Original Research Article

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Nephroprotective activity of husk extract and fractions of *Zea mays* against alloxan-induced oxidative stress in diabetic rats

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Abstract

Zea mays L. (Poacae), an annual grass, traditionally used to treat various diseases was evaluated for antioxidative stress and renoprotective potentials against alloxan-induced injuries in diabetic rats. Antioxidative stress and renoprotective activities of husk extract and fractions (187-748 mg/kg) were assessed by determining levels of oxidative stress markers, kidney function tests and histopathology of kidney. Hematological parameters were determined using hematology Analyser. The husk extract and fractions caused significant ($p < 0.05$) increases in the levels of oxidative stress markers (SOD, CAT, GPx, GSH) in the kidney and MDA level was decreased in the treated diabetic rats. The extract and fractions caused significant ($p < 0.05$) reduction of elevated serum levels of creatinine, urea and chloride in the diabetic rats. The extract/fractions caused increases in WBC, PCV, monocyte, neutrophil, platelet and eosinophil counts without affecting other parameters. Histology of kidney revealed absence or significant reductions in pathological features in the treated diabetic rats compared to untreated diabetic rats. The GC-MS analysis of n-hexane fraction revealed the presence of phytochemical compounds of pharmacological importance. The results showed that the husk extract and fractions of *Zea mays* has antioxidative and nephroprotective potentials which may be due to the antioxidant activities of their phytochemical constituents.

Keywords: *Zea mays,* Oxidative stress, Nephroprotective, Antioxidant, Histology

1. Introduction

Zea mays L. (Poacae), also known as maize or corn, is an annual grass plant with a fibrous root system and long narrow leaves. It bears ears that are enclosed in modified leaves known as husks [1]. It is cultivated mainly for human consumption and animal feed. Various parts of the plant such as maize grains, leaves, cornsilks, stalk, husk and inflorescence 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, 3, 4]. The ash of the cob is used for the treatment of cough [3] and inflammatory diseases [5]. The husks are used for the treatment of pains and arthritis [6]. Warm tea of the husks is used for the treatment of malaria and diabetes in Ibibio traditional medicine [7]. Biological activities reported on the husk extract include, analgesic, anti-inflammatory [6], antioxidant [8], antidepressant [5], antimalarial and antiplasmodial [7] activities. The median lethal dose (LD_{50}) of the ethanol husk extract was determined to be 1874.83 mg/kg [5]. Arabinoxylan, which has immunological effects, has been isolated from the husk extract [9], while eight phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, caffeic 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 anthocyanins [10]. Information on the biological activities of the husk extract is scarce. In this study, we report the antioxidative stress and kidney protective activities of the husk extract and fractions to confirm its use in the treatment of kidney diseases in Ibibio ethnomedicine.

2. Materials and Methods

2.1. Collection of plant materials

Fresh husks of *Zea mays* were collected in August, 2016 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.

2.2. 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 was divided into two parts; one part (1.5 kg)

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was macerated in 50% ethanol (5.0 L) for 72 hr. While the other part, (1.5 kg) was successively macerated for 72 hr in 5.0 L each of these solvents, n-hexane, dichloromethane, ethyl-acetate and n-butanol to give corresponding fractions of these solvents. The liquid filtrates obtained were concentrated and evaporated to dryness in *vacuo* at 40 °C using rotary evaporator. The crude extract and fractions were stored in a refrigerator at -4 °C until they were used for the experiments reported in this study. The percentage yield of the extract and fractions were calculated; Crude 50% ethanolic extract - 2.83% w/w, n-hexane fraction - 0.15% w/w, dichloromethane fraction - 0.25% w/w, ethyl acetate fraction - 0.56% w/w, n-butanol fraction - 1.28% w/w.

2.3. Phytochemical screening

Phytochemical screening of the crude husk extract was carried out employing standard procedures and tests [11, 12].

2.4. Animals

Fifty four (54) albino rats (135 – 160g) of either sex divided into nine groups of 6 rats each per model were used for these experiments. The animals were housed in standard cages and were maintained on a standard pellet feed (Guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.5. Induction of experimental diabetes using alloxan monohydrate

Fifty four (54) healthy Albino Wistar rats (male and female) of known weights were fasted for 24 hr. They were reweighed before the induction by a single intra peritoneal injection of freshly prepared solution of alloxan monohydrate (150 mg/kg) in ice cold 0.9% saline (NaCl solution). According to the method of Pari and Saravanan [13], the animals were given 2 mL of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycaemia. A rest period of 72 hr was allowed during which the rats were allowed access to food and water and the diabetes to be fully developed during these 72 hr. After the rest period, rats with moderate diabetes, having persistent glycosuria, and hyperglycaemia (i.e with blood glucose levels 200 mg/dl and above) [14] were considered diabetic and selected for the experiments.

The diabetic animals were randomised and divided into nine (9) treatment groups of 6 rats each. Based on the value of median lethal dose (LD_{50}) previously determined in our laboratory, suitable dose regimens were selected and the rats were treated as follows.

2.6. Experimental design and treatments

Group 1: 10 mL/kg/day of normal saline orally for 14 days

Group 2: 5 mg/kg/day of Glibenclamide orally for 14 days

Group 3: 187 mg/kg/day of *Zea mays* husk 50% ethanolic crude extract orally for 14 days

Group 4: 374 mg/kg/day of *Zea mays* husk crude 50% ethanolic crude extract orally for 14 days

Group 5: 748 mg/kg/day of *Zea mays* husk 50% ethanolic crude extract orally for 14 days

Group 6: 374 mg/kg/day of n-hexane fraction of *Zea mays* husk orally for 14 days

Group 7: 374 mg/kg/day of dichloromethane fraction of *Zea mays* husk orally for 14 days

Group 8: 374 mg/kg/day of ethyl acetate fraction of *Zea mays* husk orally for 14 days

Group 9: 374 mg/kg/day of n-butanol fraction of *Zea mays* husk orally for 14 days.

The 50% ethanolic crude extract and fractions were administered to the animals in aqueous suspension.

2.7. Effect of administration of husk extract and fractions of Zea mays on Fasting blood glucose of alloxan-induced diabetic rats.

The fasting blood glucose (FBG) of all the rats was measured after 14 days of administration of the husk extract and fractions. The tail-tipping method was employed. The blood obtained from the tail vein of the rats was dropped on the dextrostix reagent pad and the pad was inserted into a microprocessor digital blood glucometer and the readings were recorded [15].

All the treatments were administered between 7.00 - 8.00 am daily throughout the experimental period and food was withdrawn from the experimental animals 12 hr before measurement of FBG to create the necessary fasting period for measurement of the fasting blood glucose concentrations.

2.8. Determination of the body weight changes of the treated diabetic rats

Throughout the experimental period, the body weights of the experimental animals were monitored and recorded at the following points; just before the fasting in preparation for induction of the diabetes, after induction, on stabilization of diabetes and after the prolonged study.

2.9. Collection of blood samples and organs

After 14 days of treatment (24 hr after the last administration), 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 1500 rpm for 15 min to separate serum at room temperature to avoid haemolysis and used for biochemical assays. Blood that was collected into EDTA bottles were taken for haematological analysis. The kidneys of the diabetic rats were surgically removed, weighed and fixed in 10% formaldehyde for histological process.

2.10. Hematological study

After the animals were sacrificed under diethyl ether anesthesia, blood samples were collected from each rat 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 of hematological parameters such as red blood cell count (RBC), 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 (Sysmex Hematology – Coagulation system, Model MO-1000 I, Trans Asia, Japan).

2.11. 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.

2.12. Determination of the protective effect of the husk extract and fractions on biochemical parameters and histology of kidney of alloxan-induced diabetic rats

The various serum samples collected from the sacrificed treated rats were analyzed according to standard methods for effect of the extract on various biochemical parameters of rats such as urea, creatinine, sodium, potassium and chloride. These analyses were done at Department of Chemical Pathology, University of Uyo Teaching Hospital, (UUTH), Uyo using various diagnostic kits like Randox Laboratory kits, Dialab diagnostic kits, HUMAN diagnostic kits and TECO analytical kits. The kidneys surgically removed from the animals were weighed and a part of each was fixed in 10% formaldehyde, processed, sectioned and stained with heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo.

2.13. Preparation of renal homogenate

The other part of each kidney removed was dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at −8 °C. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4 °C. The resulting supernatant was used for the determination of malondialdehyde (MDA) content [16, 17], superoxide dismutase (SOD) [18], catalase (CAT) [19], glutathione peroxidase (GPx) [20], and reduced glutathione (GSH) [21] activities using colorimetric assay.

2.14. Histopathological examination

The kidneys of each animal that was 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 were observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

2.15. Gas chromatography-mass spectrometry analysis

Quantitative and qualitative data were determined by GC and GC-MS, respectively. The fraction was dissolved in methanol and injected into a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/splitless injector. The column used was an DB-5 (Optima-5), 30 m, 0.25 mm i.d., 0.25 µm df, coated with 5 % diphenyl-95 % polydimethylsiloxane, operated with the following oven temperature programme: 50 °C, held for 1 min, rising at 3 °C/min to 250 °C, held for 5 min, rising at 2 °C/min to 280 °C, held for 3 min; injection temperature and volume, 250 °C and 1.0 µl, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280 °C; hydrogen, flow rate, 50 ml/min; air flow rate, 400 ml/min; make-up (H_2/air), flow rate, 50 ml/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu). Agilent 6890N GC was interfaced with a VG Analytical 70-250s double -focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250 0C. The GC was fitted with a 30 m × 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

2.16. Identification of the compounds

The identification of components present in the active fraction of the plants' extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley and Nist Libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literatures [22, 23].

2.17. Statistical analysis and data evaluation

Data obtained from this work were analysed statistically using one way ANOVA followed by a posthoc test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% and 0.1% level of significance i.e. $p \leq 0.05$ and 0.001.

3. Results

3.1. Phytochemical screening

The qualitative phytochemical screening of the ethanol extract of *Zea mays* revealed the presence of alkaloids, tannins, saponins, flavanoids, terpenes and glycosides.

3.2. Effect of husk extract and fractions on body weight and fasting blood glucose of rats

There were observable changes in the body weights of the treated and untreated alloxan-induced diabetic rats (Table 1). Treatment of the diabetic rats with the husk extract and fractions produced a non-dose dependent increases in the body weight of the diabetic rats which were significant ($p < 0.05$-0.001) when compared to control. The low dose (187 mg/kg) and dichloromethane fraction produced 20.83 and 17.47% increase in body weights respectively (Table 1). The husk extract and fractions caused significant reduction ($p < 0.05$-0.001) in FBG with the n-hexane having the highest activity (Table 1).

3.3. Effect of extract and fractions on weight of organs

Treatment of alloxan-induced diabetic rats with husk extract and fractions of *Zea mays* did not cause any significant ($p > 0.05$) effect on the weight of kidneys of the diabetic rats compared to control (Table 1). Though there were considerable decreases in the weight of kidneys especially in the group treated with n-hexane fraction, these decreases were not significant ($p > 0.05$) when compared to control (Table 1).

3.4. Effect of husk extract and fractions on haematological indices

Administration of the husk extract and fractions caused significant ($p < 0.05$-0.001) increases in the levels of white blood cells, neutrophils, monocytes, eosinophils, platelets and packed cell volume when compared to control. While no significant ($p > 0.05$) effect was observed in red blood cells, hemoglobin, basophils and lymphocytes levels when compared to control (Table 2).

3.5. Effect of husk extract and fractions on kidney function test of diabetic rats

The elevated serum levels of creatinine, urea and chloride in the alloxan-induced diabetic rats were significantly ($p < 0.05$-0.001) reduced by treatment with the extract and fractions when compared to control. Bicarbonate and potassium levels were not affected by the extract and fractions administration, while the serum level of sodium was significantly ($p < 0.01$-0.001) reduced by ethyl acetate and butanol fractions administration when compared to control (Table 3).

3.6. Effect of husk extract and fractions on kidney antioxidant enzymes

Administration of husk extract and fractions significantly ($p < 0.05$-0.001) caused elevations in kidney antioxidant enzymes (SOD, CAT) and GSH levels when compared to control. The treatment also caused significant ($p < 0.001$) reduction in the level of MDA of the treated diabetic rats when compared to control (Table 4).

3.7. Histological studies

Histologic sections of kidneys of untreated diabetic rats revealed areas of glomerular inflammation, vascular congestion, cellular degeneration against hyperchromatic background. Kidneys of diabetic rats treated with glibenclamide (10 mg/kg), husk extract (187 - 748 mg/kg) and fractions (n-hexane, dichloromethane, ethyl acetate and n-butanol) at magnification B(x400) revealed normal cyto-architecture profile against the normochromic background of renal histomorphologies demonstrating a strong reversibility effect (Figure 1).

Table 1. Effect of ethanol husk extract and fractions of *Zea mays* on fasting blood glucose, body weight and weight of organs of alloxan- induced diabetic rats

Treatment	Dose	Body Weight (g)			Weight of kidney	Fasting blood glucose (mg/dL)	
	mg/kg	Day 0	Day 15	% Increase	Kidney (g)	0 hr	14th Day
Control	-	181.0 ± 25.0	174.0 ± 23.0	-3.86	1.12 ± 0.17	375.5 ± 83.43	132.0 ± 5.85
Glibenclamide	10	152.0 ± 7.55	158.33 ± 8.45	4.16	1.01 ± 0.07	343.75 ± 93.03	73.66 ± 27.76[b]
Crude extract	187	153.6 ± 19.78	185.6 ± 18.09	20.83	1.14 ± 0.11	366.75 ± 99.84	67.66 ± 6.56[b]
	374	162.25 ± 1.65	168.5 ± 31.20	3.85	1.12 ± 0.04	337.5 ± 76.73	80.45 ± 21.29 [a]
	748	160.25 ± 27.37	166.75 ± 31.20	4.05	1.07 ± 0.13	369.25 ± 73.66	71.25 ± 5.40[b]
n-hexane fraction	374	152.3 ± 20.92	157.0 ± 24.61	3.08	0.98 ± 0.07	346.25 ± 90.04	53.0 ± 3.39[c]
Dichloromethane fraction	374	128.75 ± 2.98	151.25 ± 10.84	17.47	1.01 ± 0.07	342.5 ± 97.04	64.25 ± 6.90[b]
Ethyl acetate fraction	374	164.33 ± 15.60	168.33 ± 16.02	2.64	1.14 ± 0.05	340.5 ± 87.30	82.33 ± 15.77[a]
Butanol fraction	374	175.66 ± 13.17	179.33 ± 15.16	3.60	1.16±0.14	357.5±58.25	68.25±9.56[b]

Data is expressed as mean ± SEM, Significant at [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, when compared to control (n=6)

Table 2. Effect of *Zea mays* husk extract and fractions on heamatological parameters in alloxan-induced diabetic rats

Treatment	Dose (mg/kg)	WBC (L)	Neut. (%)	Lym. (%)	Mono. (%)	Eosino. (%)	Baso. (%)	RBC (L)	HGB (g/dL)	PCV (%)	Platelets (L)
Control	-	7.20 ± 0.27	26.13 ± 1.02	68.66 ± 0.48	3.23 ± 0.22	0.40 ± 0.04	1.00 ± 0.23	6.61 ± 0.72	11.73 ± 1.06	39.86 ± 1.32	110.23 ± 8.21
Gliben-clamide	10	16.21 ± 0.91^{c}	25.5 ± 1.97	68.26 ± 1.87	4.76 ± 0.68	0.76 ± 0.29	0.70 ± 0.26	7.38 ± 0.27	13.53 ± 0.57	48.73 ± 1.57^{b}	769.66 ± 21.07^{C}
Crude extract	187	10.84 ± 0.54^{b}	32.3 ± 1.06^{b}	59.76 ± 1.07	6.20 ± 0.38^{a}	1.83 ± 0.41^{c}	0.60 ± 0.11	6.08 ± 0.63	10.93 ± 1.11	36.93 ± 1.64	761.0 ± 45.18^{C}
	374	11.35 ± 0.84^{b}	37.82 ± 0.14^{c}	54.72 ± 1.00	4.92 ± 1.05	1.37 ± 0.08^{a}	1.15 ± 0.13	7.60 ± 0.21	13.60 ± 0.34	49.0 ± 1.59^{b}	800.5 ± 35.81^{C}
	784	12.50 ± 0.52^{c}	33.0 ± 0.94^{c}	61.12 ± 5.70	3.87 ± 0.98	1.37 ± 0.24^{a}	0.62 ± 0.08	7.42 ± 0.24	12.95 ± 0.33	45.82 ± 1.36	772.0 ± 96.67^{C}
n-hexane fraction	374	12.16 ± 0.82^{c}	27.35 ± 0.42	65.97 ± 3.51	4.62 ± 0.37	1.17 ± 0.11	0.87 ± 0.16	7.76 ± 0.35	13.35 ± 0.39	46.35 ± 1.79^{a}	992.5 ± 24.45^{C}
Dichloro-methane fraction	374	10.29 ± 0.79^{a}	26.32 ± 0.58	67.77 ± 2.74	4.07 ± 0.34	0.75 ± 0.15	1.07 ± 0.12	6.46 ± 0.51	11.92 ± 0.62	40.65 ± 1.30	868.5 ± 54.34^{C}
Ethyl acetate fraction	374	11.91 ± 0.34^{c}	29.20 ± 1.59	65.70 ± 1.83	3.26 ± 0.36	1.03 ± 0.08	0.80 ± 0.05	7.37 ± 0.23	13.13 ± 0.40	44.33 ± 1.55	816.6 ± 9.82^{C}
Butanol fraction	374	8.15 ± 0.21	34.36 ± 0.79^{c}	60.8 ± 1.66	3.53 ± 0.14	0.50 ± 0.25	0.80 ± 0.10	5.95 ± 1.26	11.0 ± 1.65	38.40 ± 1.80	841.6 ± 92.37^{C}

Data is expressed as mean ± SEM; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, when compared to control (n=6).

Table 3. Effect of *Zea mays* husk extract and fractions on renal function parameters in alloxan-induced diabetic rats

TREATMENT	DOSE (mg/kg)	CREATININE (mg/kg)	UREA (mg/dl)	BICARBONATE (mMol/L)	SODIUM (mMol/L)	POTASSIUM (mMol/L)	CHLORIDE (mMol/L)
Control	-	0.69 ± 0.02	24.20 ± 0.80	25.66 ± 1.20	142.66 ±1.45	3.63 ± 0.18	119.66 ± 1.76
Glibenclamide	10	0.42 ± 0.02^{c}	17.26 ± 1.51^{a}	27.66 ± 1.20	146.0 ± 1.73	4.13 ± 0.12	101.00 ± 1.15^{c}
Crude extract	187	0.43 ± 0.03^{c}	19.8 ± 0.81	27.66 ± 1.85	142.33 ± 1.76	3.70 ± 0.10	94.33 ± 0.88^{c}
	374	0.46 ± 0.02^{b}	14.47 ± 0.36^{c}	27.50 ± 2.50	141.5 ± 1.70	4.20 ± 0.22	105.0 ± 0.40^{c}
	748	0.46 ± 0.04^{b}	16.98 ± 1.33^{a}	26.75 ± 1.10	142.75 ± 1.25	4.40 ± 0.17	104.25 ± 1.10^{c}
n-hexane fraction	374	0.43 ± 0.02^{C}	19.14 ± 1.54	29.00 ± 1.68	139.25 ± 1.70	4.28 ± 0.07	102.7 ± 1.93^{c}
Dichloromethane fraction	374	0.46 ± 0.02^{b}	19.99 ± 1.35	30.25 ± 0.75	141.5 ± 2.59	4.72 ± 0.17	105.25 ± 0.75^{c}
Ethyl acetate fraction	374	0.54 ± 0.05	17.6 ± 1.36 ^{a}	29.66 ± 0.88	127.0 ± 2.08^{b}	4.06 ± 0.12	98.33 ± 1.45^{c}
Butanol fraction	374	0.61 ± 0.01	22.73 ± 1.42	32.8 ± 1.67	116.3 ± 3.71^{c}	3.30 ± 0.15	103.33 ± 5.23^{c}

Data is expressed as mean ± SEM; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, when compared to control group (n=6).

Table 4. Effect of *Zea mays* husk extract on kidney antioxidative stress markers in alloxan-induced diabetic in rats

PARAMETERS/ TREATMENT	Dose (mg/kg)	SOD (U/mg of protein)	CAT (U/mg of protein)	MDA (U/mg of protein)	GSH (µg/mg of protein)
Control normal saline	10 mg/ml	9.18 ± 0.11	20.44 ± 3.14	68.22 ± 1.28	0.10 ± 0.01
Glibenclamide	10	15.10 ± 0.16^{c}	45.27 ± 0.48^{c}	50.24 ± 1.27^{c}	0.26 ± 0.01^{c}
Crude extract	187	12.27 ± 0.36^{c}	25.48 ± 0.17^{a}	54.28 ± 1.20^{c}	0.15 ± 0.01^{a}
	374	15.42 ± 0.20^{c}	32.56 ± 2.02^{c}	45.27 ± 1.18^{c}	0.20 ± 0.01^{c}
	748	17.44 ± 0.25^{c}	34.22 ± 1.06^{c}	43.97 ± 2.48^{c}	0.24 ± 0.01^{c}
n-hexane fraction	374	19.70 ± 0.11^{c}	38.02 ± 2.16^{c}	45.36 ± 1.32^{c}	0.32 ± 0.01^{c}
Dichloromethane fraction	374	20.18 ± 0.16^{c}	32.02 ± 1.38^{c}	42.04 ± 2.38^{c}	0.36 ± 0.02^{c}
Ethyl acetate fraction	374	15.26 ± 0. 28^{c}	35.11 ± 1.41^{c}	44.60 ± 2.46^{c}	0.28 ± 0.02^{c}
n-butanol	374	17.78 ± 0.19^{c}	41.12 ± 1.22^{c}	47.32 ± 1.54^{c}	0.22 ± 0.01^{c}

Data were expressed as mean ± SEM; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to diabetic control (n = 6).

3.8. Gas chromatography-mass spectroscopy (GC-MS) analysis

The phytochemical analysis of the most active antidiabetic fraction (n-hexane) of *Zea mays* revealed the presence of 32 compounds (Table 5). The compounds present include 2, 3-dihydro-benzofuran, dodecanoic acid, dodecanoic acid methyl ester, p-hydroxycinnamic acid ethyl ester, hexadecanoic acid methyl ester, stigmast-5-en-3-ol (3-beta)-, stigmasterol, gamma-sitosterol among others.

4. Discussion

Zea mays parts are used in the treatment of malaria, diabetes, dyslipidemia, liver and kidney diseases [2, 3, 4, 6, 7]. In Ibibio traditional medicine, tea made from the husk is employed in the treatment of these diseases. This work was focused on the evaluation of *Zea mays* husk extract and fractions for antioxidative and nephroprotective potentials in alloxan-induced diabetic rats. The body weights of diabetic rats were found to increase significantly following treatment with the husk extract and fractions. Diabetes is associated with a severe loss in body weight due to loss or degradation of structural proteins [24]. Treatment with the husk extract and fractions remedied this situation perhaps due to the alleviation of hyperglycemic state and stimulation of protein synthesis.

The husk extract and fractions treatments were observed in this study to cause significant increases in WBC and platelets counts, neutrophils, monocytes, eosinophils and PCV percentages. The increases maybe due to immunological responses by the body defense mechanism to heal or repair the injuries caused by alloxan [25]. The increases in the blood parameters further portray that the extract and fractions contain active compounds that can stimulate haemopoitins (erythropoietins and thrombopoetins) synthesis and release.

In this study, *Z. mays* husk extract and fractions were observed to demonstrate sustained significant antidiabetic activities with the n-hexane fraction exerting the highest activity after 14 days of treatment. The fasting blood glucose (FBG) levels of the treated diabetic rats were significantly reduced when compared to those of the untreated diabetic rats (control). The antidiabetic results observed in this study corroborate to that of Brobbey *et al.* [26] who reported significant antidiabetic effect of tea from dried husk of *Z. mays* in diabetic human subjects. The observed antidiabetic activity may be attributed to the activity of the phytoconstituents.

The husk extract and fractions were observed in this study to cause significant decrease in weight of organs. Generally, internal organ weights are considered as important indicator of injury and toxicities [27]. Hypertrophy of organs often indicates toxicity and damaged to organ [28]. This often results from oedema due to inflammation of the organs. Alloxan is known to generate free radicals in the body which attack and cause destruction of hepatic, pancreatic and kidney cells and tissues [29]. The decreases in weight of kidneys especially in n-hexane fraction-treated group, is a result of protective role of the fraction in alleviating the effect of free radicals generated by alloxan and diabetic condition perhaps due to its hypoglycemic and free radical scavenging activities of the phytoconstituents such as phenolic compounds earlier isolated [8] in the husk extract and fractions.

Diabetes mellitus is a chronic metabolic disorder and is always accompanied by an increased generation of free radicals especially ROS [30]. Alloxan, a hydrophilic compound, is biotransformed to dialuric acid. This leads to the generation of H_2O_2, •OH and superoxide radicals via iron catalyst which attack organs like kidney, liver and pancreas etc [29] and cause oxidative stress which is implicated in the pathogenesis of diabetes complications in animals or humans [31]. Reactive oxygen species (ROS) produce cellular and tissue injury through covalent binding, DNA strand breaking, lipid peroxidation (LPO) and augment fibrosis which is also implicated in other disease conditions [32, 33].

The results of this study show that oxidative stress was duly induced by alloxan in the diabetic rats as reflected in the marked reductions in the levels of SOD, CAT, GSH and GPx in both hepatic and renal tissues as well as significant increase in MDA levels in the kidney of the diabetic rats. This finding agrees with earlier findings that the activities of these antioxidants are known to reduce during diabetes [34, 35, 36]. The oxidative stress status of the diabetic rats is further supported by marked elevations in the serum levels of urea and creatinine levels of the diabetic rats. Reduction in the tissue (kidney) levels of these oxidative markers following treatments with the husk extract and fractions strongly suggest the great potentials of husk extract and fractions in attenuating oxidative stress associated with type II diabetes mellitus which was probably mediated via free radical scavenging activities and improving glutathione status in the tissues by its phytochemical constituents.

Kidney function is often compromised in uncontrolled diabetes mellitus. Glycosuria, one of the cardinal diagnostic features of diabetes imposes dehydration via glucose osmotic diuresis. This dehydration is accompanied by severe loss of electrolytes like potassium, sodium, chloride, calcium, and phosphates [37]. Ketoacidosis, a prevalent

Table 5. GC-MS analysis of n-hexane fraction of *Zea mays* husk

PEAK	RT	COMPOUND NAME	FORMULA	MOL. MASS
1.	8.856	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144
2.	11.210	2,3-dihydro-benzofuran	C_8H_8O	120
3.	12.320	2H-pyran-2-one, tetrahydro-4-hydroxy-4-methyl-	$C_6H_{10}O_3$	130
4.	13.257	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
5.	15.698	Ethyl .beta.-d-riboside	$C_7H_{14}O$	178
6.	17.005	Alpha.-d-Lyxofuranoside, methyl	$C_6H_{12}O_5$	164
7.	17.537	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	$C_{12}H_{16}O_5$	240
8.	18.257	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214
9.	19.654	Dodecanoic acid	$C_{12}H_{24}O_2$	200
10.	22.730	7-methyl-oxa-cyclododeca-6,10-dien-2-one	$C_{12}H_{18}O_2$	194
11.	23.750	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180
12.	24.353	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256
13.	25.884	p-Hydroxycinnamic acid, ethyl ester	$C_{11}H_{12}O_3$	192
14.	27.147	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
15.	27.446	Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate	$C_{12}H_{14}O_4$	222
16.	27.943	3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210
17.	28.867	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
18.	29.557	Perhydrothiaxanthene	$C_{13}H_{22}S$	210
19.	31.940	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
20.	32.751	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242
21.	33.539	Ethyl (9Z,12Z)-9,12-octadecadienoate	$C_{20}H_{36}O_2$	308
22.	33.651	9-octadecenoic acid (Z)-, ethyl ester	$C_{19}H_{36}O_2$	296
23.	34.152	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
24.	38.030	2-hydroxy-3-[(9E)-9-octadecenoyloxy]propyl (9E)-9-octadecenoate	$C_{39}H_{72}O_5$	620
25.	38.784	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390
26.	39.452	Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	$C_{27}H_{50}O_6$	470
27.	40.173	10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz(b,f) oxepin	$C_{16}H_{16}O_4$	272
28.	41.403	Oleic acid, 3-hydroxypropyl ester	$C_{21}H_{40}O_3$	340
29.	42.361	2-Docosanone, 4,21,21-trimethyl-, L-(-)-	$C_{25}H_{50}O$	366
30	46.599	Stigmast-5-en-3-ol, (3.Beta.)-	$C_{29}H_{50}O$	414
31.	50.831	Stigmasterol	$C_{29}H_{48}O$	412
32.	52.624	Gamma.-Sitosterol	$C_{29}H_{50}O$	414

feature leads to ketonuria. Attempt by the kidney to buffer the urine decreases the alkaline metals including potassium and sodium [38]. In this study, significant reduction of chloride levels and in some groups, sodium levels, was observed following treatment with the husk extract and fractions, while potassium and bicarbonate levels were not affected. These reductions could have resulted from the renoprotective effect of the extract/fraction to alleviate the effect of diabetes by reducing diabetic acidosis which usually leads to increased retention of sodium in the serum and decreased excretion of potassium in urine [39]. Another possibility is that as the extract treatment clears glucose from the blood and urine, water loss (dehydration) gradually disappears and ketoacidosis stimulus hence decreases. Besides, corn silk is reported to exert diuretic effect on the kidney by promoting potassium-induced natriuresis [40]. The husk extract and some fractions may likely possess this effect thereby leading to loss of sodium and chloride, thereby protecting the kidney.

In this study, the serum levels of urea and creatinine in the treated diabetic rats were significantly reduced compared to the untreated rats. Insulin deficiency and inability of glucose to reach extra-hepatic tissues stimulates gluconeogenesis as an alternative route of glucose supply [41]. So glucogenic amino acids circulate in plasma and are deaminated in the liver with the resultant increased in blood urea. As glucose is cleared from the blood and insulin effect is reintroduced, proteolysis declines via hormonal stimulation [41], hence decreased urea concentration in blood. Similar decrease was observed in creatinine level. Creatinine is a metabolite of muscle creatine, whose amount in serum is proportional to the body's muscle mass. Elevated levels of serum creatinine indicate diminished renal function, as it is excreted by the kidney [42].

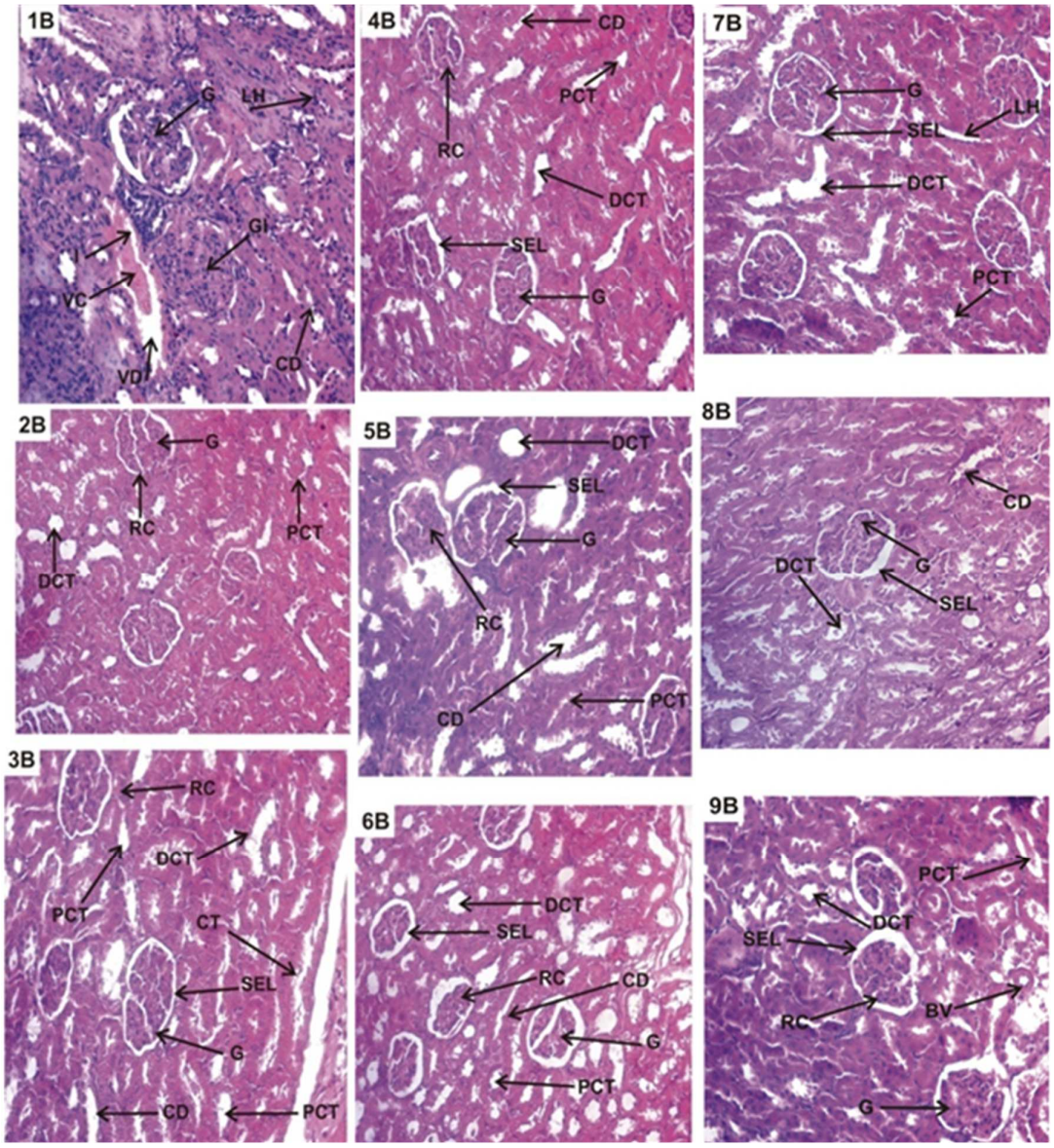

Figure 1. Histological sections of kidneys of alloxan-induced diabetic rats treated with Normal saline (Control) 10 ml/kg(1), Glibenclamide 10 mg/kg bw (2), husk extract 187 mg/kg bw (3), husk extract 374 mg/kg bw (4), husk extract 748 mg/kg bw (5), n-hexane fraction 374 mg/kg bw (6), dichloromethane fraction 374 mg/kg bw (7), ethyl acetate fraction 374 mg/kg bw (8), n-butanol fraction 374 mg/kg bw (9) at Magnification B(x400), stained with H&E Method. **Keys:** Cortex (**C**), Renal corpuscle (**Rc**), Medulla (**M**), Collecting duct (**CD**), Distal convulated tubules (**DCT**), Proximal convulated tubules (**PCT**), Glomerulus (**G**), (**Bc**), (**LH**) and Squamous epithelial lining (**SEL**). Collecting duct (**CD**), Distal convulated tubules (**DCT**), Proximal convulated tubules (**PCT**), Glomerulus (**G**), Connective tissue (**CT**), Blood vessel (**BV**), Adipocytes (**Ad**) and Squamous epithelial lining (**SEL**).

Administration of the husk extract/fraction exerted a reduction in the serum level of creatinine suggesting a protection of the kidney against damage due to diabetes. The reductions in levels of serum creatinine and urea portray kidney protective potential of the extract and fractions. These results further corroborate the histologic findings which had demonstrated significant kidney protective effects. This finding agrees with that reported on corn silk which also possess renal protecting activity [43].

Reports have indicated that persistent hyperglycemia causes increased production of oxidative stress in alloxan-induced diabetes [44]. Hence, excessive ROS produced leads to oxidative damage and increased LPO. The results of this study showed a significant increase in LPO levels resulting in high level of MDA in alloxan-induced diabetic rats' kidneys with accompanying reduction in the levels of oxidative stress markers (SOD, CAT, GPx and GSH) in the kidney. This reduction in the levels of antioxidant enzymes in this study corroborates those of earlier reports [34, 36, 45].

In *in vivo* experimental models, tissue oxidative stress markers such as SOD, CAT and GSH are useful and reliable markers of antioxidant status while MDA is a sensitive and reliable marker for lipid peroxidation [46, 47]. The antioxidant enzyme levels were found to be reduced in untreated alloxan-induced diabetic rats. Hence, the changes

of these biomarkers is in accordance with the decrease in antioxidant state in the body as other reports [48, 49]. In the present study, administration of the extract and fractions significantly counteract the changes of oxidative stress biomarkers in alloxan-induced rats thus preventing the accumulation of excessive oxidative stress with corresponding increases in the levels of antioxidant enzymes/oxidative stress markers. This activity is due to the antioxidant activities of the phytochemicals such as flavonoids and other phenolic compounds reported to be present in the husk extract as reported by Dong et al. [8]. Similarly, stigmasterol present in the extract/fractions has been reported to cause reduction in hepatic lipid peroxidation and elevation in the activities of catalase, superoxide dismutase and glutathione [50]. Also, β-sitosterol has been reported to stimulate antioxidant enzymes by activation of estrogen receptor/PI3- kinase-dependent pathway [51, 52]. These compounds may have contributed to the observed antioxidative stress activity of the extract and fractions and consequently, elevation of enzymatic and non-enzymatic antioxidants thus suggesting cellular antioxidant activity.

Administration of the husk extract and fractions was observed in this study to protect the kidneys of diabetic rats from alloxan-induced oxidative stress. Dialuric acid, a metabolite of alloxan is known to generate free radicals which attack organs like kidney etc [29, 36]. Glomerular inflammation, vascular congestion and cellular degeneration found in the untreated diabetic rats were absent in the kidneys of extract and fractions-treated rats. This suggests significant kidney protective effect. This finding corroborates that of chemical pathology and earlier findings of Karami et al. [43] who reported similar activity on the corn silk extract. This effect could be due to the antioxidant/free radical scavenging activities of flavonoids [8], p-hydroxycinnamic acid, stigmasterol, sitoterol, anthocyannins and octadecanoic acid [47, 50, 51, 52, 53, 54].

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