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HEPATO-NEPHROPROTECTIVE, HEMATOPOIETIC AND ANTI-SPERMATOGENIC EFFECT OF THE ETHANOLIC EXTRACT OF JATROPHA TANJORENSIS USING MALE ALBINO RATS

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ABSTRACT
This study was conducted to ascertain the genotoxic, sperm boosting, hematopoietic, hepato-nephron-protective, properties of the ethanolic extract of *Jatropha tanjorensis* (EEJT) using male Wistar albino rats. The animals were randomly assigned to five groups of six animals each. Animals in group 1 were administered 10mgkg^{-1} water, groups 2-4 received 200, 400 and 800mgkg^{-1} EEJT respectively while groups 5 received 300mgkg^{-1} of Addyzoa for 64 days. Micronucleus assay, sperm cell count, hematological, biochemical, and antioxidant potentials were evaluated using standard methods. At 800mgkg^{-1}, there was significant ($p \leq 0.05$) increase in the level of red blood cell (RBC), Haemoglobin (HGB) and Hematocrit (HCT) and significant ($p \leq 0.05$) decrease in ALP compared to Addyzoa. The extract dose dependently attenuated the concentrations of AST, ALP, total bilirubin, cholesterol, LDL, triglyceride and MDA compared to control and Addyzoa groups. Platelet, granulocytes, SOD, HDL, total protein RBC, HGB and HCT were dose dependently increased in the groups administered the extract compared to the control and Addyzoa groups. At 200mgkg^{-1}, EEJT had the lowest number of binucleated and micronucleus cells. The highest number of abnormal sperm cells were seen in the group administered 800mgkg^{-1} of the extract. This experiment shows that EEJT dose-dependently improved erythropoietin promoting potential and hepatoprotective activity in the experimental animal. There was marked reduction in sperm count with sperm head abnormality at very high dosages. However, more research should be carried out to ascertain its mechanism of action and the dose to be administered over a long period.

KEYWORDS: Genotoxic, Sperm Booster, Hematopoietic, Hepato-Nephron-Protective, Micronucleus Assay, Antioxidant.

INTRODUCTION
Jatropha tanjorensis, commonly referred to as "hospital-too-far" or Ugu-Oyibo in Igbo language belongs to the family Euphorbiaceae. It is widely grown and used as a leafy vegetable and a medicinal plant in South Eastern Nigeria. It is used locally in treatment of malaria, ailments associated with the liver and kidney, infertility and improvement of hematologic indices. It is reported to have antidiabetic, hepatoprotective, hypoglycaemic and antioxidant properties and enhances the function of the bone marrow (Olayiwola, et al., 2004; Orhue *et al*., 2008; Madubuike, et al., 2015). Phytochemical screening of *J. tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (Ehimwenma and Osagie, 2007).

The testes (testicles) are two oval shaped organs located in the scrotum (Osuchukwu *et al*., 2016) and are the most essential organs of the male reproductive system. They are the glands where sperm and testosterone are produced (Keith et al., 2013). This implies that whatever affects the testes can influence sexual characteristic and fertility. Some plants have been used to improve semen quality e.g. Korean red ginseng (Hong, *et al*., 2002), *Lepidium meyenii* (Lee *et al*., 2016), while some have been used to improve hematopoiesis e.g. *Sanguisorba officinalis* (Chen *et al*., 2017). In the case of hepatic diseases, several species such as *Silybum marianum*, *Phyllanthus niruri*, and *Panus giganteus* have been shown to ameliorate hepatic lesions (Cacciapuoti *et al*., 2013).

Plants used in traditional Complementary and Alternative medicine (CAM) for the treatment of different ailments and disorders are believed to be safe

nevertheless, adequate pharmacological assays should be performed so as to ascertain their safety and efficacy. In many cases, the mechanisms and modes of action of these plants as well as their therapeutic effectiveness have been confirmed in clinical studies.

In South Eastern Nigeria, it is used traditionally in enhancing male fertility, hepatoprotection and as a blood nourishing tonic. It is believed to facilitate production of blood cells and platelets in the body. Therefore, this study is aimed at determining the genotoxic, hematopoietic, hepatoprotective, antioxidant and sperm improving properties of the ethanolic extract of *Jatropha tanjorensis* using male Wistar albino rats for 64 days.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

Fresh *Jatropha tanjorensis* leaves were collected from a residential farmyard in Abaranje, Ikotun Local Government Area, Lagos Nigeria. They were subsequently identified and authenticated at the Department of Botany, University of Lagos, Nigeria and was allocated a voucher specimen number LUH:7446. The leaves were air dried at room temperature and finely ground using Corona® hand grinder. The dried powdered leaves were soaked in 50 % ethanol for 48 hours. The crude extract was weighed and the stock solution was prepared using water.

Experimental Animals

Thirty adult male Swiss albino rats weighing an average of 175.5 ± 5g were purchased from the Laboratory animal centre of the College of Medicine, University of Lagos Nigeria. They were maintained under standard laboratory conditions at the Experimental Animal House of the Department of Cell Biology and Genetics, Faculty of Science, University of Lagos with dark and light cycle (12/12 hrs.) and fed with standard rat chow bought from Ladokun feeds, Ibadan, Nigeria and clean tap water *ad libitum*. All animal experiments were carried out in accordance with the recommendations of the Guide for the care and use of laboratory animals.

Drug Treatment Protocol

After an adaptive period of 1 week, the animals were randomized and divided into five groups of six animals each. The animals were weighed before and after experiment.

Group 1: serve as normal control received distilled water (10 ml kg^{-1} body weight p.o.) for 64 days.
Group 2: were treated with 200 mg kg^{-1} body weight of the extract for 64 days.
Group 3: were treated with 400 mg kg^{-1} body weight of the extract (GOV) for 64 days.
Group 4: were treated with 800 mg kg^{-1} body weight of the extract (GOV) for 64 days.
Group 5: received a sperm boosting reference drug Addyzoa (300 ml kg^{-1} body weight) for 64 days.

After the experimental period, the animals were anesthetized mildly with ether and blood was collected from the retro-orbital plexus. They were sacrificed and more blood samples were collected by cardiac puncture for evaluating the biochemical parameters and oxidative stress. The testes were harvested and used for antioxidant and epididymal sperm analysis. The livers and kidneys were also dissected out for histology assay.

Haematologic Indices

An auto Hematology Analyzer, Mindray BC3200 was used to determine Haemoglobin (HGB), Hematocrit level, blood cell (RBC), mean corpusclar volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), Granulocytes, red blood cell distribution width - Coefficient of variation (RDW-CV), red blood cell distribution width - Standard deviation (RDW-SD), Mean Platelet Volume (MPV), platelet count (PLT), Platelet Distribution Width (PDW) and plateletcrit (PCT).

Determination of Antioxidant Activity

Estimation of enzymic and nonenzymic antioxidants activities e.g. Catalase (Sinha 1972), Reduced Glutathione (Ellman, 1959), Glutathione-S-Transferase (Habig and Jakoby, 1974), Superoxide Dismutase (Kakkar *et al.*, 1984) and lipid peroxidation level (thiobarbituric acid reactive substances) (Niehaus and Samuelsson, 1968) were determined using testes samples.

Assessment of Biochemical Parameters

Assessment of hepato- and nephro-protective activity was performed by determining the activities of some biochemical parameters e.g. Alkaline phosphatases (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activity, while the chemical analytes were assessed by determining the total bilirubin albumin, creatinine, cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL), total protein, triglyceride and urea concentrations in serum. These assays were carried out using Randox® reagent kits and the procedures were followed as those described in the literature available with the kits.

Epididymal Sperm Analysis

Epididymis was separated carefully from each testis and divided into 3 segments; head, body and tail. The epididymal tail was trimmed with scissors and placed in Petri dishes containing 1.0 ml of 0.1 M phosphate buffer of pH 7.4. The dishes gently swirled for homogeneity and allowed sperm diffusion in the solution for 10 min under 37^{o}C for dispersion of sperm cells. Sperm samples were assessed for gross morphology of sperm cells.

Micronulear Assay

The animals were sacrificed, lower abdomen and limbs were incised and the femora were cleaned and separated from the hip joint. The ends of the femur were trimmed

and a blunt needle was pushed to pierce the marrow cavity. Bone marrow was flushed into a tube containing 0.9% saline. Smears were made on sterile coded slides using a drop of the suspension. The slides were air dried, fixed in absolute methanol and stained using May-Grunwald Giemsa method (D'Souza *et al*., 2002). Micronuclei were identified as dark blue staining bodies in the cytoplasm of polychromatic and normochromatic erythrocytes. One thousand polychromatic and normochromatic erythrocytes each were scored per animal for the presence of Micronucleus using light microscope. The frequency of micro-nucleated cells was expressed in percentage (Marzouk *et al*., 2012)

Histopathology. (Mallory, 1961)
A small chunk of liver and kidney were taken from the sacrificed experimental rats used for hepatotoxicity studies and were preserved in 10% formal saline for histological studies. The tissues were processed and sectioned in paraffin. The paraffin sections of buffered formalin- fixed tissue samples (3 µm thick) were dewaxed and rinsed in alcohol and also water. It was stained with Harris' haematoxylin (Sigma) for 10 minutes, washed in running tap water for 1 minute, differentiated in acid alcohol for 10 seconds and washed again in running tap water for 5 minutes. The tissues were stained with eosin for 4 minutes and washed in running tap water for 10 seconds. It was dehydrated and mounted for photomicroscopic observations of the histological architecture of the different groups. The general structure of the livers and kidneys of the normal control group (group 1) was compared with those of the treated groups (groups 2-5).

Statistical Analysis
The results were expressed as mean + SEM for six rats. Statistical analysis of the data was performed using ANOVA statistical SPSS package (15.0) version. The significance of differences among all groups was determined by the Tukey HSD test. P – values less than 0.05 ($p \leq 0.05$) were considered to be statistically significant.

RESULTS

Hematological Results
Table 1 shows the effect of *Jatropha tanjorensis* on the haematologic indices in rat. There was significant ($p \leq 0.005$) increase in HGB, RBC and HCT level of the extract at 800 $mgkg^{-1}$ compared to addyzoa, 200 $mgkg^{-1}$ and 400 $mgkg^{-1}$ groups. At 400 and 800 $mgkg^{-1}$, the RBC level was statistically ($p \leq 0.005$) high compared to control group. The extract at 800 $mgkg^{-1}$ increased the concentrations of HGB, RBC and HCT compared to all the other groups. The MCV value of the control group was significantly ($p \leq 0.005$) increased compared to the extract at 200 and 800 $mgkg^{-1}$.

Table 1: Effect of *Jatropha* on the hematologic indices in rats.

GROUPS	HGB (g/dl)	RBC (10^6/µl)	HCT (%)	MCHC (%)	MCV (fl)	MCH (pg)
CONTROL	$16.6\pm0.2^{(b,c)}$	$7.4\pm0.13^{(b,e)}$	$41.2\pm1^{(b,c,d)}$	40.4 ± 0.7	$55.3\pm2.1^{(c,e)}$	$22.5\pm0.42^{(c)}$
ADDYZOA	$13.3\pm0.3^{(a,e)}$	$6.5\pm0.15^{(a,d,e)}$	$33.8\pm1^{(a,e)}$	39.3 ± 0.6	52.0 ± 1	20.4 ± 0.1
Extract 200	$12.3\pm1.2^{(a,e)}$	$7.1\pm0.03^{(e)}$	$31.6\pm2^{(a,e)}$	38.1 ± 2.8	$48\pm1.7^{(a)}$	$19.6\pm1^{(a)}$
Extract 400	$14.3\pm0.2^{(e)}$	$7.2\pm0.08^{(b,e)}$	$35.2\pm0.7^{(a,e)}$	40.73 ± 1	49.3 ± 1	20 ± 0.4
Extract 800	$19.1\pm0.7^{(b,c,d)}$	$9.4\pm0.24^{(a,b,c,d)}$	$45.4\pm0.5^{(b,c,d)}$	42.1 ± 1.8	$48.6\pm1.1^{(a)}$	20.4 ± 0.94

Values are expressed as Mean ± SEM for six rats. The Mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Addyzoa group. (c) = $p \leq 0.05$ as compared with the 200 mg kg^{-1} group. (d) = $p \leq 0.05$ as compared with the 400 mg kg^{-1} group. (e) = $p \leq 0.05$ as compared with the 800 mg kg^{-1} group. The significance of differences among all groups was determined by the Tukey HSD test.

KEY:	**MCH**: Mean Corpuscular Haemoglobin	**RBC**: Red Cell Count	**HCT**: Hematocrit
	MCHC: Mean Corpuscular Haemoglobin Concentration	**MCV**: Mean Corpuscular Volume	**HGB**: Haemoglobin

In Table 2, the extract at 200 $mgkg^{-1}$ significantly ($p \leq 0.005$) attenuated the level of RDW-CV, RDW-SD, MPV, PDW and PCT compared to the control group. There was a significant ($p \leq 0.005$) increase in the number of granulocytes in the 400 $mgkg^{-1}$ group compared to Addyzoa and the control groups. It also significantly ($p \leq 0.005$) increased MPV and PCT compared to Addyzoa. At 800 $mgkg^{-1}$, there was a significant ($p \leq 0.005$) increase in the percentage of Granulocytes compared to control, Addyzoa and the 200 $mgkg^{-1}$ groups.

Table 2: Effect of *Jatropha* on the hematologic indices in rats.

GROUPS	GRAN (%)	RDW-CV (%)	RDW-SD (fl)	MPV (fl)	PLT (%)	PDW (%)	PCT (%)
CONTROL	$11.4\pm0.5^{(d,e)}$	$16.4\pm0.1^{(b,c,d,e)}$	$30.7\pm0.3^{(b,c,d,e)}$	$6.6\pm0.03^{(b,c,e)}$	961 ± 16	$16.14\pm0.06^{(c)}$	$0.6\pm0.008^{(b,c,d,e)}$
ADDYZOA	$9.7\pm2^{(d,e)}$	$15.740^{(a,c,d,e)}$	$27.8\pm0.3^{(a,e)}$	$5.63\pm0.05^{(a,d)}$	1181 ± 11	$15\pm0.07^{(c)}$	$0.12\pm0.02^{(a,c,d,e)}$
Extract 200	$12.94\pm0.1^{(e)}$	$15.06\pm0.08^{(a,b)}$	$26.3\pm0.4^{(a,e)}$	$5.8\pm0.35^{(a)}$	1095.4 ± 28	$6.9\pm3.3^{(a,b,de)}$	$0.06\pm0.001^{(a,b,d,e)}$
Extract 400	$18.6\pm2.2^{(a,b)}$	$14.8\pm0.1^{(a,b)}$	$26.4\pm0.14^{(a,e)}$	$6.4\pm0.1^{(b)}$	1189.8 ± 266.7	$15.4\pm0.1^{(c)}$	$0.24\pm0.015^{(a,b,c,e)}$
Extract 800	$20\pm0.7^{(a,b,c)}$	$14.7\pm0.2^{(a,b)}$	$24.4\pm0.7^{(a,b,c,d)}$	$5.8\pm0.04^{(a)}$	1316.6 ± 46	$14.4\pm0.2^{(c)}$	$0.5\pm0.02^{(a,b,c,d)}$

Values are expressed as Mean ± SEM for six rats. The Mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Addyzoa group. (c) = $p \leq 0.05$ as compared with the 200 mg kg^{-1} group. (d) = $p \leq 0.05$ as compared with the 400 mg kg^{-1} group. (e) = $p \leq 0.05$ as compared with the 800 mg kg^{-1} group. The significance of differences among all groups was determined by the Tukey HSD test.

KEY:	**RDW-CV:** Red Cell Distribution Width (Coefficient of Variation)	**PLT:** Platelet Count
GRAN: Granulocytes	**RDW-SD:** Red Cell Distribution Width (Standard Deviation)	**MPV:** Mean Platelet Volume
PCT: Plateletcrit	**PDW**: Platelet Distribution Width	

Biochemical Results

Table 3 shows the biochemical result after pre-treatment of rats with different doses of *Jatropha tanjorensis* extract and Addyzoa. There was significant ($p \leq 0.005$) dose dependent attenuation in the concentrations of ALT and AST on administration of the extract compared to Addyzoa and the control group. At 800 $mgkg^{-1}$, the level of ALP was attenuated significantly ($p \leq 0.005$) compared to Addyzoa, 200 and 400 $mgkg^{-1}$. Compared to all other groups, the extract at 200 and 400 $mgkg^{-1}$ showed the lowest levels of total bilirubin and AST and ALT respectively.

Table 3: Serum levels of ALP, ALT, AST and TBIL (Liver Function Enzymes) of rats treated with *Jatropha.*

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)	TOTAL BIL
CONTROL	372 ±8.4$^{(b,c,d)}$	61 ±6$^{(d)}$	667.2 ±3.1$^{(b,c,d,e)}$	2.1 ±0.5
ADDYZOA	506.8 ±5$^{(a,c,d,e)}$	52.5 ±1$^{(d)}$	252.5 ±3$^{(a,e)}$	1.4 ±0.1
Extract 200	649.08 ±17.7$^{(a,b,e)}$	63.78 ± 0.7$^{(d)}$	299.82 ± 7.8$^{(a,e)}$	1.12 ± 0.3
Extract 400	609. 4±32.4$^{(a,b,e)}$	38.26 ±2.5$^{(a,b,c,e)}$	220.5 ±51$^{(a,e)}$	1.22 ±0.15
Extract 800	400.4 ±5.2$^{(b,c,d)}$	62.44 ±3.3$^{(d)}$	434.4 ±47.6$^{(a,b,c,d)}$	1.4 ±0.13

Values are expressed as Mean ± SEM for five rats. The Mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Addyzoa group. (c) = $p \leq 0.05$ as compared with the 200 mg kg^{-1} group. (d) = $p \leq 0.05$ as compared with the 400 mg kg^{-1} group. (e) = $p \leq 0.05$ as compared with the 800 mg kg^{-1} group. The significance of differences among all groups was determined by the Tukey HSD test.

KEY:	**AST:** Aspartate amino transferase	**ALP**: Alkaline phosphatase
	ALT: Alanine amino transferase	**TOTAL BIL:** Total bilirubin

In table 4, there was significant ($p \leq 0.05$) increase in albumin (ALB) in the groups administered 200 and 400 $mgkg^{-1}$ of the extract compared to the control group. There was significant ($p \leq 0.005$) decrease in the concentrations of cholesterol, and triglyceride in the group administered 400 $mgkg^{-1}$ of the extract compared to the control group. LDL was significantly ($p \leq 0.005$) attenuated in the Addyzoa, 200 and 800 $mgkg^{-1}$ groups compared to the 400 $mgkg^{-1}$ group. The extract at 400 $mgkg^{-1}$, presented the lowest and highest concentrations of LDL and total protein respectively while at 800 mg kg^{-1}, the group had the highest concentration of HDL and the lowest level of cholesterol and triglyceride. The extract significantly ($p \leq 0.005$) attenuated the concentration of triglyceride and cholesterol compared to the control group.

Table 4: Serum levels of biochemical analytes in rats pre-treated with *Jatropha.*

Groups	ALB (g/L)	CHO (mmol/L)	HDL CHO (mmol/L)	LDL CHO (mmol/L)	CREA (mmol/L)	TOTAL PROTEIN (g/L)	TRIG (mmol/L)	UREA (mmol/L)
CONTROL	26 ±1.3$^{(b,c,d)}$	3 ±0.1$^{(b,c,d,e)}$	0.34±0.04$^{(e)}$	0.09±0.01$^{(b,c,e)}$	36 ±2.6$^{(b,e)}$	61 ±4	3.7±0.4$^{(b,c,d,e)}$	5.4±0.13$^{(b,d)}$
ADDYZOA	33.7 ±1$^{(a,e)}$	2.3 ±0.1$^{(a)}$	0.4 ±0.02	0.24 ±0.02$^{(a,d)}$	25 ±0.8$^{(a,c,d)}$	66 ±0.6	2±0.1$^{(a)}$	4.3±0.1$^{(a,c,d,e)}$
Extract 200	32.2 ±0.4$^{(a)}$	2.2 ±0.05$^{(a)}$	0.39 ±0.02	0.17 ±0.01$^{(a,d)}$	35.02±0.6$^{(b,e)}$	62.8 ±2	2.06±0.08$^{(a)}$	5.3 ±0.2$^{(b,d)}$
Extract 400	33.5±0.64$^{(a)}$	2.14±0.06$^{(a)}$	0.44 ±0.02	0.07±0.025$^{(b,c,e)}$	37.34±1.5$^{(b,e)}$	67.32±1.4	2.5±0.3$^{(a,e)}$	6.2±0.15$^{(a,b,c)}$
Extract 800	29.3±1.5$^{(b)}$	2.13±0.13$^{(a)}$	0.5±0.01$^{(a)}$	0.2 ±0.01$^{(a,d)}$	25.9±1.9$^{(a,c,d)}$	60.6±1.6	1.2±0.1$^{(a,d)}$	5.7±0.2$^{(b)}$

Values are expressed as Mean ± SEM for five rats. The Mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Addyzoa group. (c) = $p \leq 0.05$ as compared with the 200 mg kg^{-1} group. (d) = $p \leq 0.05$ as compared with the 400 mg kg^{-1} group. (e) = $p \leq 0.05$ as compared with the 800 mg kg^{-1} group. The significance of differences among all groups was determined by the Tukey HSD test.

KEY:

CHO: Cholesterol **LDL CHO:** Low density protein **HDL CHO:** High density protein **ALB:** Albumin **TRIG:** Triglyceride **CREA**: Creatinine

Table 5 shows the sperm count of the rats exposed to the ethanolic extract of *Jatropha tajorensis* for the duration of sixty-four (64) days. There was a significant ($p \leq 0.005$) increase in the sperm count of the control and addyzoa groups compared to the groups administered the extract. The extract at 200 $mgkg^{-1}$, showed a higher number of sperm cells compared to all other extract groups while the group administered the extract at 800 $mgkg^{-1}$, showed a lower count compared to all the groups.

Table 5: Sperm count of the rats exposed to the ethanolic extract of *Jatropha tajorensis* and Addyzoa.

GROUPS	Sperm count (10^6)
CONTROL	$7.52 \pm 0.69^{(c,d,e)}$
ADDYZOA	$6.07 \pm 0.52^{(c,d,e)}$
Extract 200	$2.62 \pm 0.34^{(a,b)}$
Extract 400	$2.46 \pm 0.39^{(a,b)}$
Extract 800	$2.26 \pm 0.2^{(a,b)}$

Values are expressed as Mean ± SEM for five rats. The Mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Addyzoa group. (c) = $p \leq 0.05$ as compared with the 200 mg kg^{-1} group. (d) = $p \leq 0.05$ as compared with the 400 mg kg^{-1} group. (e) = $p \leq 0.05$ as compared with the 800 mg kg^{-1} group. The significance of differences among all groups was determined by the Tukey HSD test.

Sperm Head Abnormality

Analysis of the head abnormality was made after 64 days of exposure to the ethanolic extract of *Jatropha tajorensis*. The result of the sperm head abnormality showed that there was high level of abnormality in the sperm head of the rats exposed to the extract compared to the control group. The major abnormality were pin head, hook head and banana shaped head. The extract (800 mg kg^{-1}) showed higher forms of sperm head abnormality.

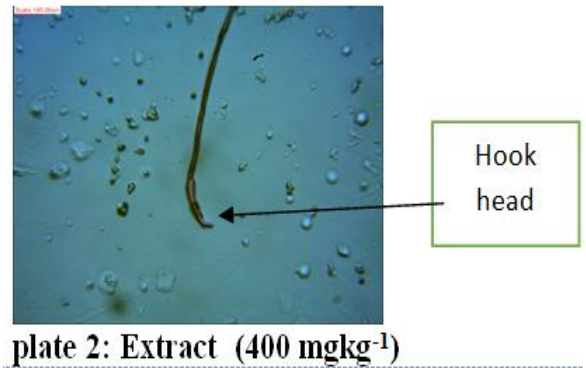

plate 2: Extract (400 $mgkg^{-1}$)

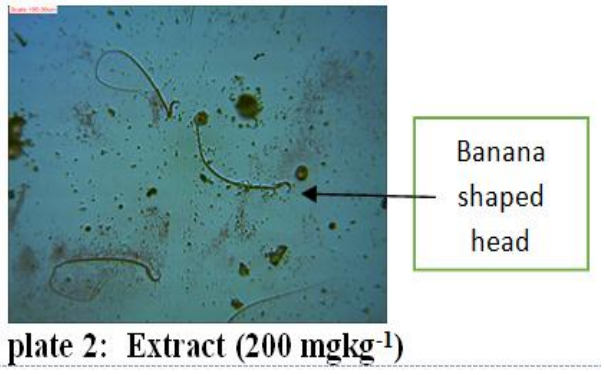

plate 2: Extract (200 $mgkg^{-1}$)

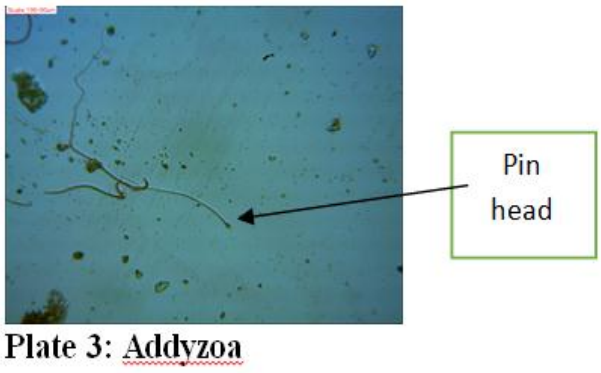

Plate 3: Addyzoa

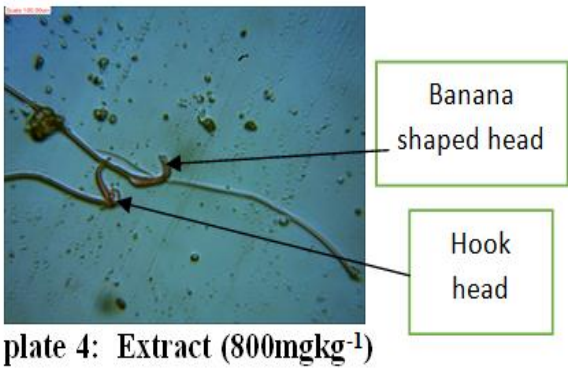

plate 4: Extract (800$mgkg^{-1}$)

Micronucleus assay

A number of fragmented nucleus and binucleated were observed under the microscope at X40 magnification as shown in table 6. The group administered 800 $mgkg^{-1}$ of the extract, had the highest number of both binucleated cells and micronucleus cells. Binucleated cells are cells that contain two nuclei and this type of cells are found mostly in cancer cells. Fragmented nucleus, which can also be related to Karyorrhexis, is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis.

Table 6: The Total Number of Binucleated and Micronucleus cells.

Group	Binucletated Cells	Micronucleus
Control	104	72
Addyzoa	136	140
Extract (200 mgkg^{-1})	64	64
Extract (400 mgkg^{-1})	164	168
Extract (800 mgkg^{-1})	304	260

The histological section of liver of the control, Addyzoa and 800 mgkg^{-1} groups showed hepatic lobules consisting of branching hepatocyte sheets, the liver architecture were mildly distorted revealing intra cytoplasmic vacuolation which is an indication of mild liver toxicity. The central veins of these groups were congested with red blood cells; the portal veins were dilated and filled with red blood cells. Few inflammatory cells infiltrated the areas of haemorrhage and reacting endothelial cells were seen. The 200 and 400 mgkg^{-1} groups showed moderate to severe intra cytoplasmic vacuolation respectively, which resulted to severe liver toxicity.

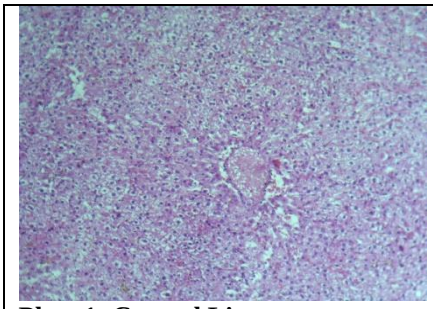

Plate 1: Control Liver
Showing hepatic lobules consisting of branching hepatocyte sheets with slightly distorted liver architecture

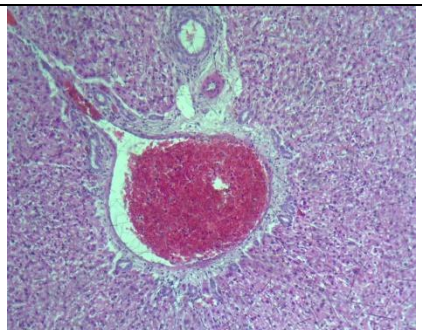

Plate 2: Addyzoa Liver
Showing portal veins dilated and filled with red blood cells

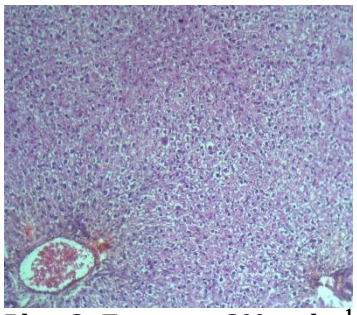

Plate 3: Extract at 200 mgkg^{-1}
Showing moderate intra cytoplasmic vacoulation

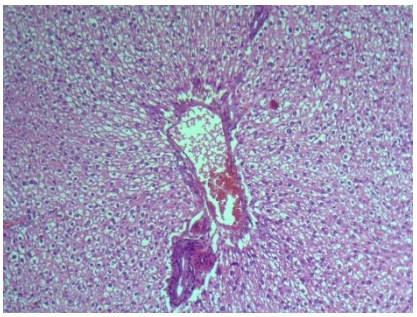

Plate 4: Extract at 400 mg/kg
Showing severe intra cytoplasmic vacoulation

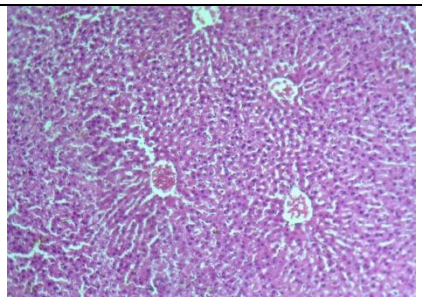

Plate 5: Extract at 800 mg/kg
Showing central veins congested with red blood cells and mild intra cytoplasmic vacoulation.

The histological section of kidneys of the control, Addyzoa, 200, and 800 mgkg^{-1} groups showed partially normal histologic features to mild tubular toxicity of the kidney architecture. Mild haemorrhage, mild tubular and renal corpuscle distortion as well as inflammatory cells infiltrates were seen more in the 800 mgkg^{-1} group of the extract. The 400 mgkg^{-1} group however showed marked distortion of kidney architecture which revealed increased tubular destruction.

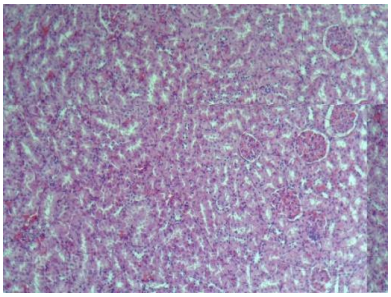

Plate 6: Control Kidney Showing partially normal histologic features and mild tubular toxicity

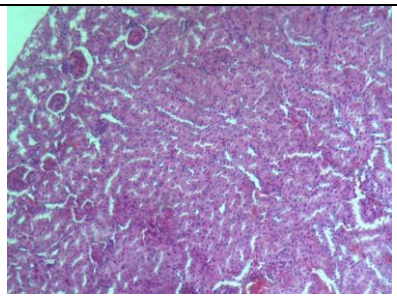

Plate 7: Addyzoa Kidney: Showing partially normal histologic features and mild tubular toxicity

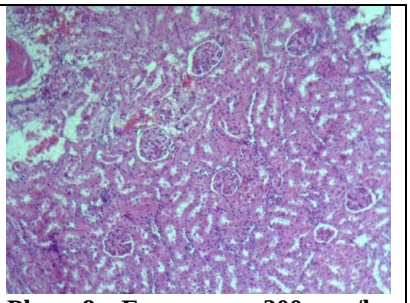

Plate 8: Extract at 200 mg/kg Showing mild tubular toxicity, mild haemorrhage, mild tubular and renal corpuscle distortion

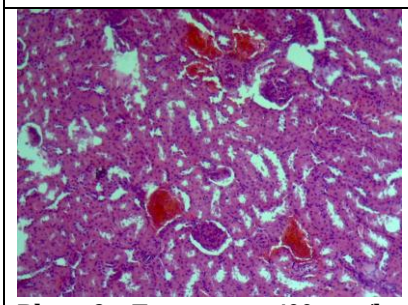

Plate 9: Extract at 400 mg/kg Showing mild haemorrhage, mild tubular and renal corpuscle distortion as well as inflammatory cells infiltrates

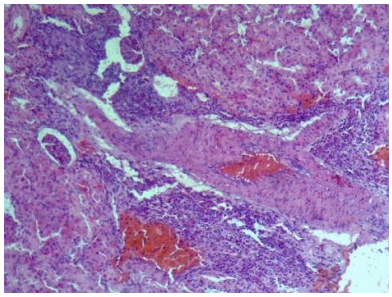

Plate 10: Extract at 800 mg/kg Showing marked distortion of kidney architecture which revealed increased tubular destruction

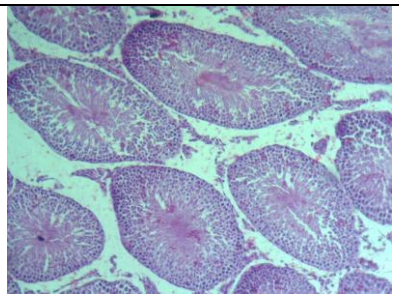

Plate 11: Control

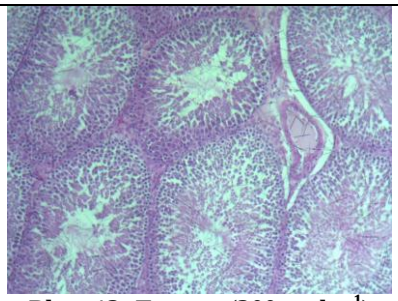

Plate 12: Extract (200 mgkg^{-1})

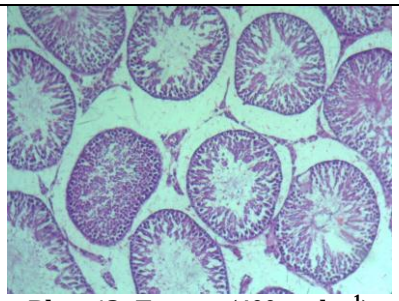

Plate 13: Extract (400 mgkg^{-1})

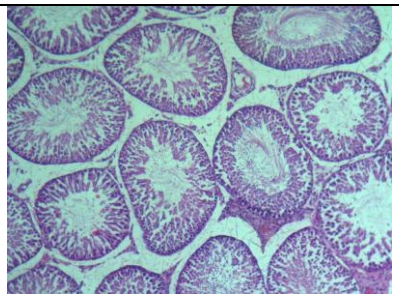

Plate 14: Addyzoa group

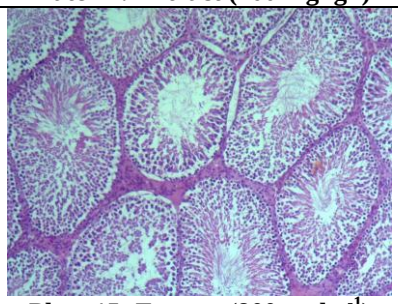

Plate 15: Extract (800 mgkg^{-1})

Plates 11 – 15 Shows the histological section of the testis of the experimental groups. Histological sections of testicular specimens of all the groups showed normal histologic images.

The antioxidant effect on the testes of the rats exposed to the ethanolic extract of the *Jatropha tajorensis* for sixty-four (64) days are shown in table 7. There was significant ($p \leq 0.005$) increase in the level of Malondialdehyde (MDA) in the control group compared to all groups while the extract at 800 mgkg^{-1} significantly ($p \leq 0.005$) attenuated it compared to all groups. The level of total protein was significantly ($p \leq 0.005$) reduced in the groups that received 200 and 400 mgkg^{-1} of the extract compared to the Addyzoa and control groups. Addyzoa showed a significant ($p \leq 0.005$) increase in catalase concentration compared to all the other groups while the extract at 200 mgkg^{-1} demontrated a significant ($p \leq 0.005$) decrease in catalase level compared to

Addzoa, 400 and 800 mgkg^{-1} of the extract. The level of GST was significantly ($p \leq 0.005$) increased in the extract group administered 200 mgkg^{-1} compared to 400 and 800 mgkg^{-1}. The control group showed a significant ($p \leq 0.005$) decreases in GSH compared to Addyzoa, 200 and 800 mgkg^{-1}. The SOD concentration at 400 mgkg^{-1} was significantly ($p \leq 0.005$) high compared to all the groups.

Table 7: The antioxidant effect on the serum of the rats exposed to the ethanolic extract of the *Jatropha tajorensis* for a duration of sixty four (64) days.

GROUPS	MDA (nmol/ml)	Total protein (g/L)	CAT (µmol/min/mg protein)	GST (µmol/ml)	GSH (µmol/ml)	SOD (µmol/ml)
CONTROL	16.96± 0.95$^{(b,c,d,e)}$	9.36± 0.97$^{(c,d)}$	3.26 ±0.05$^{(b,d,e)}$	4.42±0.09$^{(b,c,d,e)}$	2.24 ±0.1$^{(b,c,e)}$	2.74±0.05$^{(b,c,d,e)}$
ADDYZOA	6.26 ±0. 1$^{(a,c,e)}$	9.24 ±0.17$^{(c,d)}$	11.46± 0.15$^{(a,c,d,e)}$	2.82 ±0.06$^{(a,d,e)}$	12 ±0.84$^{(a,c,d,e)}$	6.36 ±0.1$^{(a,c,d,e)}$
EXTRACT 200	10.12± 0.06$^{(a,b,d,e)}$	6.56 ± 0.25$^{(a,b)}$	3.32 ±0.18$^{(b,d,e)}$	3.04 ±0.08$^{(a,d,e)}$	6.7± 0.2$^{(a,b)}$	7.16±0.11$^{(a,b,d,e)}$
EXTRACT 400	7.4 ±0.51$^{(a,c,e)}$	6.98 ±0.12$^{(a,b)}$	4.66 ±0.11$^{(a,b,c,e)}$	1.78 ±0.1$^{(a,b,c)}$	4.6±0.2$^{(b)}$	10.2 ±0.07$^{(a,b,c,e)}$
EXTRACT 800	2.8± 0.23$^{(a,b,c,d)}$	8.46 ±0.46	7.28 ±0.12$^{(a,b,c,d)}$	2.04 ±0.12$^{(a,b,c)}$	8.12 ±1.76$^{(a,b)}$	3.7 ±0.14$^{(a,b,c,d)}$

Values are expressed as Mean ± SEM for six rats. The Mean difference is significant at the 0.05 level. (a) = $p \leq 0.05$ as compared with the normal control group. (b) = $p \leq 0.05$ as compared to Addyzoa group. (c) = $p \leq 0.05$ as compared with the 200 mg kg^{-1} group. (d) = $p \leq 0.05$ as compared with the 400 mg kg^{-1} group. (e) = $p \leq 0.05$ as compared with the 800 mg kg^{-1} group. The significance of differences among all groups was determined by the Tukey HSD test.

DISCUSSION

Red blood cells (RBC) carry most of the body's iron that is vital for haemoglobin synthesis. There was a dose dependent increase in hemoglobin level on administration of the extract. Decreased haemoglobin level is indicative of anaemia. The significant ($p \leq 0.05$) dose-dependent increase in the red blood cell count shows that the extract has hematopoietic properties and might have increased the rate of production of corpuscles. Erythropoietin affects the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and hemoglobin are very important in transferring respiratory gases (Oyedeji and Bolarinwa, 2013). The Addyzoa group however showed significant decrease in RBC levels. The platelet count was increased suggesting that the extract had a good effect on the oxygen-carrying capacity of the blood and also on glycoprotein which is the hormone responsible for the production of platelets by the bone marrow (McLellan *et al.*, 2003).

MCV and RDW are indices used to measure average size of RBC's in the blood and the variation in size of RBC's respectively, while MPV is used to measure the average size of the platelets found in the blood. Low MPV levels can be indicative of blood disorders or diseases. HCT is the ratio of the volume of RBC's to the volume of the whole blood. The HCT levels were significantly ($p \leq 0.05$) high in the extract groups at 800 mgkg^{-1}. The grup administered the extract dose dependently caused an increase in HGB, HCT and RBC levels compared to control and Addyzoa groups. Normally, local tissue anoxia apparently leads to the formation of erythropoietin, thereby stimulating increased production of erythrocytes (Bowman and Rand, 1980). Erythropoietin is a glycoprotein hormone that stimulates stem cells in the bone marrow producing red blood cells (Ohlsson and Aher, 2009). It is most likely the extract contains erythropoietin-like agent(s), compounds and or phytochemicals that stimulate the formation or secretion of erythropoietin in the stem cells of the animals that are responsible for increased production of erythrocytes. Previous studies by Okpuzor *et al*., (2009) and Kuppast *et al*., (2009) indicated that an increase in the count of erythrocytes and PCV is suggestive of polycythermia and positive erythropoiesis. This is an indication of an improved bone marrow function. This also implies that the extract probably possesses anti-anemic potential and can cause polychethermia.

The ALT, AST and ALP and serum bilirubin are common biochemical parameter for detecting liver injury (Girish *et al*., 2009). They are cytoplasmic in location and released into circulation after cellular damages indicative of development of hepatotoxicity. In this study, treatment with ethanolic extract of *Jatropha tajorensis* significantly ($p \leq 0.05$) attenuated the increase in ALP, AST and ALT levels in the test groups compared to the control. This is an indication that the extract improved plasma membrane stabilization and protected against liver injury. The increase in albumin activity shows high activity in the proliferation of cells and high productivity of protein in the body. Cholesterol is a critical fat that is a structural component of cell membrane and plasma lipoproteins, and is important in the synthesis of steroid hormones, glucocorticoids, and bile acids. Decrease in the level of cholesterol is an indicative of malnutrition, liver insufficiency,

malignancies, anaemia and infection. There was also increase in the level of HDL. The triglyceride level was also reduced while the level of creatinine was increased except from the Addyzoa group. The decreased triglyceride level may be present in chronic obstructive pulmonary disease, brain infarction, hyperthyroidism and malnutrition. This indicates that the kidneys have sufficient capacity to retain water content.

Based on the sperm count, the control and Addyzoa groups, showed a significant increase, compared to the groups administered the extract, which signifies that the extract was not effective enough in boosting sperm count. The group, administered the highest dose of 800 $mgkg^{-1}$ showed a high reduction in the number of sperm count compared to all groups. This might be an indication that the highest dose of the extract decreased the number of spermatogonium, spermatocytes, spermatozoids and leydig cells. Sperm count is often used as a measure of sperm production, testicular function and male fertility. Low sperm count has been associated with reduced fertility (Raji *et al*., 2003). For the sperm abnormality, both the Addyzoa and the extract groups showed abnormality. The highest extract of 800 $mgkg^{-1}$ showed more abnormality compared to all groups. More of banana shaped head and hook head was found in the group administered 800 $mgkg^{-1}$ of the extract compared to other groups. Both sperm abnormality and low sperm count is the major reason for infertility in male reproduction process (Raji *et al*., 2003).

In high quantities, endogenous reactive oxygen species (ROS) overwhelm the innate antioxidant defence system causing oxidative stress. Reduction in the level of free radicals or antioxidation activity is important in the protection against chemical substances that cause testicular damage. The improved fertility observed in male rats might be attributed to the antioxidant effect of the extract. The level of Malondialdehyde (MDA) was significantly high in the control group compared to all groups. MDA a toxic and reactive metabolite produced when increased reactive oxygen (ROS) reacts with polyunsaturated fatty acids. It is a marker of lipid peroxidation and oxidative stress. Superoxide dismutase catalyses the dismutation of superoxide radicals to hydrogen peroxide. The level of superoxide dismutase was high in the group that received 400 $mgkg^{-1}$ of the extract compared to all groups. Elevated level in the amount of superoxide dismutase is also a feature of cancer properties. Addyzoa showed significant increase ($p \leq 0.005$) in the level of catalase compared to all groups. Catalase is an enzyme produced when a living organism is exposed to oxygen. It catalyzes the decomposition of water and oxygen. It is a very important enzyme in protecting the cells from oxidative damage by reactive oxygen species (ROS). One catalase molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second (Boom *et al*., 1978). This is an indicative that Addyzoa is more effective in boosting the catalase level compared to all groups. The control group also showed increase in the level of glutathione compared to all groups. A high attenuation was noticed in the extract 400 $mgkg^{-1}$. Glutathione is a naturally occurring tripeptide in the body. It is important to intermediary metabolism, immune response and overall health. It repairs cellular damage from harmful free radical. Low glutathione level indicates cellular damage while increased levels are effective in combating free radical induced cellular damage.

Micronucleaus assay, is a test used in identifying the toxicological potential of a genotoxic compounds. In this study, more of binucleated and fragmented nucleus was found. The extract at 800 $mgkg^{-1}$ had more of this aberration compared to all groups. This showed that high dose of this plant extract results in more of the aberrated nucleus. Based on all these parameters, the ethanolic extract of *J. tajorensis* showed increase in sperm count, with sperm head abnormality basically at high dosage concentration. The toxicity rate of the extract is not high, only except at high concentration. The extract also showed improvement in the protection of cells from oxidative damage, especially in reducing the level of MDA which is a toxic radical.

CONCLUSION

The results of this study show that *Jatropha tanjorensis* probably possess hepatoprotective and hematopoietic properties. The ethanolic extract of *J. tajorensis* also showed diminution in sperm count, with sperm head abnormality basically at high dosage concentration. Also, it doesn't support its use in enhancing male fertility at high doses for a long duration. The use of this plant in South Eastern Nigeria as a blood nourishing tonic and a hepatoprotective drug has been corroborated by this study using Wistar albino rats. More studies should be carried out so as to ascertain the appropriate dose that is safe and will produce the desired results.

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