusk extract significantly and dose-dependently protected the basement membrane with intact membrane architecture at maximum dose. The effect observed may be due to the antioxidant activity of the plants. 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 they contain.[46]This is in agreement with the findings of this study. The phenolic and flavonoid components of this plant 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.[47] Plant flavonoids which show an antioxidant activity *in vitro* also function as antioxidants *in vivo*.[48] Phenolic compounds function as high-level antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species.

Conclusion

The results of this study show that the husk extract of *Zea mays* possesses hepatoprotective and nephroprotective activity which is due to the activities of its phytochemical constituents.

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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