GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Toxicological study ofchronic administrationof *Zea mays* husk extract onmale rats

Jude E. Okokon [1,*], Mandu E. Nyong [1], Chinyelu C. Osigwe [2], Uwaeme Ugonma Florence [2] and Grace E Essien [1]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.*
[2] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele, Rivers State.*

GSC Biological and Pharmaceutical Sciences, 2026, 34(02), 027-043

Publication history: Received on 18 December 2025; revised on 01 February 2026; accepted on 03 February 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.34.2.0039

Abstract

Zea mays L (Family-Poaceae),used locally in ethnomedicine as a remedy for malaria, diabetes and fever among others, was evaluated for possible effect on hematological indices, liver and kidney functions as well as lipid profile and organs histologies of male Wistar rats following chronic administration of the husk extract. The husk extract (187, 374 and 561 mg/kg body weight) was orally administered to male Wistar rats daily for 100 days and the rats were sacrificed under light diethyl ether anesthesia at the completion of the administration. Chronic administration of *Zea mays* husk extract resulted in insignificant ($p>0.05$) decrease/increase in body weights of rats without any significant ($p>0.05$) effect on the organs weights relativeto control. The husk extract treatment did not alter any blood indices of the malerats significantly ($p>0.05$) except significant ($p<0.001$) reduction of clotting time of male rats at all doses. The husk extract did not cause any significant ($p>0.05$) effect on liver function parameters (total protein, albumin, AST,ALT, ALP, GGT, total and combined bilirubin)and kidney function indices (urea, creatinine, bicarbonate, potassium, sodium and chloride) of male rats. The extract did not alter the lipid profile of treated maleratsexcept significant ($p<0.01$) increase in VLDL of male rats at high dose (561 mg/kg) when compared to control.The husk extract exerted mild to moderate effect on the histologies of brains, hearts, livers and kidneys ofthe malerats. No pathological effect was observed on testis and sperm morphology. The extract should be taken with caution and high dose should be avoided.

Keywords: *Zeamays*; Haematological Parameters; Kidney Function; Liver Function

1. Introduction

Zea mays L.(Poacae) also called maize or corn, is a grass and food plant withmodified leaves known as husks. The plant parts are employed in traditional medicine for the treatment of several ailments such as diabetes [1,2,3,4,5], cough [2], inflammatory diseases [6], pains and arthritis [7] and ulcer [8]. Documented biological activities of the husk extract include; analgesic, anti-inflammatory [7], antioxidant [9], antidepressant [6], antimalarial and antiplasmodial [5], hepatoprotective [10,11,12], antidiabetic and hypolipidemic[13] and nephroprotective [14,15], antiulcer [16], antiobesity [17], *in vivo* alpha amylase and alpha glucosidase inhibitory [18], genotoxic and cytotoxic [19] activities. Okokon *et al.* [6] had reported the median lethal dose (LD_{50}) of the ethanol husk extract to be 1874.83 mg/kg. Phytochemical compounds such as arabinoxylan [20], phenolics (gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, femlic acid, rutin, resveratrol, and kaempferol) [9], anthocyannins [21], stigmasterol, stigmasteryl stearate and stigmasteryl palmitate [17] have been isolated from the husk extract. In this study, we investigated the effects of chronic administration of the husk extract of *Zea mays* on male rats.

*Corresponding author: Jude E. Okokon

2. Materials and methods

2.1. Plant collection and extraction

Fresh husks of *Zea mays* were collected in August 2020 from farmland in Uruan in Uruan LGA, Akwa Ibom State, Nigeria. The husks were identification and authentication of *Zea mays* was done 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.

Fresh husk of *Zea mays* were washed, cut into smaller pieces and dried under shade for two weeks. The husks were further pulverized to powder using electric grinder. The powdered leaves material was divided into two parts; one part (1.5 kg) was macerated in 50% ethanol (7.5 L) for 72 hours at room temperature (28 ±2 40°C). This wasthereafter filtered, and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40^0C using a rotary evaporator (BuchiLab, Switzerland) and stored in a refrigerator at -4°C, until used for the proposed experiments.

2.2. Animals

Swiss albino male rats (123 – 138 g) used for these experiments were gotten from 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 obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo (UU/CHS/IHREC/2023/VOL.1/57).

2.3. Experimental design

Adult Wistar male rats were used in this study. They were weighed, randomly divided into four groups of 5 rats each andtreated as follows; groups I, II, and III were administered 187, 374 and 561 mg/kg of the husk extract respectively on alternate days for 100 days. Group IV was administered with distilled water (10 mL/kg) for the same period of time. The weights of the animals in all the treatment groups were weighed periodically to monitor the weight increases. At the end of the treatment period (100 days), the animals were again weighedand sacrificed under light ethyl ether vapour. Blood samples were collected by cardiac puncture into EDTA-bottles and plain bottles. Blood samples in EDTA bottles were used for haematological study such as bleeding time, clotting time, full blood counts etc. Sera were separated from the blood samples in plain bottles and stored at -20˚C until used for biochemical determinations such as liver function test, kidney function test, and lipid profile

The effects of the extract on some organs were studied. The organs; liver, kidney, spleen, brain, testis, and heart of rats wereremoved surgically and fixed in 10% buffered formalin. The organs were weighed, processed, sectioned and stained using hematoxylin and eosin (H&E) according to standard procedures.

2.3.1. Haematological analysis

Blood samples collectedfrom each diethyl ether euthanized ratinto different Ethylene Diamine Tetra-acetic Acid (EDTA)-coated sample bottles were analysed for all the haematological indices (RBC, HGB, PCV, platelets, WBC and differentials – neutrophils, eosinophils, basophils, lymphocytes and monocytes). These parameters were analysed using automated Haematology analyser according to manufacturer's protocols (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan) at the University of Uyo Teaching Hospital. Also, the bleeding and clotting times of each rat were also determined.

2.3.2. Biochemical analysis

Using a centrifuge (Nikon optical Co., Japan), whole blood of each sacrificed rat collected through cardiac puncture into different plain sample bottles were centrifuged at 2500 rpm for 15 min at 10 °C to obtain the serum. The obtained sera were analysed for the concentrations and activities of certain biomarkers of liver and kidney functions. The determinations were done spectrophotometrically using Randox Analytical Kits® according to standard procedures of manufacturer's protocols [22] at the Chemical Pathology Department of University of Uyo Teaching Hospital.

2.3.3. Liver function test

The following parameters were determined; Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total Cholesterol, Alkaline phosphatase (ALP), Total plasma protein, Total and combined bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols [22] at the Chemical Pathology Department of University of Uyo Teaching Hospital.

2.3.4. 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 urea.

2.3.5. Determination of the effect of the crude extract on the lipid profile (Serum TG, TC, HDL, LDL, VLDL levels) of the treatedrats

Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels of the treated rats were measured by enzymatic colorimetric methods using Randox diagnostic kits. The low and very low-density lipoprotein (LDL and VLDL) levels were estimated from the formula of Friedwald *et al.* [23].

2.3.6. Histopathological examination

The kidneys, livers, pancreas, hearts, brains, spleens and testes 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) 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.

2.3.7. Seminal morphometry and analysis

Sperm motility: The caudal epididymis was incised to expose fluid. Using a micro-pipette, 5 µL of the epididymal fluid exposed in 1000 µL physiological saline. The fluid was delivered onto a glass slide and covered with a 22 x 22 mm cover slip. This was viewed under a light microscope at magnification of X400. Motility estimation was carried out at room temperature between 24 – 28 ºC. Microscopic field was scanned systematically and each spermatozoon encountered was assessed. Motility was recorded in percentage and classified as Motile, Non – motile, Actively motile (progressive) and Sluggish. The procedure was repeated and average taken [24].

Sperm Cell Count and Concentration: The harvested epididymis was cut with an anatomical scissors and minced in a petri dish. The epididymal spermatozoa (50 µL) was diluted in 950 µL of diluent and mixed well with pipette into both chamber of hemocytometer and placed on the stage of the microscopewith objectiveadjusted to a X40 magnification. Hemocytometer was viewed and counting done. Values of counts were recorded. Counting was repeated in each chamber and average count recorded [25]. Average number of cells and cell concentration were calculated using the formular;

$$\text{Average number of cells } = \frac{\text{sum of cells in each squares}}{\text{umber of squares}}$$

Sperm morphology: Caudal epididymis was incised to expose fluid. The epididymal fluid (5 µL) was collected with a micro pipette, placed on a glass slide and covered with a 22 x 22 mm cover slip. Glass slide was taken to light microscope with magnification of X400. Morphological assessment was carried out at room temperature between 24 -28 ºC. Microscopic field was scanned systematically and each spermatozoon encountered was assessed. Morphology was recorded in percentage and classified as Normal, Bent neck and Curve. Procedure was repeated and average taken [24,25].

2.4. Statistical analysis and data evaluation

Data obtained from this work were analysed statistically using Instat Graphpad (California, USA). ANOVA (one –way) followed by a post test (Tukey-Kramer multiple comparison test) were employed in the analysis. 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. Body weight

The effect of the extract on body weights of male rats treated chronically with husk extract of *Zea mays* for 100 days is shown in Table 1. The extract administration produced a non dose-dependent increases in body weights of male rats with the middle dose (374 mg/kg) producing the most significant ($p<0.01$) weight gain when compared to the controls statistically.

3.2. Effect of extract on organs weights

Administration of husk extract of *Zea mays* (187-561 mg/kg) to male rats for 100 days did not cause any significant ($p>0.05$) effect on the weights of heart, brain, liver, kidney, spleen, pancreas and testes of the animals when compared to control. However, slight decreases in the weights of all the organs of male rats treated with the low dose (187 mg/kg) were observed but these decreases were not significant ($p>0.05$) when compared statistically to the control group. Similarly, insignificant ($p>0.05$) increases in pancreas weights of male rats treated with higher doses (374 and 561 mg/kg) of the husk extract were observed when compared to control (Table 2).

3.3. Effect on haematological parameters

The results of the effect of chronic administration of ethanol husk extract of *Zea mays* on haematological parameters of male rats are shown in Table 3. Chronic administration of husk extract of *Zea mays* to male rats for 100 days did not cause any significant ($p>0.05$) effect on the hematological parameters of male rats when compared to control. However, RBC and WBC counts, PCV and lymphocytes percentages in male rats were insignificantly ($p>0.05$) decreased by the extract treatment when compared to control.

Administration of husk extract (187-561mg/kg) to male rats for 100 days caused significant ($p<0.01$-0.001) non dose-dependent reduction in clotting time of male rats when compared to control, while the bleeding time was not affected significantly ($p>0.005$) when compared to control (Figure 1).

Table 1 Effect of chronic administration of *Zea mays* husk extract on body weights of male rats

Treatment R&G /Extract	Dose (mg/kg)	Initial body weight (g)	Final body weight (g)	Weight gain (g)
Control	0.2ml	127.6± 3.18	207.6±5.69	80.0±3.67
Zea mays husk	187	125.5± 6.39	201.0±17.00	75.5±2.31
	374	134.25±4.58	232.0±8.50	97.75±3.11[b]
	561	138.0± 8.87	209.6±2.40	71.6 ±2.74

Data are expressed as mean ± SEM. Significant at[a]$p>0.001$ when compared to control .n = 5.

Table 2 Effect of chronic administration of *Zea mays* husk extract on organs weights ofmale rats

Treatment	DOSE (mg/ kg)	Heart (mg)	Brain (mg)	Liver (mg)	Kidney (mg)	Spleen (mg)	Pancreas (mg)	Testes (mg)
Control	10 mg/ml	0.75± 0.03	1.59±0.04	8.32±0.90	1.44±0.20	0.62±0.11	0.77±0.02	2.28±0.22
Husk extract	187	0.65±0.05	1.47±0.03	6.86±0.44	1.24±0.03	0.58±0.04	0.75±0.05	1.46± 0.42
	374	0.82±0.03	1.68±0.03	8.55±0.59	1.48±0.06	0.76±0.06	1.11±0.15	2.51± 0.10
	561	0.76±0.04	1.56±0.02	6.78±0.68	1.13±0.07	0.66±0.05	0.80±0.24	2.33± 0.04

Data are expressed as MEAN ± SEM, Significant at [a]$p<0.05$, [b]$p< 0.01$, [c]$p< 0.001$, when compared to control. (n=5).

3.4. Effect of extract on liver function indices of rats

Administration of husk extract of *Z. mays* (187-561 mg/kg) to malerats for 100 daysdid not affect the levels of total protein, albumin and combined bilirubin of the male rats significantly ($p>0.05$) when compared to control (Table 3).

However, dose-dependent and insignificant (p>0.05) reductions in the levels of ALT, ALP, AST, GGT and total bilirubin of malerats when compared to controlwere observed following the extract administration (Table 3).

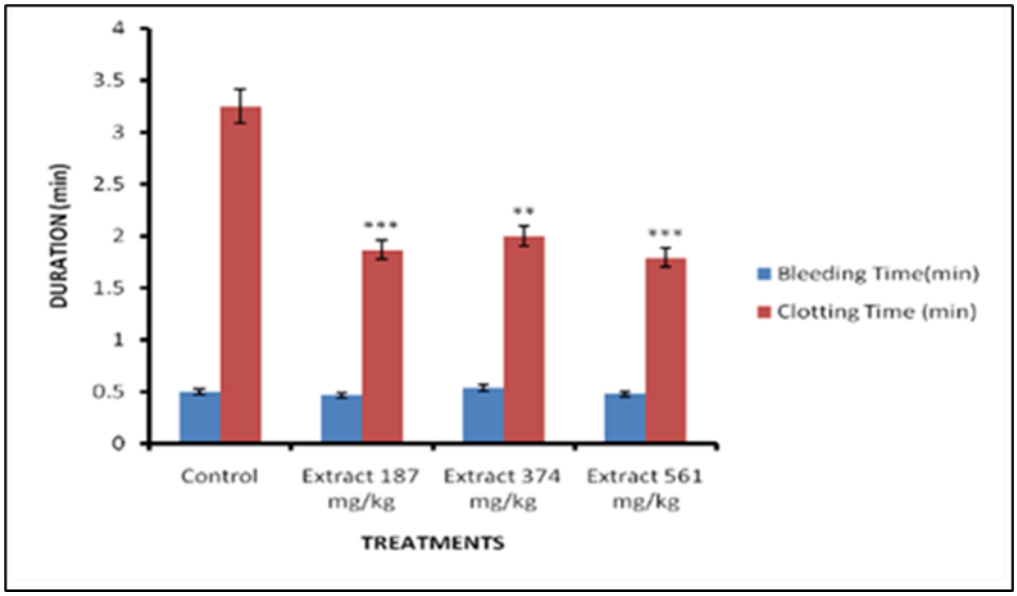
3.5. Effect on kidney function parameters of rats

Treatment of malerats for 100 days with husk extract of *Zea mays* (187-561 mg/kg) did not cause any significant (p>0.05) effect on the levels of creatinine, urea, bicarbonate, sodium, potassium and chloride of the male rats when compared to control (Table 4). However, higher doses (374 and 561 mg/kg) of the husk extract were found to cause insignificant (p>0.05) and non dose-dependent increases in the level of sodium ion of male rats when compared to control (Table 4).

Table 3 Effect of chronic administration of *Zea mays* husk extract on heamatological parameters of male rats

Treatment	Dose	WBC (10^9/L)	NEUT. (%)	LYM (%)	MONO (%)	ESINO (%)	BASO (%)	RBC (10^{12}/L)	HGB (g/dL)	PCV (%)	PLATELETS. (10^9/L)
Control normal saline	10 mg/ml	12.72±3.47	27.93±1.69	68.0±1.50	2.33±0.29	0.86±0.14	0.86±0.08	8.21±0.14	14.0±0.49	51.03±2.27	828.0± 139.08
Crude extract	187	10.78±1.01	32.60±2.92	62.92±2.78	2.42±0.28	1.12±0.19	0.92±0.17	7.54±0.23	13.02±0.45	46.70±1.80	979.75± 93.79
	374	9.09±0.92	31.96±1.91	63.80±1.51	2.13±0.97	1.33±0.21	0.76±0.14	7.43±0.45	13.13±0.56	48.03±2.38	769.66± 84.16
	561	10.90±1.44	33.50±2.05	63.95±2.06	1.07±0.17	0.67±0.13	0.80±0.13	7.74±0.11	12.80±0.10	44.47±0.23	834.25±105.22

Data are expressed as MEAN ± SEM,(n=5).



Data are expressed as MEAN ± SEM, Significant at **p<0.01, ***p<0.001, when compared to control. (n=5).

Figure 1 Effect of chronic administration of *Zea mays* husk extract on clotting and bleeding times of male rats

Table 4 Effect of chronic administration of *Zea mays* husk extract on liver function parameters of male rats

Treatment	Dose (mg/ kg)	Total Protein (mg/dL)	Albumin (mg/dL)	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	Total Bilirubin (µmol/L)	Combined Bilirubin (µmol/L)	GGT (IU/L)
Control	10 mg/mL	65.66±2.02	42.0±1.52	60.66±1.20	55.33±1.45	22.00±1.52	11.83±0.52	5.56±0.40	2.13±0.42
Crude extract	187	65.0±3.58	40.0±1.78	65.00±4.50	51.25±1.79	20.75±0.62	12.05±0.65	6.95±0.25	2.12±0.42
	374	64.66±2.18	38.0±1.52	57.33±3.18	48.33±4.91	17.66±0.88	11.16±0.60	5.83±0.20	1.86±0.28
	561	63.50±1.50	40.75±1.75	54.25±2.97	47.25±5.39	19.75±2.01	10.62±0.48	6.17±0.80	1.45±0.11

Data are expressed as MEAN ± SEM,(n=5).

Table 5 Effect of chronic administration of *Zea mays* husk extract on kidney function parameters of male rats

TREATMENT	DOSE (mg/kg)	CREATININE (mg/kg)	UREA (mg/dL)	BICARBONATE (mMol/L)	SODIUM (mMol/L)	POTASSIUM (mMol/L)	CHLORIDE (mMol/L)
Control normal saline	10 mg/ml	119.0± 12.05	8.33± 0.71	25.33± 2.40	139.0±14.43	5.10± 0.46	69.0± 1.15
Crude extract	187	126.5± 9.88	8.72± 0.58	27.0± 0.57	126.5±10.94	4.62± 0.39	67.75± 0.85
	374	131.3± 11.62	8.90± 0.58	25.33± 2.40	162.6± 16.34	5.90± 0.55	70.0± 1.15
	561	112.7± 7.60	7.90± 0.37	27.5±0.95	143.7±14.48	5.30± 0.52	64.75± 0.85

Data are expressed as MEAN ± SEM,(n=5).

3.6. Effect of husk extract on lipid profile indices of malerats

Treatment of malerats with husk extract of *Zea mays*(187-561 mg/kg) for 100 days caused dose-dependent but insignificant ($p>0.05$) decreases in the levels of total cholesterol, triglyceride and HDL of male rats treated for 100 days when compared to control. However, LDL and VLDLlevels were dose-dependently increased with significant ($p<0.01$) increase recorded only in VLDL level of the male group treated with the highest dose (561 mg/kg) when compared to control (Table 6).

3.7. Effect on histology of organs

Figures 2 - 7 show the effects of chronic administration of ethanol husk extract of *Zea mays* to male rats for 100 days on histology of some organs. The husk extract (187-561 mg/kg) exerted varying effect on the histo-structures of the different organs of male rats. Moderate effect was observed on the histo-structure of cerebral cortex with abnormal presentation of the layer cells showing proliferating astrocytes, shrinkage of neural cells and micro-cerebral blood vessels, perinuclear vacoulatory astrocytes, shrinkage of neural cells and micro-cerebral blood vessels (Figure 2). The histo-structure of the heart tissues ofmale rats were moderately affected showing cardiac myometrium with abnormal cardiomyocytes array, wide spread interstitial fibrocytes and fibroblast nuclei, vacoulatory myocyte nuclei, and micro-hemorrhages, area of inflammatory infiltrates and degenerating myocyte nucleiwithin the cardiac tissue (Figure 3).The extract treatment exerted a mild effect on the kidney histo-structure of male rats with section showing glomeruli with occluding bowman's space, glomeruli having widened bowman's space (Figure 4). The liver tissue was moderately affected with hepato-architecture showing infiltrated micro-vesicular steatosis, areas of degenerating hepatocytes and vacuolated hepatocytes within the hepatic lobule (Figure 5). The male rats splenic tissues and testicular cyto-structures were not affected by the extract's treatment for 100 days. These tissues had normal histo-structures (Figures 6 and 7).

3.8. Effect of chronic administration of husk extract of *Zea mays* on seminal analysis

Table 6 Effect of chronic administration of *Zea mays* husk extract on lipid profile of male rats

Treatment	Dose mg/kg	Total cholesterol (mMol/L)	TRIGLYCERIDE (mMol/L)	HDL-C (mMol/L)	LDL-C (mMol/L)	VLDL (mMol/L)
Control	10 mL/kg	2.06± 0.17	0.79± 0.14	1.20± 0.27	1.22± 0.07	0.18± 0.01
Crude extract	187	2.10±0.09	0.94± 0.10	1.28± 0.14	1.24± 0.15	0.20± 0.01
	374	2.10± 0.15	0.93± 0.13	1.22± 0.08	1.30± 0.13	0.22± 0.02
	561	2.02± 0.14	0.73± 0.17	0.90± 0.12	1.48± 0.21	0.25± 0.01[b]

Data are expressed as MEAN ± SEM, Significant at [a]$p<0.05$, [b]$p< 0.01$, [c]$p< 0.001$, when compared to control. (n=5).

Table 7 Effect of chronic administration of husk extract of Zea *mays* on semen of rats

	Motility				Concentration				Morphology				
Treatment	% Motile	% Non-motile	% Prog. motile	% Non-prog. motile	1st	2nd	3rd	4th	Average (Cells X 106)	% Normal	% Bent neck	% Curve	% Slender
Control	75	25	70	30	84	95	78	89	89.50	80	10	10	-
Ext 187 mg/kg	70	30	70	30	91	75	83	77	81.50	50	10	30	10
Ext 374 mg/kg	85	15	80	20	91	86	83	110	92.50	75	15	10	-
Ext 561 mg/kg	70	30	65	35	83	86	79	81	82.25	70	5	25	-

Table 7 shows the effect of chronic administration of husk extract of *Zea mays* on the semen from rats. The semen in all treatment groups was found to be milky in appearance,the percentage of motile sperm cells in the control was 75 %

while 25% of the cells were non motile. The motile cells in the extract-treated groups were 70%, 85% and 70% respectively for 187, 374 and 561 mg/kg treated groups. The average cell concentrations were 89.0, 81.50, 92.50 and 82.25 cells x10^6 for control, 187, 374, and 561 mg/kg treated groups respectively. However, morphological analysis of the semen revealed that 80% of cells in the control group were normal and abnormal cells were 20%. In the low dose (187 mg/kg) and high dose (561 mg/kg) treated groups, 50% normal cells and 50% abnormal cells were recorded, while the middle dose (374 mg/kg) treated groups recorded 75%normal cells and 25% abnormal cells (Table 7).

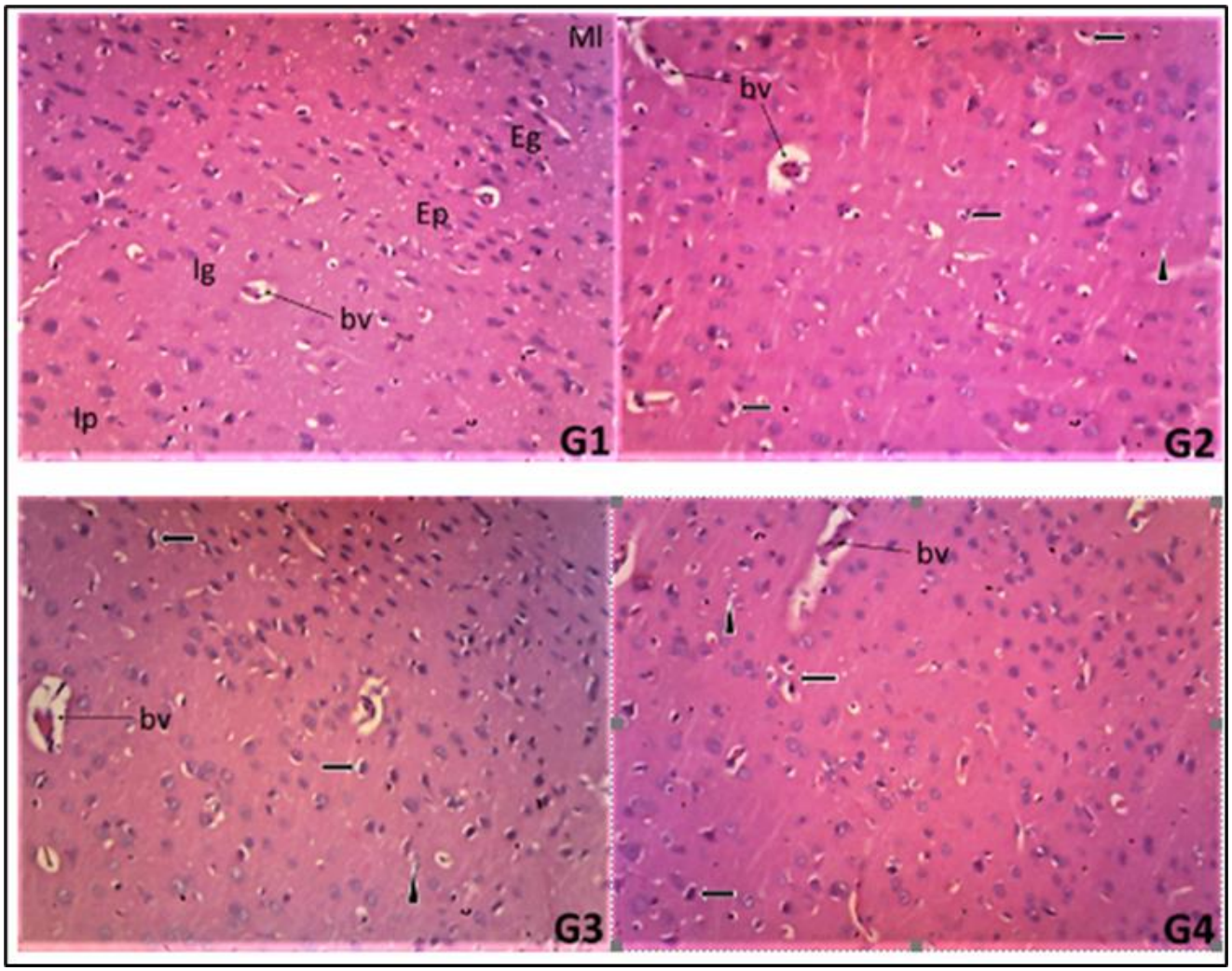


Figure 2 Photomicrograph of the transverse sections of cerebral cortex of male rats treated with distilled water (G1), husk extract of *Zea mays* at 187 mg/kg (G2), 374 mg/kg (G3) and 561 mg/kg (G4) liver tissue showing external pyramidal layer (EPL), external granular layer (Ep),internal granular layer (Ig), Pyramidal cells (Pc), blood vessels (bv),Molecular layer (ML),pyramidal cells (Pc) and granular cells (Gc), astrocytes (As), showing moderate effect on extract treated rats tissue withperinuclear vacoulatory astrocytes (arrow head), shrinkage of neural cells (arrow) and micro-cerebral blood vessels (bv). (x100).

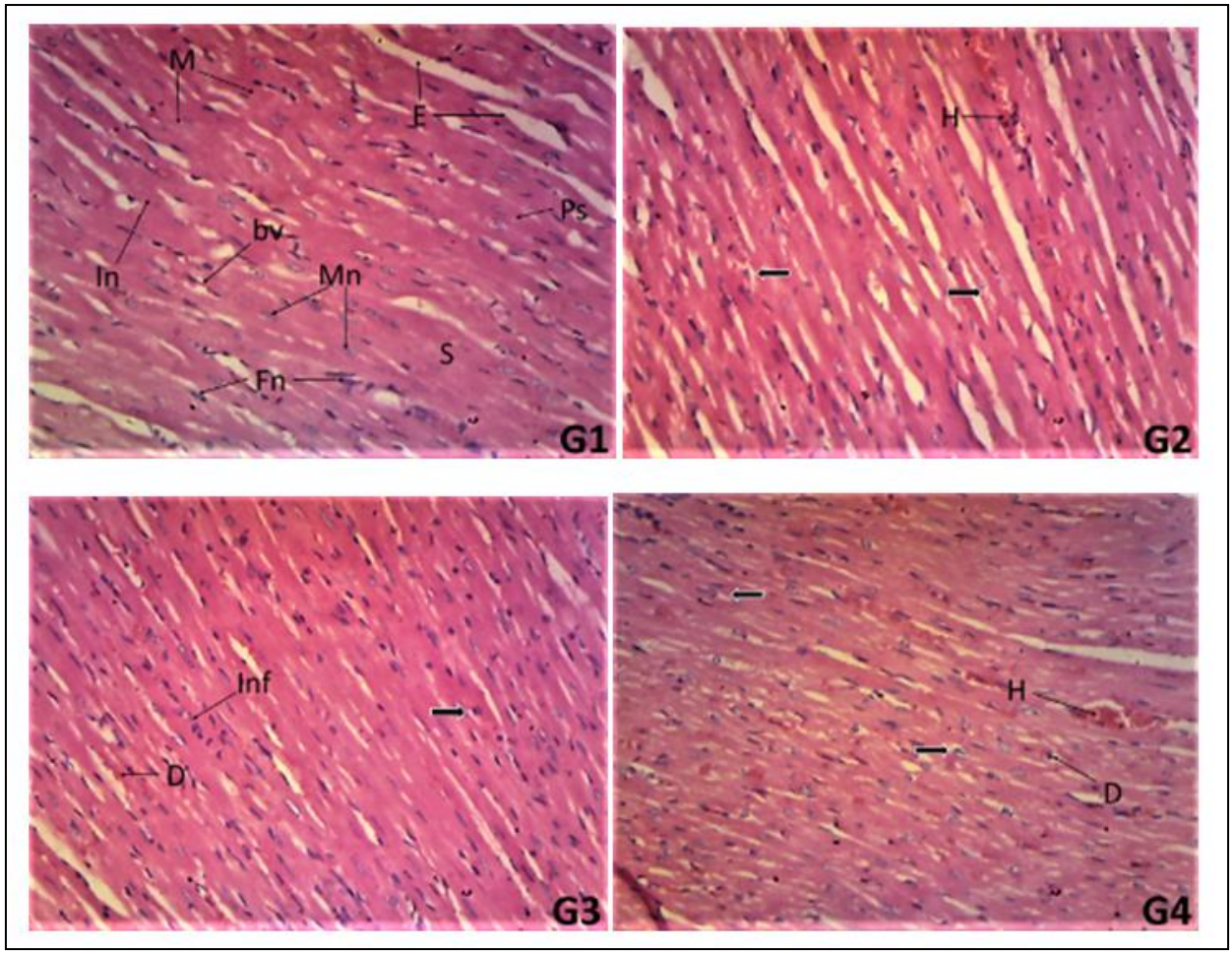


Figure 3 Photomicrograph of the transverse sections of hearts of male rats treated with distilled water (G1), husk extract of *Zea mays* at 187 mg/kg (G2), 374 mg/kg (G3) and 561 mg/kg (G4) liver tissue showing sarcoplasm (S), wide spread interstitial fibrocytes and fibroblast nuclei (Fn), well stained myocyte central nuclei (Mn), perinuclear sarcoplasm (Ps), interstitial endomysium (E),wide spread interstitial fibrocytes and fibroblast nuclei (F), vacoulatory myocyte nuclei (arrow), area of inflammatory infiltrates (Inf) and degenerating myocyte nuclei (D) within the cardiac tissue.

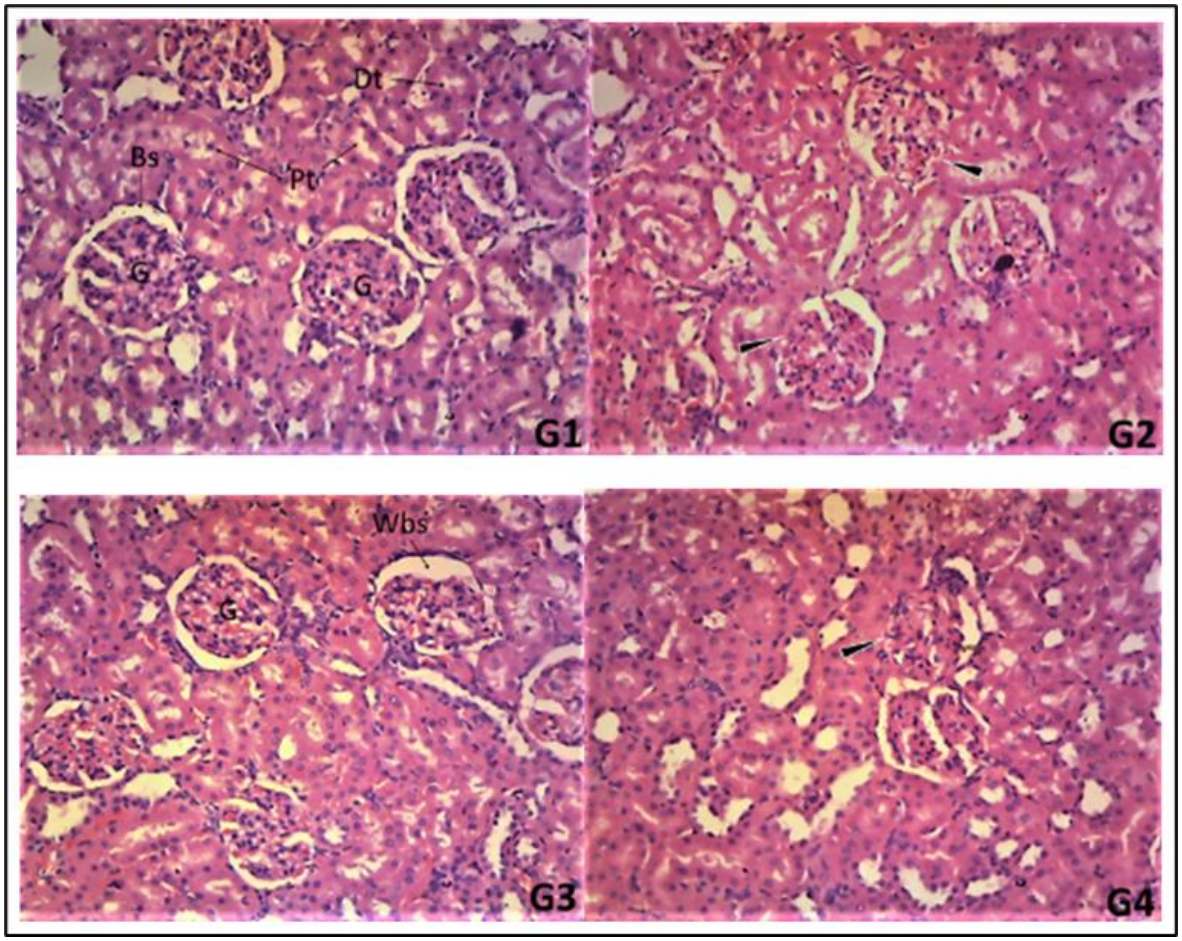


Figure 4 Photomicrograph of the transverse sections of kidneys of male rats treated with distilled water (G1), husk extract of *Zea mays* at 187 mg/kg (G2), 374 mg/kg (G3) and 561 mg/kg (G4) liver tissue showing glomeruli (G),Bowman's space (Bs), proximal convulated tubules (Pt), distal convulated tubules (Dt) and infiltrating interstitial connective tissues (Ic), widened bowman's space (Wb),and widening bowman's space (Wb), (x 100)

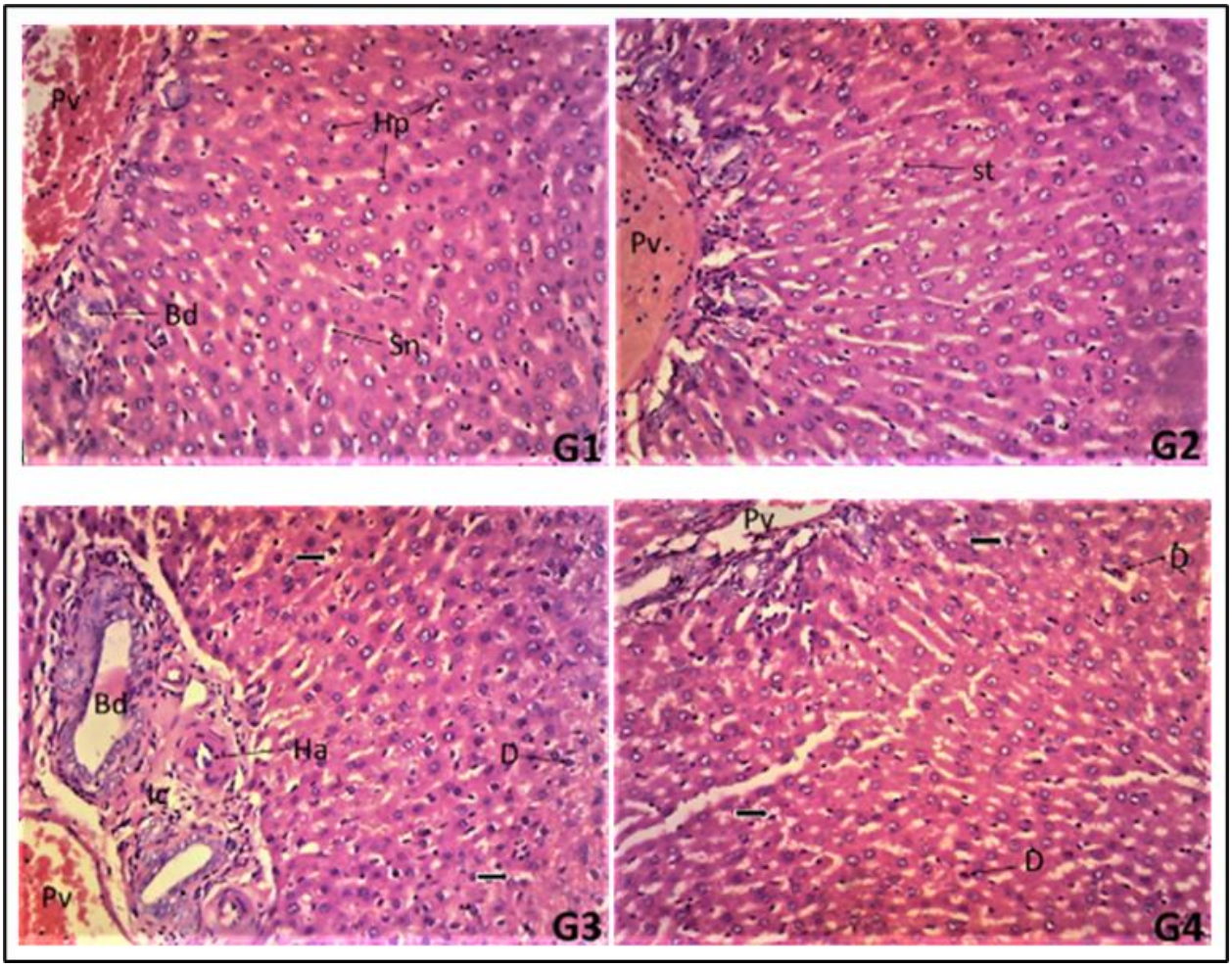


Figure 5 Photomicrograph of the transverse sections of livers of male rats treated with distilled water (G1), husk extract of *Zea mays* at 187 mg/kg (G2), 374 mg/kg (G3) and 561 mg/kg (G4) liver tissue showing portal vein (Pv), bile duct (Bd) and hepatic artery (Ha) within the connective tissue (Ct) of the portal area, well populated hepatocytes (Hp), kupffer cells (Kc) and arrays of sinusoidal spaces (Sn) ,areas of vacoulatory hepatocytes (arrow), Interstitial connective tissue (Ic) (x 100).

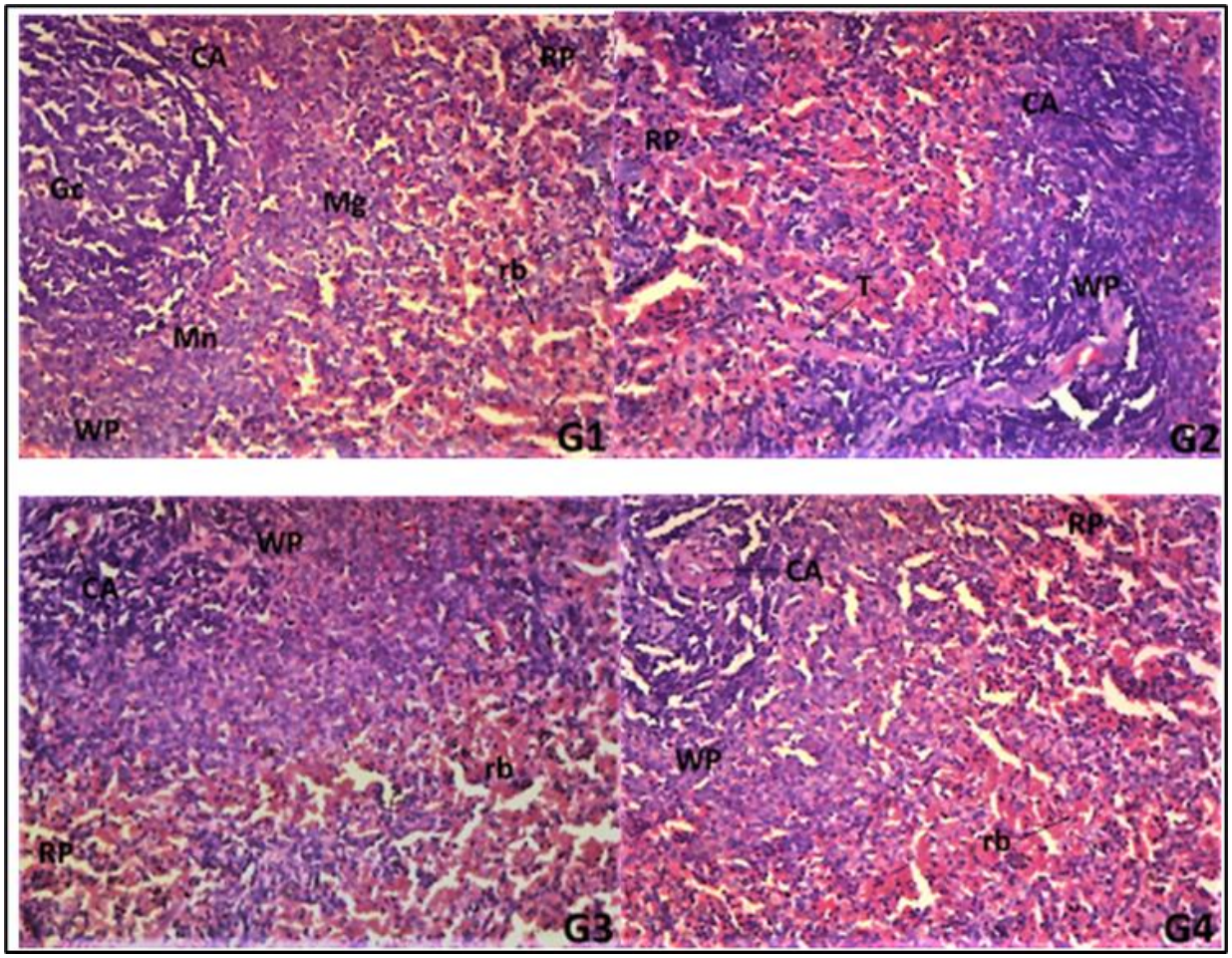


Figure 6 Photomicrograph of the transverse sections of spleens ofmale rats treated with distilled water (G1), husk extract of *Zea mays* at 187 mg/kg (G2), 374 mg/kg (G3) and 561 mg/kg (G4) liver tissue showing white pulp (WP), germinal center (Gc), central artery (C), central vein (Cv),ramified trabecula (T), mantle layer (Mn),marginal zone (Mz), the red pulp (RP), red blood cells (Rc) and tubercular tissue (Tb), degenerative immunal cells (black arrow) and the central vein (Cv).

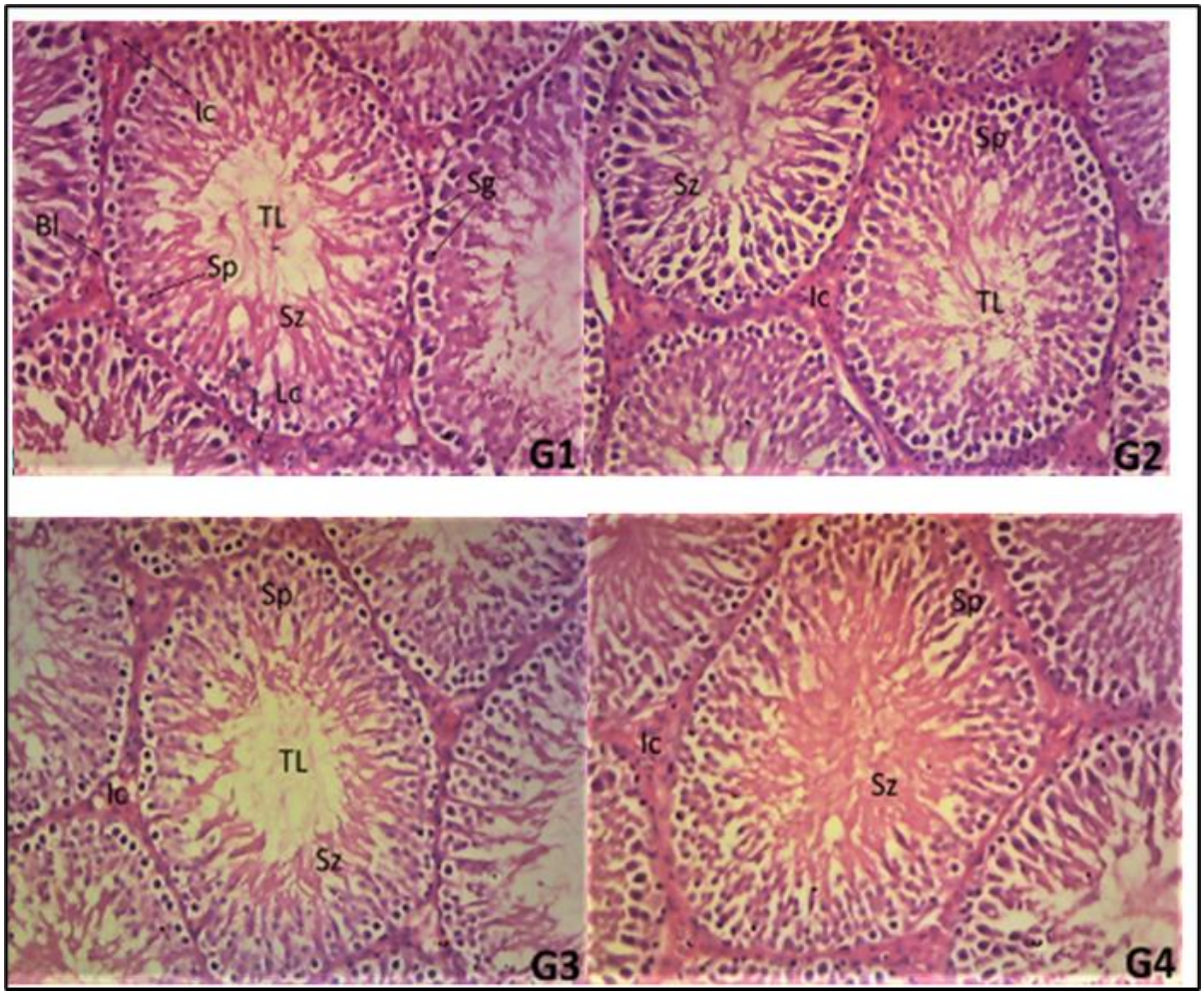


Figure 7 Photomicrograph of the transverse sections of testis ofrats treated with distilled water (G1), husk extract of *Zea mays* at 187 mg/kg (G2), 374 mg/kg (G3) and 561 mg/kg (G4)testis tissue showing basement layers (Bl), spermatogonia cells (Sg), radiating spermatozoa (Sz) within the tubular lumen (TL), spermatids (Sp), Leydig cells (Lc), blood vessels (Bv) with erythrocytes (E), spermatozoa (Sz), tubular lumen (TL),interstitial connectives tissues (Ic). (x 100)

4. Discussion

In this study, chronic administration of the husk extract caused significant increases in the body weights ofmale rats compared to untreated control, as observed in the weight gain by all the treated groups. Alterations in body weights are indicators of toxic effects of drugs and it is considered to be severe ifthe body weight loss is more than 10% [26]. The increases in body weights of male rats in all the extract treated groups which was significant ($p<0.05$-0.01) at middle dose (374 mg/kg) indicates that extract treatment did not interfere with the feeding habit of the rats and probably must have stimulated the appetite of the male rats. Furthermore, the administration of the extract did not exertnegative effects on the body growth processes of rats.

Treatment of rats with the husk extract (187-561 mg/kg) for 100 days did not affect the weights of heart, brain, liver, kidney, spleen, pancreas, testes and ovary when compared to control, though with a slight decreases in pancreas weights of treated male rats. Internal organs weights are generally considered as important indicator to injury and toxicities [27]. Hypertrophy of organs resulting from inflammation of the organs leads to weight increases of the affected organs is an indication of toxicity and damaged to organ [28].The insignificant decreases in weights ofpancreas do not portray a serious harmful effect and maybe a reflection of the moderate effect observed in the histopathology of these organs.

Assessment of haematological parameters is commonly used to determine the extent of deleterious effect of foreign compounds including plant extract on the blood [29] . It can also be used to explain blood-relating functions of a chemical compound including those contained in plant extracts [30]. Chronic administration of husk extract of *Zea mays*

to male rats for 100 days did not affect RBC, WBC and platelets counts, hemoglobin concentration, PCV, neutrophils, lymphocytes, basophils, monocytes and eosinophils percentages significantly. This might be an indication that there was no destruction of matured RBC's and no change in the rate of production of erythrocytes (erythropoiesis) [31] as well as leucocytosis. This also indicates that the extract did not inhibit erythropoeitin potential, the humoral regulator of RBC production [32]. The lack of significant effect on the RBC and Hb also implies that the oxygen-carrying capacity of the blood was not hindered. The non-definite effect of the extract on the platelets can be attributed to adaptation by the animals to the effect of the extract. However, the clotting times of male ratswere observed to be significantly reduced in this study. This may be due to the extract ability to facilitate blood clotting mechanisms in the rats.

In this study, administration of husk extract for 100 days to malerats did not exert any significant effect on all the liver function parameters. This suggests that the husk extract did not cause any harm to the liver as both the synthetic and excretory functions indicated by total protein, albumin and bilirubin levels were not affected [33,34]. Also, the fact that the levels of liver enzymes (AST, ALT and ALP) were not affected significantly further confirms the non hepatotoxic and/or hepatoprotective effect of the huskearlier reported [10,11,12].

In this study, the administration of husk extract of *Zea mays* to male rats for 100 days did not cause any significant effect on any of the kidney function parameters (urea, creatinine, bicarbonate, potassium and chloride) except a slight elevation of sodium levelin male rats which was not significant. This does not suggest a serious effect on the glomerular filtration rate as was seen in the mild effect on the histo-structure of the renal tissues in this study. However, this does not portray a serious harmful effect as other parameters were not affected. Blood urea nitrogen (BUN) produced in the liver is derived from the diet or tissue sources and is excreted in the urine via the kidney. Serum urea accumulates in the serum in renal disease when the rate of production exceeds that of excretion [35]. Serum creatinine is basically derived from endogenous sources by tissue creatinine breakdown [35].Therefore, elevation of urea and creatinine levels in the serum had been taken as the index of nephrotoxicity [36,37]. In this study, there was no significant effect on creatinine and urea levels in rats exposed to prolonged administration of the husk extract. This suggests that the extract does not exert a serious nephrotoxic effect and further support previously reported nephroprotective potentials of the husk extract [14,15].

In this study, administration of husk extract to male rats for 100 days was found to exert hypolipidemic effect on all major lipids in the rats except the LDL and VLDL levels of male rats at the highest dose (561 mg/kg) which was found to be raised significantly. Alterations in the concentrations of major lipids like total cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides provide useful information on the lipid metabolism and its attendant effect on heart related diseases [38]. High blood cholesterol concentrations are an important risk factor for cardiovascular disease [39]. Therefore, the reduced levels of serum cholesterol by the husk extract may be a beneficial effect to the animalsas the extract is unlikely to be associated with cardiovascular risk except at very high doses in the male rats. Decreased serum level of triacylglycerides by the extract may be explained as an inhibitory effect on lipolysis [38]. The reduction in the levels of total cholesterol, tryglycerides and HDL in this study further support the strong hypolipidemic and antiobesity potentials of the husk extract [17] which perhaps is due to inhibitory activity of its phytoconstituents on lipolysis.

On the histology, chronic administration of ethanol husk extract of *Zea mays* (187-561 mg/kg) to male rats for 100 days produced mild to moderate defects on the histology of the brain, heart, liver, kidney, ovary and spleen tissues, indicating that chronic administration of the extract may produce toxic effect though not serious effect on these organs. However, the testis was not affected as observed in this study suggesting that the extract has no effect on male reproductive system. These mild to moderate effects observed in this study corroborate the chemical pathology results observed as only a slight increase in sodium concentration were observed, which suggest a mild toxic effect of the extract. However, no mortality was recorded throughout the period of chronic study.

The semen analysis revealed that the extract-treated groups had high percentages of motile active cells (70 - 85%), high concentration (81.50 - 92.50 x10^6 cells) and 50 - 75% normal cells. This suggests that the extract did not affect the process of spermatogenesis or cause any testicular cell damage. This was further supported by the histological findings on the testes which showed no pathological effecton the testis in all extract-treated groups.

5. Conclusion

The results of this investigation suggests that the husk extract of *Zea mays* when taken for a long time could have mild to moderate effects on the brain, heart, liver and kidney. However, the husk extract exerted no toxic effect on the spleen, testis and sperm cells and provided hypolipidemic effect. It recommended that high doses should be avoided.

Compliance with ethical standards

Acknowledgments

This study was privately funded by the authors and no special financial support was obtained from any funding agencyin the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

All the authors hereby declare that no conflicts of interest exist related to this article.

Statement of ethical approval

This study was approved and ethically cleared by College of Health Sciences Animal Ethics Committee, University of Uyo(UU/CHS/IHREC/2023/VOL.1/57).

References

[1] Foster S, Duke JA. Field Guide 10 Medical Plants: Eastern and Central North America. Houghton MifAin, Boston.1990.

[2] Gill LS. Ethnomedical Uses of Plants in Nigeria*.* Uniben Press, Benin, Nigeria, 1992;p 249.

[3] Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AE. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. Journal of Ethnopharmacology, 2008;IS: 67-71. ·DOI: 10.1016/j.jep.2007.09.005

[4] Brobbey AA, Somuah-Asante S, Asare-Nkansah S, Boateng FO, Ayensu I. Preliminary phytochemical screening and scientific validation of the anti-diabetic effect of the dried husk of Zea mays L. (Corn, Poaceae). International Journal of Phytopharmacy. 2017; 7 (1): 01-05.DOI: https://dx.doi.org/10.7439/ijpp.

[5] Okokon JE, Bassey S, Dinesh M, Dinkar S. Antimalarial and anti-plasmodial activity of husk extract and fractions of Zea mays. Pharmaceutical Biology, 2017;55(1): 1394 – 1400.·DOI: 10.1080/13880209.2017.1302966

[6] Okokon JE, Nelson E, Sunday M.Antidepressant activity of ethanol husk extract of Zea mays. Adv Herbal Med. 2016;2(4): 22 -28.

[7] Owoyele BV, Negedu MN, Olaniran SO, Onasanwo SA, Oguntoye SO, Sanya JO, Oyeleke SA, Ibidapo AJ,Soladoyel AO.Analgesic and anti-inflammatory effects of aqueous extract of Zea mays husk in male wistar rats. Journal of Medicinal Foods, 2010;13:343-347.doi: 10.1089/jmf.2008.0311.

[8] Jadhav SM. Protective effect of Zea mays on experimentally induced gastric ulcer in rats. Imperial Journal of Interdisciplinary Research, 2016;2: 8.

[9] Dong J, Cai L, Zhu X, Huang X, Yin T, Fang H, Ding Z. (2014). Antioxidant activities and phenolic compounds of cornhusk, corncob and Stigma maydis. Journal of Brazilian Chemical Society. 2014;25: 1956-1964.https://doi.org/10.5935/0103-5053.20140177

[10] Okokon JE, Nyong ME, Essien GE, Nyong E. Nephroprotective activity of husk extract and fractions of Zea mays against alloxan-induced oxidative stress in diabetic rats. Journal of Basic Pharmacology and Toxicology. 2017;1(3):1-10.

[11] Okokon JE, Udobang JA, Bassey AL, Edem UA, Agu CE. Hepatoprotective and nephroprotective activities of Zea mays husk extract against paracetamol-induced liver and kidneys injuries in rats.Tropical Journal of Natural Products Research2020;4(3): 69-76.DOI: 10.26538/tjnpr/v4i3.2

[12] Udobang JA, Okokon JE, Obot D, Agu CE. Hepatoprotective activity of Zea mays husk extract against carbon tetrachloride induced liver injury in rats. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences. 2019;5(5):82-94. DOI: 10.26479/2019.0505.08

[13] Okokon JE, Nyong M. Antidiabetic and hypolipidemic activities of Zea mays husk extract and fractions. Journal of Herbs Spices and Medicinal Plants2018; 24(3):1-17.

[14] Okokon JE, Nyong ME, Essien GE,Nyong E. Hepatoprotective activity of husk extract and fractions of Zea mays against alloxan-induced oxidative stress in diabetic rats. International Journal of Herbal Medicine. 2017;5(4):43-50.

[15] Okokon JE, Udobang JA, Obot D, Agu CE. Nephroprotective activity of Zea mays husk extract against gentimicin-induced kidney injury in rats. Journal of Medicinal Plant Studies2019;7(6): 156-160.

[16] Okokon JE, Obot J,Amazu LU. Antiulcerogenic activity of husk extract of Zea mays. African Journal of Pharmacology and Therapeutics2018;7(2): 41-45.

[17] Okokon JE, Nyong ME, Thomas P, Udobang JA, Enin G. Antiobesity activities of extract, fractions and pure compounds from husk of Zea mays. Tropical Journal of Natural Products Research 2021;5(10): 1868-1875.doi:10.26538/tjnpr/v5i10.27

[18] OkokonJE, Enin GU, Nyong ME, Anyanwu BC, Dick GF.Inhibitory effect of husk extract and fractions of Zea mays on alpha amylase and alpha glucosidase enzymes. Advance Pharmaceutical Journal.2021;6(6): 150-156.

[19] Akpan EE, Johnny II, Anagboso MO, Ebong OO, Okokon JE. Genotoxic and cytotoxic potentials of cornhusk extracts of Zea mays and leaf extract of Saccharum officinarum.Biology Medicine and Natural Product Chemistry 2024;13(2):531-540. doi:10.14421/biomedich.2024.132.531-540

[20] Ogawa K, Takeuchi M, Nakamura N.Immunological effects of partially hydrolysed arabinoxylan from corn husk in mice. Biosciences, Biotechnology and Biochemistry 2005; 69:19-25.DOI: 10.1271/bbb.69.19

[21] LiC, Kim H, Won S, Min H, Park K, Park J, Ahn M, Rhee H. Corn husk as a potential source of anthocyanins. Journal of Agricultural Food and Chemistry, 2008; 56 (23): 11413–11416.DOI: 10.1021/jf802201c

[22] Tietz, N. W. Fundamentals of Clinical Chemistry. 2nd Edition. W. B. Saunders, Philadelphia, pp335-337, 1976.

[23] Friedewald WT, Levy RI, Fredrickson DS.Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, 1972;18(6): 499 – 502. PMID: 4337382

[24] Azu OO, Duru FIO, Osinubi AA, Oremusu AA, Norohna CC, Okanlawon AO, Elseha SO. Long-term treatment with Kigelia africana fruit extract ameliorates the testicular toxicity following cisplatin administration in male Sprague-Dawley rats. Journal of Medicinal Plants Research, 2011;5(3): 388-397.

[25] Osinubi AA, Daramola AO, Noronha CC, Okanlawon AO, Ashiru AO. Effect of quinine and ascorbic Acid on rat testes. West African Journal of Medicine, 2007; 26(3): 217-221.

[26] Tepongning RN, Mbah JN, Avoulou FL, Jerme MM, Ndanga E-K, Fekam FB. Hydroethanolic extracts of Erigeron floribundus and Azadirachta indica reduced Plasmodium berghei parasitaemia in Balb/c Mice. Evidence-based Complementary and Alternative Medicine. 2018: 5156710. doi: 10.1155/2018/5156710.

[27] Farah AO, Nooraain H, Noriham A, Azizah AH, Nurul HR. Acute and oral subacute toxicity study of ethanolic extract of Cosmos caudatus leaf in Sprague Dawley Rats. International Journal of Biosciences, Biochemistry and Bioinformatics. 2013; 3(4): 301-305.doi:10.7763/IJBBB.2013.V3.218

[28] Ping KY, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats. Biomedical Research International.2013;2:1-14.doi: 10.1155/2013/182064.

[29] Lawal IO, Grierson DO, Afolayan AI. Phytochemical and antioxidant investigations of ma Clause a Aniston Hook; A South African medicinal plant. African Journal of Traditional complimentary Alternative Medicine. 2015; 12(1): 28-37.DOI:10.4314/ajtcam.v12i1.5

[30] Bashir L, Shittu OK, Busari MB, Sani S,Aisha MI. Safety evaluation of giant African land snails (Archachatina maginata) haemolymph on hematological and biochemical parameters of albino rats. Journal of Advances in Medical and Pharmaceutical Sciences, 2015;3(3), 122-30.doi:10.9734/JAMPS/2015/16393.

[31] Berinyuy EB, Lawal B, Olalekan AA, Olalekan IA, Yusuf AA, Sakpe S, Ossai PC. Hematological status and organs/body-weight parameters in Wister rats during chronic administration of Cassia occidentalis. International Blood Research and Review,2015; 4(3), 1-7. doi:10.9734/IBRR/2015/22021

[32] Shittu OK, Lawal B, Alozieuwa BU, Haruna GM, Abubakar AN, Berinyuy EB. Alteration in biochemical indices following chronic administration of methanolic extract of Nigeria bee propolis in Wistar rats. Asian Pacific Journal of Tropical Disease, 2015;5(8): 654-657.https://doi.org/10.1016/S2222-1808(15)60907-0

[33] Kaplan A, Szabo LV, Opheim KE. Clinical Chemistry: Interpretation and Techniques: Lea &Febiger Philadelphia; 1979.

[34] Yakuba MT, Bilbis LS, Lawal M, Akanji MA. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. Biokemistri 2003;15 (2): 50-6.

[35] Mayne PD. The kidneys and renal calculi. In: Clinical chemistry in diagnosis and treatment. 6th ed. London: Edward Arnold Publications, pp.2-24,1994.

[36] Ali BH, Ben TH, Basheer AA.Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity: influence of gonad-ectomy and hormonal replacement therapy. Industrial Journal of Pharmacology 2001;33: 369-373.

[37] Flaoyen A, Hove K, Wilkins AL. Tolerance to nephrotoxic component of Nartheciumossi fragumin sheep: The effects of repeated oral doses of plant extracts. Veterinary Research Communication. 2001;25: 127-136.doi: 10.1023/a:1006456919052

[38] Yakubu MT, Akanji MA, Oladiji AT. Alterations in serum lipid profile of male rats by oral administration of aqueous extract of Fadogia argrestis stem. Res. J. Med. Plant. 2008; 2: 66-73.doi=rjmp.2008.66.73

[39] Abolaji AO, Adebayo AH, Odesanmi OS. Effect of ethanolic extract of Parinari polyandra(Rosaceae) on serum lipid profile and some electrolytes in pregnant rabbits. Research Journal of Medicinal Plants 2007;1: 121- 127. doi=rjmp.2007.121.127