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Hepatoprotective 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 hepatoprotective potentials against alloxan-induced injuries in diabetic rats. Antioxidative stress and hepatoprotective activities of husk extract and fractions (187-748 mg/kg) were assessed by determining oxidative stress markers levels, liver tests and histopathology of liver. The husk extract and fractions caused significant ($p<0.05 – 0.001$) increases in the levels of oxidative stress markers (SOD, CAT, GPx, GSH) in the liver of the treated diabetic rats. The extract/fractions treatment caused reduction in liver enzymes (ALT and ALP), GGT, total bilirubin, and also increased total protein and albumin levels as well as AST. Histology of liver revealed absence or significant reductions in pathological features in the treated diabetic rats compared to untreated diabetic rats. The GCMS analysis of n-hexane fraction revealed the presence of phytochemical compounds of pharmacological importance. The results show that the husk extract and fractions of *Zea mays* has antioxidative stress and hepatoprotective potentials which may be due to the antioxidant activities of their phytochemical constituents.

Keywords: *Zea mays,* medicinal plant, liver-protective, kidney protective, antioxidant

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 through for human consumption and animal feed. Various parts of the plant; maize grains, leaves, corn silks, 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 [2-4]. 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, filmic 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. We report in this study the antioxidative stress, liver and kidney protective activities of the husk extract and fractions to confirm its use in the treatment of diabetes 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) was macerated in 50% ethanol for 72 hours. While the other part, (1.5 kg) was successively and gradiently macerated for 72 h in 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 (yield 2.83%) and fractions were stored in a refrigerator at -4°C until they were used for the experiments reported in this study.

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 56 rats each per model were used for these experiments. 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 obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.5 Induction of experimental diabetes using alloxan monohydrate
Sixty (60) healthy Albino Wistar rats (male and female) of known weights were fasted for 24 hours, 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 Saravana [13], the animals were given 2 ml of 5% dextrose solution using monogastric tube immediately after induction to overcome the drug induced hypoglycaemia. A rest period of 72 h was allowed during which the rats were allowed access to food and water and the diabetes to be fully developed during these 72 h. After the rest period, rats with moderate diabetes, having persistent glycosuria, and hyperglycemia (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 crude extract orally for 14 days

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

Group 5: 748 mg/kg/day of *Zea mays* husk 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.

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 method that was employed was "the tail-tipping method". The blood obtained from the tail vein of the rats was dropped on the dextrostix reagent pad and the pad 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 h before measurement of FBG to create the necessary fasting period for measurement of the fasting blood glucose concentrations.

2.8 Determination of the body weights 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 hours after the last administration) the rats were weighed again and sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture into plain centrifuge tubes and used immediately. The blood in the centrifuge tubes were centrifuged immediately at 1500 rpm for 15 mins to separate of serum at room temperature to avoid haemolysis and used for biochemical assays. The livers of the diabetic rats were surgically removed, weighed and fixed in 10% formaldehyde for histological process.

2.10 Liver function test
The following parameters were determined;
Aspartate transaminase (AST), Alanine aminotransferase (ALT), Total Cholesterol, Alkaline phosphatase (ALP), Total plasma protein, Gamma-glutamyl transpeptidase, (GGT), 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.

2.11 Evaluation of the protective effect of the Husk extract and fractions on biochemical parameters and histology of Livers of alloxan-induced diabetic rats
Serum was separated from the blood of each animal sacrificed and the sera were stored at -20°C until used for biochemical determinations such as total protein, albumin, aspartate aminotransferases (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl

transpeptidase (GGT), total cholesterol, direct and total bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols [16, 17].

The livers of the animals were surgically removed, weighed and a part of each fixed in 10% formaldehyde for histological processes, while the other part was washed with ice cold 0.9% NaCl and homogenates were made in a ratio of 1 g of wet tissue to 9 ml of 1.25% KCl by using motor driven Teflon-pestle. The homogenates were centrifuged at 7000 rpm for 10 min at 4˚C and the supernatants were used for the assays of superoxide dismutase (SOD) [18], catalase (CAT) [19], glutathione peroxidase (GPx) [20], and reduced glutathione (GSH) [21].

2.12 Histopathological examination

The liver of each animal that was used in the study was surgically harvested and fixed in buffered formalin. They were then processed and stained with haematoxylin 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.

2.13 Gas chromatography-Mass spectrometry analysis

Quantitative and qualitative data were determined by GC and GC-MS, respectively. The fraction was injected onto 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 % polydimethyl siloxane, 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 (H2/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 x 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

2.14 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].

2.15 Statistical analysis and data evaluation

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% and 0.1% level of significance ie $P \leq 0.05$ 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 weights 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 weights 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 weights of liver of the diabetic rats compared to control (Table 1). Though there were considerable decreases in the weights of liver, these decreases were not significant ($p>0.05$) when compared to control (Table 1).

3.4 Effect of husk extract and fractions on liver function test parameters of diabetic rats.

Treatment of the diabetic rats with husk extract and fractions of *Zea mays* caused significantly ($p<0.05$-0.001) increases in the levels of total protein and albumin of the diabetic rats when compared to control (Table 2). However, the extract and fractions administration significantly ($p<0.001$) caused reductions in the level of total bilirubin, ALT, ALP and GGT when compared to control. Direct bilirubin level was not affected by treatment with the extract and fractions, while AST level was significantly ($p< 0.01$-0.001) increased by the highest extract dose (784 mg/kg), n-hexane, ethyl acetate and n-butanol fractions when compared to control (Table 2).

3.5 Effect of husk extract and fraction on Liver antioxidant enzymes.

Treatment of alloxan-induced diabetic rats with corn husk extract and fractions caused significant ($p<0.05$-0.001) dose-dependent elevation in the levels of the antioxidant enzymes (SOD, CAT, GPX) when compared to control. Similarly, GSH level was significantly ($P<0.001$) elevated following treatment with the extract and fractions when compared to control. Similar elevations of the enzymes and GSH levels were observed with the standard drug, glibenclamide (Table 3).

3.6 Histological studies

Histological sections of livers of untreated diabetic rats revealed numerous hepatocytes with pyknotic nucleus, vascular degeneration, vascular congestion and periportal inflammation. Livers of diabetic rats treated with glibenclamide (10 mg/kg) revealed normal cellular profile indexed with normochromic pyknotic nucleus. There was no visible cellular abnormality (Figure 1). Liver of rats treated with husk extract (187 – 748 mg/ kg) revealed hyperchromic cellular profile with vascular congestion and numerous pyknotic nucleus. There was no visible cellular abnormality

(Figure 1). Liver of diabetic rats treated with n-hexane, dichloromethane, ethyl acetate and n-butanol fractions (374 mg/kg) revealed normochromic cellular profile containing periportal inflammation and pyknotic nuclei with no obvious cellular abnormality. Thus could be considered slightly affected. (Figure 1).

3.7 Gas chromatography-Mass Spectroscopy (GCMS) analysis

The phytochemical analysis of the most active antidiabetic fraction (n-hexane) of *Zea mays* revealed the presence of 32 compounds (Table 4). 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.

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

Treatment	Dose	Body weight (g)			Weights of organs	Fasting blood glucose (mg/dl)	
	mg/kg	Day 0	Day 15	% Increase	LIVER (g)	0 HR	14TH DAY
Control	-	181.0 ± 25.0	174.0 ± 23.0	-3.86	8.03±0.98	375.5±83.43	132.0±5.85
Glibenclamide	10	152.0 ± 7.55	158.33 ± 8.45	4.16	5.75±0.30	343.75±93.03	73.66±27.76^{b}
Crude extract	187	153.6 ± 19.78	185.6 ± 18.09	20.83	7.94±0.73	366.75±99.84	67.66±6.56^{b}
	374	162.25 ± 1.65	168.5 ± 31.20	3.85	7.30±0.82	337.5±76.73	80.45±21.29 ^{a}
	748	160.25 ±27.37	166.75 ±31.20	4.05	6.87±1.31	369.25±73.66	71.25±5.40^{b}
n- hexane fraction	374	152.3 ± 20.92	157.0 ± 24.61	3.08	6.69±0.90	346.25±90.04	53.0±3.39^{c}
Dichloromethane fraction	374	128.75 ± 2.98	151.25 ±10.84	17.47	6.97±0.46	342.5±97.04	64.25±6.90^{b}
Ethyl acetate fraction	374	164.33 ±15.60	168.33 ±16.02	2.64	7.39±0.86	340.5±87.30	82.33±15.77^{a}
Butanol fraction	374	175.66 ±13.17	179.33 ±15.16	3.60	5.96±0.36	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 the liver function parameters of alloxan-induced diabetic rats

Treatment	Dose (mg/ kg)	Total protein (g/ dl)	Albumin (g/dl)	Total bilirubin (mg/dl)	Alt (iu/l)	Alp (iu/l)	Ast (iu/l)	Direct bilirubin (mg/ dl)	Ggt (u/l)
Control	-	3.50±0.28	3.30±0.41	0.25±0.01	55.0± 3.21	156.0±0.57	81.33± 0.88	0.08±0.01	0.91±0.02
Glibenclamide	10	5.70±0.56^{b}	3.86±0.14	0.09± 0.02^{c}	30.33±1.85^{c}	66.33± 3.71^{c}	89.33± 3.52	0.06±0.01	0.48±0.03^{c}
Crude extract	187	5.80±0.46^{b}	3.93±0.24	0.07± 0.01^{c}	32.33±3.84^{c}	70.66 ±1.76^{c}	98.33 ± 1.45	0.06±0.01	0.55±0.07^{b}
	374	5.85±0.42^{b}	4.05±0.06	0.06± 0.01^{c}	29.75±1.49^{c}	73.25 ±2.72^{c}	117.0± 11.06	0.05±0.01	0.58±0.05^{b}
	784	6.02±0.25^{b}	4.17±0.16	0.08 ±0.01^{c}	31.75±1.54^{c}	69.5 ± 3.92 ^{c}	158.25±15.60^{b}	0.05±0.01	0.62±0.01^{a}
N- hexane Fraction	374	6.82±0.08^{c}	4.21±0.17	0.085±0.01^{c}	35.75±2.01^{c}	76.75± 3.25^{c}	172.75±10.22^{c}	0.05±0.01	0.68± 0.06
Dichloromethane fraction	374	6.68±0.34^{c}	4.60±0.11^{b}	0.10 ±0.01^{c}	37.0 ±0.91^{c}	87.25 ±4.49^{c}	119.5 ± 17.24	0.05±0.01	0.68± 0.04
Ethylacetate fraction	374	6.52±0.36^{c}	4.66±0.13^{b}	0.09± 0.01^{c}	38.33±1.45^{c}	98.33 ±6.17^{c}	165.33±14.19^{b}	0.05±0.01	0.75 ±0.04
Butanol Fraction	374	7.12±0.11^{c}	4.84±0.22^{b}	0.11 ±0.01^{c}	40.0 ±1.52^{c}	107.66±9.95^{c}	162.33±11.66^{b}	0.06±0.01	0.84 ±0.05

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 3: Effect of *Zea mays* husk extract on Liver antioxidative stress markers in alloxan-induced diabetic in rats.

Parameters/ Treatment	Dose Mg/Kg	Sod (U/Mg Of Protein)	Cat (U/Mg Of Protein)	Gpx (U/Mg Of Protein)	Gsh (µg/Mg Of Protein)
Diabetic Control	-	10.28±0.30	30.15± 0.16	12.23±0.45	0.13±0.01
Glibenclamide	10	18.14 ±0.22^{b}	45.34±1.14^{c}	23.27±0.48 ^{c}	0.33±0.01^{c}
Crude extract	187	12.38±0.11	33.32±0.99^{a}	17.99±0.88 ^{a}	0.24±0.01
	374	15.17±0.14	39.16±1.12^{b}	19.73 ±0.46^{a}	0.24±0.01^{c}
	748	16.16± 0.18^{b}	46.38±2.19 ^{c}	21.39±0.18 ^{b}	0.27±0.02^{c}
n -hexane fraction	374	19.02±0.15^{b}	50.47± 1.92^{c}	22.11±0.81^{c}	0.31±0.01^{c}
Dichloromethane fraction	374	21.14 ±0.22^{c}	55.34±1.14^{c}	23.27±0.48 ^{c}	0.35±0.01^{c}
Ethyl acetate fraction	374	16.6 ± 3.72	47.98±0.85^{c}	16.4±2.65	0.27± 0.07^{c}
n-butanol	374	18.2 ± 1.77 ^{b}	47.28±0.41^{b}	19.8±3.21^{a}	0.23± 0.65^{c}

Table 4: GCMS 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

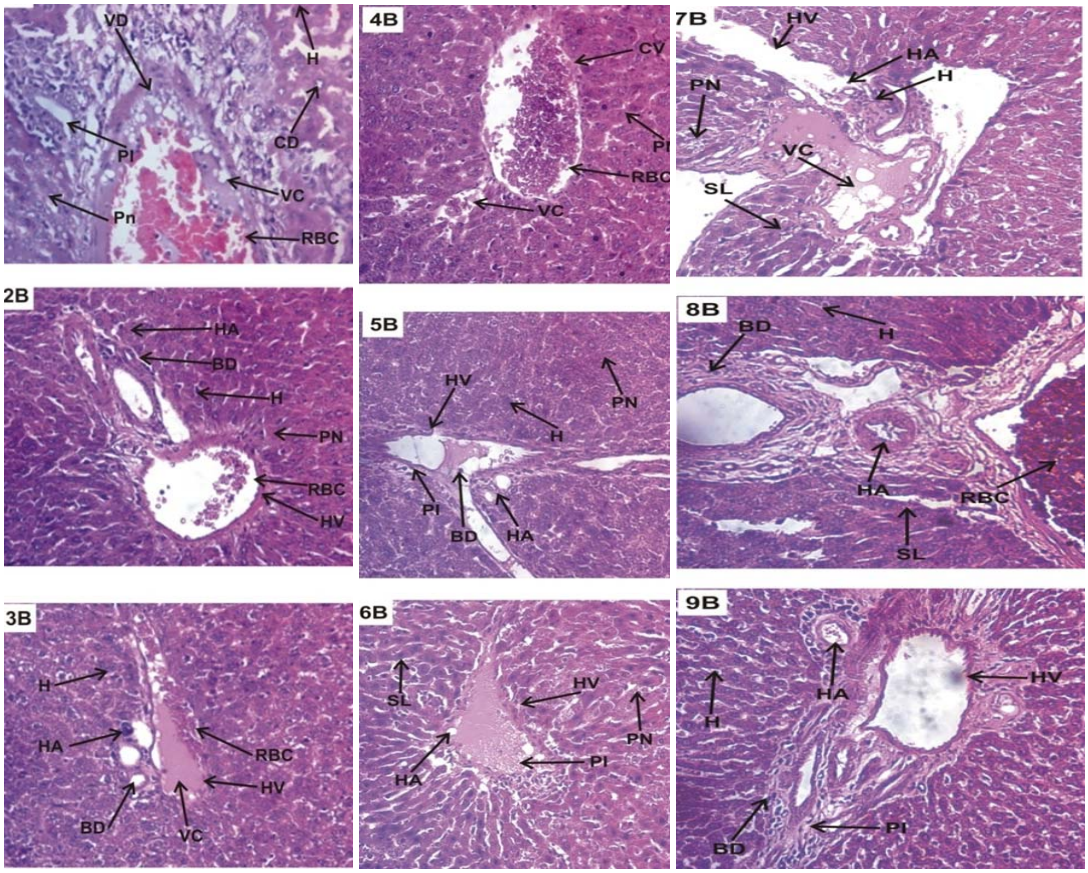

Fig 1: Histological sections of livers of alloxan-induced diabetic rats treated with Normal saline (Control) 10 ml/kg(1), Glibenclamide 10 mg/kg bw (2), husk extract 187 mg/kg bw (3) husk extract 374 mg/kg bw (4), husk extract 748 mg/kg bw (5), n-hexane fraction 374 mg/kg bw (6), dichloromethane fraction 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: Hepatic artery (HA), Hepatic vein (HV), Portal triad (PT), Bile duct (BD) and Hepatocytes (H) and Periportal inflammation (PI), Keys: Hepatic artery (HA), Hepatic vein (HV), Sinusoidal lining (SL), Bile duct (BD), Red blood cell (RBC),Vascular congestion (VC), Hepatocytes (H) and Red Blood Cells (RBC), Vascular congestion (VC), Vascular degeneration (VD), Cellular degeneration (CD), Periportal inflammation (PI), Pyknotic nucleus (Pn), Red blood cell (RBC) and Hepatocytes (H). Hepatic artery (HA), Hepatic vein (HV), Periportal inflammation (PI), Bile duct (BD), Central vein (CV), Sinusoidal lining (SL), Hepatocytes (H) and Pyknotic nucleus (Pn).

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 stress and hepatoprotective 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.

In this study, *Z. mays* husk extract and fractions were observed to demonstrate sustained significant antidiabetic activities with the dichloromethane fraction exerting the highest activity after 14 days of treatment. The fasting blood glucose (FBG) levels of the treated diabetic rats were non-dose dependently and significantly reduced when compared to those of untreated diabetic rats (control). The antidiabetic results observed in this study corroborate that of Brobbey *et al.,* [25] who reported significant antidiabetic effect of tea from dried husk of *Z. mays* in diabetic human subjects. The antidiabetic activity is due 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 organs weights are considered as important indicator to injury and toxicities [26]. Hypertrophy of organs often indicates toxicity and damaged to organ [27]. 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 [28]. The decreases in weights of liver is as a result of protective role of the extract/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 [29]. 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 [28] and cause oxidative stress which is implicated in the pathogenesis of diabetes complications in animals or humans [30]. 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 [31, 32].

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 the hepatic tissues of the diabetic rats. This finding agrees with earlier findings that the activities of these antioxidants are known to reduce during diabetes [32, 33, 34]. The oxidative stress status of the diabetic rats is further supported by marked elevations in the serum levels of AST, ALT, ALP, and GGT levels of the diabetic rats. Reduction in the tissue (liver) 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.

Disease conditions such as diabetes affect the liver and changes the hepatic function as shown by increases in the levels of AST, ALT, ALP, GGT, which indicates liver damage which are also detected in human diabetes [36]. Damage to liver cells often results in leakage of enzymes such as Aspartate aminotransferase (AST), and alanine aminotransferase (ALT) into the blood, while blood levels of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) rise when the bile flow is slow or blocked [37].

In this study, treatment of the diabetic rats with the husk extract and fractions was found to caused significant reductions in the levels of ALT, ALP, GGT and total bilirubin, while AST level was significantly increased at higher doses and in few fractions-treated diabetic groups. The integrity of hepatocytes is assessed by levels of the serum liver enzyme markers such as AST, ALT and ALP [38]. Serum aminotransferases levels (ALT and AST) are two of the most useful measures of liver parenchymal cell injury. AST is raised in acute liver damage, but is also present in RBCs, kidney, testis, cardiac and skeletal muscles, so it is not specific to the liver, while ALT is almost exclusively found in the liver [39]. Elevated levels of AST and ALT indicate liver damage but are not good measures of liver function since they do not reliably reflect the synthetic ability of the liver and may come from tissues other than the liver such as muscles. The husk extract and fractions produced significant increase in the levels of AST in the diabetic rats. These could have resulted from extrahepatic sources as well. The significant rise in the level of AST could have been due to the constituents of the extract/fractions and further corroborate histologic findings of periportal inflammation observed in the study. In diabetic animals, the variations in the levels of AST, ALT and ALP are directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of diabetics and are also responsible for the increased gluconeogenesis and ketogenesis [40]. Diabetes and hyperlipidaemia also cause cell damage by altering the cell membrane architecture, which results in enhanced activities of ALP in diabetic rats [40]. This suggests that alloxan-induced diabetes caused lipid peroxide mediated tissue damage in the liver. The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissues and then migrating into the blood stream. The decrease in GGT, ALT and ALP levels in corn husk extract and fractions treated groups indicates the protective effect on the liver.

Reduced levels of total protein and albumin were observed in the untreated diabetic group in this study. This may be associated with the reduction in the number of hepatocytes which in turn may result in decreased hepatic capacity to synthesize protein and albumin [41]. These decreased levels of total protein and albumin were improved by the husk extract and fractions treatment suggesting increased synthetic capacity of the liver. An increase in the levels of total protein and albumin suggests the regeneration of endosplasmic reticulum and synthetic capacity which leads to protein synthesis [41].

Total bilirubin level which was increased in the untreated diabetic rats was observed in this study to be significantly reduced in groups treated with the husk extract and fractions. Bilirubin, is a metabolic product of hemoglobin which undergoes conjucation with glucuronic acid in hepatocytes to increase its water solubility. Determination of bilirubin represent an index for the assessment of hepatic function, severity of necrosis, conjugation and excretory capacity of hepatocytes and any abnormal increase in the level of

bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function [42]. Decrease in serum bilirubin level of the diabetic rats after treatment with the husk extract and fractions indicated extract/fractions effectiveness to restore normal functional status of the liver.

The above activities of the extract/fraction show that the husk extract and fractions posses hepatoprotective potential against alloxan-induced hepatic injury. These results corroborate histologic findings which show significant hepatoprotective potentials of the extract/fractions. This effect could be due to the antioxidant / free radical scavenging activities of flavonoids [8], p-hydroxycinnamic acid, stigmasterol, sitosterol, anthocyanins and octadecanoic acid [43-48].

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, 49]. SOD plays an important role in oxygen defense metabolism by intercepting and reducing superoxide to hydrogen peroxide, which in mammals is readily reduced to water principally by CAT and GPx. GPx plays a pivotal role in minimizing the oxidative stress. GPx and GST work together with GSH and decompose H_2O_2 and other organic hydroperoxides to non-toxic products. Glutathione, reduced form (GSH) is ubiquitous tripeptide thiol, is a vital intra/extra-cellular protective antioxidant against oxidative/nitrosative stress. The antioxidant enzymes levels were found to be reduced in untreated alloxan-induced diabetic rats. 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 at 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 [45]. Also, β-sitosterol has been reported to stimulate antioxidant enzymes by activation of estrogen receptor/PI3- kinase-dependent pathway [44, 48]. 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.

The liver of the extract/fractions treated diabetic rats were found to be significantly protected from the effects of free radicals generated by alloxan. This protection was visible as the livers of the treated rats lacked or had reduced pathological signs such as vascular degeneration and congestion as well as periportal inflammation observed in the untreated diabetic rats. These findings corroborated that of chemical pathology and therefore suggest hepatoprotective activity against oxidative stress induced by alloxan. The protection is due to the free radical scavenging potentials of the phytoconstituents of the extract and fractions such as anthocyanins, stigmasterol, sitosterol, p-hydroxycinnamic acid and octadecanoic acid [43, 44, 45, 46, 47, 48, 50, 51] as well as the antioxidant activity of other phenolic compounds present in the extract (Dong *et al*.,[8]. The findings of this study corroborate that of Karami *et al*., [52], who had earlier reported hepatoprotective effect of the corn silk.

This activity is attributable to the antioxidant activity of the phytochemical constituents of the husk extract and fractions by scavenging the free radicals generated by alloxan.

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