

## Hepatoprotective Effect of the Ethanolic Extract of *Jatropha tanjorensis* on Acetaminophen-Induced toxicity in Rat Model

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### Abstract

The hepatoprotective activity of ethanolic leaf extract of *Jatropha tanjorensis* (ELEJT) against acetaminophen induced hepatotoxicity using Swiss albino rats was ascertained. The animals were grouped into six with five animals each. Hepatoprotection against acetaminophen-induced toxicity was evaluated using standard methods. Effect on genotoxicity using micronucleus test and spermatogenesis was ascertained. ELEJT significantly ( $p < 0.05$ ) decreased the levels of serum liver enzymes. At  $800 \text{ mgkg}^{-1}$ , ELEJT significantly ( $p < 0.05$ ) reduced triacylglycerol and LDL levels but significantly ( $p < 0.05$ ) increased the level of HDL compared to the toxin control group. Administration of ELEJT resulted in a dose-dependent increase in the total protein level. Sperm head abnormality was observed more at  $800 \text{ mgkg}^{-1}$  while at  $400 \text{ mgkg}^{-1}$  binucleated cell and micronuclei cells were detected. ELEJT exhibited hepatoprotective activity against Acetaminophen-induced liver damage using Swiss albino rats. This validates the traditional use of *Jatropha tanjorensis* in management of liver ailments and improvement of spermatogenesis. However, more work is needed to ascertain its mode of action.

**Key words:** Spermatogenesis, Hepatoprotective, Genotoxicity, Acetaminophen

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### INTRODUCTION

*Jatropha tanjorensis* commonly called “hospital-too-far” or “iyana ipaja” belongs to the family Euphorbiaceae and is widely grown in Southern Nigeria. Locally, the plant has been used as a source of edible vegetable and as a medicinal plant. The leaf extract has hypoglycemic and antioxidant properties that make it a popular remedy for the treatment of diabetes, malaria and hypertension (Orhue *et al.*, 2008). Phytochemical screening of *J. tanjorensis* leaf revealed that it contains bioactive constituents

such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (Ehimwenma and Osagie, 2007). Administration of *Jatropha tanjorensis* leaf powder to rabbits resulted in improvement in haematological indices which revealed an enhancement of bone marrow function (Orhue *et al.*, 2008). The liver is an important organ that plays a key role in the maintenance of homeostasis. The liver is responsible for multiple metabolic functions and physiological processes such as bile production, energy generation, vitamin storage, and the

metabolism of carbohydrates, proteins, and lipids. Hepatic diseases are the primary causes of morbidity and mortality worldwide (Lam and Younossi, 2009). Liver disease is exacerbated by unhealthy lifestyles, obesity, and excessive consumption of alcohol and drugs (Lam and Younossi, 2009). Some plant species such as *Silybum marianum*, *Phyllanthus niruri*, and *Panus giganteus* have been shown to ameliorate hepatic lesions (Morales-González *et al.*, 2013). This study is aimed at determining the hepatoprotective potentials of *Jatropha tanjorensis* and its effect on spermatogenesis so as to repudiate the folkloric claim that it can attenuate the effect of hepatotoxic substances and also improve spermatogenesis.

## 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 by Mr. O.O Oyebanji 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 and extracted. The crude extract was weighed, and a stock solution was prepared using water.

### Experimental Animals

Thirty adult male Swiss albino mice weighing an average of  $25.5 \pm 1g$  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 procedures described were according to University Animals Ethical Committee.

### Experimental Protocol

After an adaptive period of 1 week, the animals were divided into six groups of 5 animals each. Animals in groups 1 and 2 served as the normal and toxin control groups respectively and were

administered distilled water ( $10 \text{ ml kg}^{-1}$  body weight p.o.) for 14 days. Groups 3 -5 received 200, 400 and  $800 \text{ mg kg}^{-1}$  (p.o.) of extract respectively while animals in group 6 were given  $300 \text{ mg kg}^{-1}$  (p.o.) of Silymarin for 14 days. On the 12<sup>th</sup> day, animals in groups 2–6 were administered  $3 \text{ g kg}^{-1}$  (p.o.) of APAP in distilled water. On the 14<sup>th</sup> day, the animals were mildly anaesthetized with ether and blood was collected from the retro-orbital plexus. They were sacrificed and more blood samples were collected by cardiac puncture. The blood samples were used for biochemical assays.

### Assessment of biochemical parameters

Blood was collected and the serum was separated by centrifuging at 3000 rpm for 10 min. The collected serum was used to assay for biochemical parameters, such as ALP, AST, ALT, albumin, total protein, total cholesterol, total bilirubin, triacylglycerol and urea concentration using Randox kit and the manufacturer's instructions were strictly followed.

### Epididymal sperm analysis

Epididymal sperm analysis was determined using the method of Hamilton (1975). Epididymis was separated carefully from 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 were gently swirled for homogeneity and allowed sperm diffusion in the solution for 10 min at 37°C for dispersion of sperm cells. Sperm samples were for assessed gross morphology of sperms.

### Micronuclear 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 as described by D'Souza, *et al.* (2005). Micronuclei were identified as dark blue stained 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.

### Statistical analysis

The results were represented as Mean  $\pm$  Standard Error of Mean of five animals per group. The statistical analysis was carried out by one-way analysis of variance (ANOVA), using SPSS package (version 15.0). Significance of difference among the groups was determined by the Turkey HSD test, with values less than 0.05 ( $p < 0.05$ ) considered to be statistically significant.

### RESULTS AND DISCUSSION

Table 1 shows the effect of acetaminophen on the liver enzymes of rats administered ELEJT. There was significant ( $p < 0.05$ ) decrease in Albumin (ALB) value of the toxin group

compared to all the other groups. There was increase in ALP activity in the toxin group compared to other groups, but there was an attenuation at 200 mg  $\text{kg}^{-1}$  compared to the toxin and control group. Compared to all other groups, the toxin group showed a significant ( $p < 0.05$ ) increase in ALT. The toxin group demonstrated significantly ( $p < 0.05$ ) high level of AST compared to the control. However, the ALT activity of rats in the toxin group was found to be non-significantly ( $p > 0.05$ ) higher compared to the AST level of rats in the 800 mg  $\text{kg}^{-1}$  and the silymarin groups. Total bilirubin (BILT) was statistically significant ( $p < 0.05$ ) in the toxin group compared to all groups.

Table 1: The effect of acetaminophen on the liver enzymes of rats administered ELEJT

Groups	ALB	ALP	ALT	AST	BILT
Control	31.04 $\pm$ 0.8 <sup>(b)</sup>	283.18 $\pm$ 4.7 <sup>(b)</sup>	53.22 $\pm$ 1.2 <sup>(b)</sup>	103.52 $\pm$ 3 <sup>(b)</sup>	1.1 $\pm$ 0.1 <sup>(b)</sup>
Toxin	25.06 $\pm$ 2.04 <sup>(a)</sup>	433.02 $\pm$ 12.6 <sup>(a)</sup>	81.12 $\pm$ 5.53 <sup>(a)</sup>	435.38 $\pm$ 5.8 <sup>(a)</sup>	1.98 $\pm$ 0.04 <sup>(a)</sup>
Ext + toxin 200	30.56 $\pm$ 1.9 <sup>(b)</sup>	142.66 $\pm$ 7 <sup>(b)</sup>	60.62 $\pm$ 1.2 <sup>(b)</sup>	394.86 $\pm$ 18.7 <sup>(a)</sup>	1.72 $\pm$ 0.2 <sup>(a)</sup>
Ext + toxin 400	31.86 $\pm$ 0.4 <sup>(b)</sup>	336.6 $\pm$ 13.3 <sup>(b)</sup>	60.96 $\pm$ 7.76 <sup>(b)</sup>	399.9 $\pm$ 0.4 <sup>(a)</sup>	1.8 $\pm$ 0.03 <sup>(a)</sup>
Ext + toxin 800	31.7 $\pm$ 0.6 <sup>(b)</sup>	313.9 $\pm$ 4.5 <sup>(b)</sup>	53.8 $\pm$ 2.6 <sup>(b)</sup>	265.5 $\pm$ 16.6 <sup>(a,b)</sup>	1.58 $\pm$ 0.2
Silymarin	32.6 $\pm$ 0.7 <sup>(b)</sup>	322.22 $\pm$ 24.5 <sup>(b)</sup>	58.36 $\pm$ 1.3 <sup>(b)</sup>	352.6 $\pm$ 9.6 <sup>(a,b)</sup>	1.82 $\pm$ 0.2 <sup>(a)</sup>

Values are expressed as mean  $\pm$  SEM for five rats. The mean difference is significant at the .05 level. (a) =  $p < 0.05$  compared with the normal control group. (b) =  $p < 0.05$  compared with the toxin control group. The significance of differences among all groups was determined by the Tukey HSD test.

**Key:** Alkaline phosphatase = ALP Alanine aminotransferase (ALT)  
 Albumin = ALB Aspartate aminotransferase =AST Total bilirubin = TBIL

There was a significant ( $p < 0.05$ ) decrease in the level of total cholesterol and LDL in all groups when compared to the toxin group (table 2). Similarly, there was a significant ( $p < 0.05$ ) increase in the level of HDL for all groups when compared to the toxin group. The toxin group demonstrated a significant ( $p < 0.05$ ) increase in the level of creatinine and Triacylglycerol (TAG) but a significant ( $p < 0.05$ ) decrease in the level of total protein compared to the control group. For the urea, there was no significant ( $p \leq 0.005$ ) increase in all groups. There was a significant ( $p < 0.05$ ) attenuation in the level of total protein in the toxin group compared to all the groups. At a dose of 800 mg/kg, the extract caused a significant ( $p < 0.05$ ) decrease in the concentration of urea compared to the toxin control group.

Table 2: The effect of ELEJT on the biochemical parameters of acetaminophen induced rats

Group	CHOL	LDL	HDL	CREA	TP	TAG	UREA
Control	1.6 ± 0.04 <sup>(b)</sup>	0.31 ± 0.05 <sup>(b)</sup>	1.09 ± 0.1 <sup>(b)</sup>	28.04 ± 1.5	55.2 ± 0.9 <sup>(b)</sup>	0.9 ± 0.07 <sup>(b)</sup>	10.8 ± 1.02
Toxin	2.4 ± 0.22 <sup>(a)</sup>	1.52 ± 0.24 <sup>(a)</sup>	0.61 ± 0.04 <sup>(b)</sup>	33.4 ± 4.7 <sup>(a)</sup>	43.4 ± 2.9 <sup>(a)</sup>	1.3 ± 0.13 <sup>(a)</sup>	11.6 ± 0.5
Ext + toxin 200	1.6 ± 0.03 <sup>(b)</sup>	0.53 ± 0.02 <sup>(b)</sup>	1.05 ± 0.12 <sup>(b)</sup>	31.1 ± 0.4	50.9 ± 0.56 <sup>(b)</sup>	0.7 ± 0.01 <sup>(b)</sup>	9.5 ± 0.16
Ext + toxin 400	1.7 ± 0.13 <sup>(b)</sup>	0.4 ± 0.01 <sup>(b)</sup>	1.2 ± 0.14 <sup>(b)</sup>	35.4 ± 0.9	58.1 ± 0.59 <sup>(b)</sup>	0.62 ± 0.04 <sup>(b)</sup>	11.5 ± 0.26
Ext + toxin 800	1.4 ± 0.3 <sup>(b)</sup>	0.32 ± 0.02 <sup>(b)</sup>	1.03 ± 0.2 <sup>(b)</sup>	28.25 ± 1.2	51.2 ± 0.3 <sup>(b)</sup>	0.6 ± 0.02 <sup>(b)</sup>	9.1 ± 0.1 <sup>(b)</sup>
Silymarin	1.4 ± 0.03 <sup>(b)</sup>	0.34 ± 0.05 <sup>(b)</sup>	1.04 ± 0.09	28.8 ± 0.9	49.9 ± 0.4 <sup>(b)</sup>	0.6 ± 0.05 <sup>(b)</sup>	10 ± 0.36

Values are expressed as mean ± SEM for five rats. The mean difference is significant at the .05 level. (a) = p < 0.05 as compared with the normal control group. (b) = p < 0.05 as compared with the toxin control group. The significance of differences among all groups was determined by the Tukey HSD test.

Key:

CHOL: Cholesterol

LDL: Low-density lipoprotein

HDL: high-density lipoprotein

TP: Total protein

TAG: Triacylglycerol

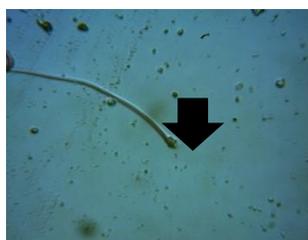


Fig 1: Normal sperm head (Control)



Fig 2: Pin head (Ext at 200 mgkg<sup>-1</sup>)



Fig 3: Hook Head (Ext at 400 mg kg<sup>-1</sup>) x40

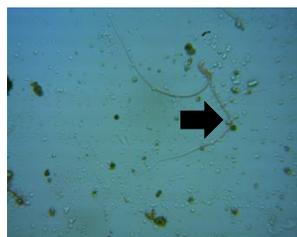


Fig 4: Two tails (800mgkg<sup>-1</sup>) x40

Table 3: The Total Number of Binucleated and Micronucleus cells

Groups	Binucleated cells	Micronucleus cells
Control	21	16
Toxin control	61	70
Extract + toxin (200 mgkg <sup>-1</sup> )	27	26
Extract + toxin (400mgkg <sup>-1</sup> )	27	31
Extract+ toxin (800 mgkg <sup>-1</sup> )	31	34

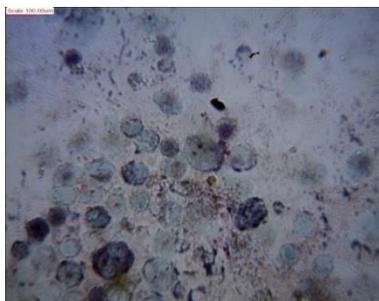


Figure 5: showing Fragmented nucleus

Liver damage can be induced using D-galactosamine or acetaminophen, carbon tetrachloride so as to ascertain the hepatoprotective properties of substances (Ferenčokova *et al.*, 2003). N-acetyl-p-benzoquinoneimine (NAPQI), the active metabolite of acetaminophen is responsible for its hepatotoxic action (Chaitanya *et al.*, 2012). The NAPQI radical covalently binds to cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation which causes damage to biomembranes (Olayiwola *et al.*, 2004). Lipid peroxidation caused by NAPQI leads to elevated levels of the cytosolic enzymes in the serum (Madubuike *et al.*, 2015).

At 200 mgkg<sup>-1</sup>, the extract reduced the activity of ALP compared to all the groups. Decrease in concentration of ALB shows there is low activity in the proliferation of cells and little synthesis of protein in the body to replace the worn-out ones. This finding agrees with the findings of Chaitanya *et al* (2012) who reported a decrease in total serum protein in rats treated with paracetamol and this decrease was associated with the decrease in the number of hepatocytes, which in turn resulted in the decreased hepatic capacity to synthesize protein and consequently decrease liver weight.

In this study, pre-treatment with the extract significantly ( $p < 0.05$ ) attenuated the increase in the levels of ALP, AST and ALT in the test groups compared to the toxin control group. This is an indication that it could cause plasma membrane stabilization as well as repair hepatic tissue damage caused by acetaminophen (Dash Deepak *et al.*, 2007). The decrease in the level of serum TAG and LDL coupled with increase in the level of HDL in the test groups when compared with the toxin and control groups suggest that *J. tanjorensis* could offer significant protection against the toxic effect of paracetamol. The significant ( $p < 0.05$ ) increase in the serum concentration of albumin and total protein in the

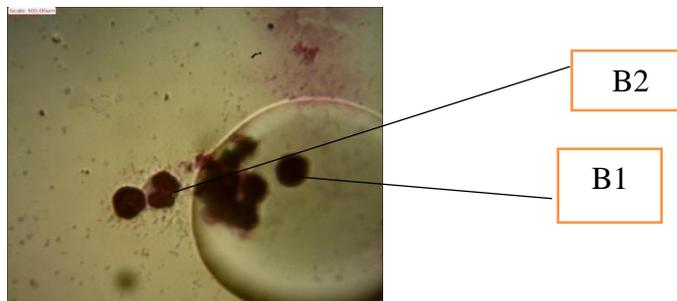


Fig 6: Shows (b1) Binucleated cells (b2) Monocytes

groups administered with different doses of the extract, may be attributed to hepatoprotective effects of the extract. The hepatoprotective activity of *J. tanjorensis* may be due to the presence of flavonoids and tannins (Madubuike *et al.*, 2015). Flavonoids prevent hepatosteatosis by increasing fatty acid oxidation in the liver (Omoriegie and Osagie, 2007). It also has positive effect on lipid metabolism on the hepatocytes, insulin resistance, inflammation and oxidative stress which are the most important pathophysiological pathways in the remediation of hepatotoxicity (Omoriegie and Osagie, 2007).

Silymarin is a known hepatoprotective compound obtained from *Silybum marianum*. It is reported to have a protective effect in plasma membrane of hepatocytes and possesses multiple mechanism of actions against different hepatotoxic agents (Omoriegie and Osagie, 2010). This knowledge informed its use as a reference drug for this study.

There was a significant decrease in the level of triacylglycerol. Triacylglycerol, stored in adipose tissues as glycerol, fatty acids and monoglycerols, are reconverted as triglycerides by the liver. Chaitanya *et al* (2012) reported a decrease in the levels of total protein, total cholesterol, and triglycerides. The level of creatinine and urea was high. The level of creatinine is usually used as a marker to determine the severity of kidney failure, while high levels of urea is an indication that there is low body water content (dehydration) and the kidney is not highly effective in the retention of water (Traynor *et al.*, 2006).

Different sperm head abnormality e.g. pin head, hook head and a single sperm with two tails were also observed. At 800 mgkg<sup>-1</sup>, there was evidence of acute sperm deformity, as both hook head and a single sperm possessing two tails was observed, and this might affect sperm transport and selection within the uterus and the

ability to fertilize the oocyte. However, at lower doses severe sperm deformities were not observed, which suggest that it is only at high dosage that it causes deformity. Osuchukwu *et al* (2016) reported that *Jatropha tanjorensis* may have the potential of enhancing spermatogenesis when consumed for a short period of time, and this was dose dependent. Micronucleus test is used in toxicological screening for potential genotoxic compounds. It is a reliable assay for genotoxic carcinogens (carcinogens that act by causing genetic damage). Fragmented nucleus, binucleated cells and micronuclei cells were discovered at the dose of 400 mgkg<sup>-1</sup>. This shows that the toxicity rate of *J. tanjorensis* is less toxic at lower doses but might be potentially toxic at high dosage. Almeida *et al* (2016) reported alterations, such as chromosome adherence, C-metaphases, chromosome bridges, nuclear buds, and the presence of micronuclei on administration of the extracts which indicated mutagenic action at the chromosome level.

The results of this study indicate that *Jatropha tanjorensis* could possess a potent hepatoprotective activity against acetaminophen induced liver damage in albino rats though it might affect sperm morphology at high doses. However, more work is needed to ascertain its mechanism of action.

#### Conflict of Interest

There is no conflict of interest regarding the manuscript.

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