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Haematological and Histopathological Studies of *Jatropha tanjorensis* (J.L. Ellis and Soroja) Leaves in Rabbits

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Abstract: This study investigated the possible toxicity of consumption of the leaf by determining a variety of serum biochemical parameters in liver and renal function tests, haematological and physical parameters. A 5 week repeated dose toxicity of *Jatropha tanjorensis* leave powder was carried out in rabbits. Forty animals, male and female, were administered feed mash plus ground *J. tanjorensis* leaf powder in graded concentrations of 0, 5, 10 and 25%. All rabbits survived at the end of the study and results showed no significant alteration in average body weight in the treatment groups when compared with the control group. The haemoglobin, hematocrit, platelets and platelet cell distribution width in the female group, showed significant increase between the control and the treated groups. This is an indication of an improved bone marrow function. No severe histopathologic indicator was recorded.

Key words: *Jatropha tanjorensis*, histopathology, haematology, rabbits

INTRODUCTION

Some plants, apart from serving as food, have also been known to exhibit medicinal properties (Borget, 1993; Ozuola *et al*., 2006). Over 400,000 species of tropical flowering plants have medicinal properties, a factor that may have made traditional medicine cheaper than modern medicine (Akpulu *et al*., 1994; Aibinu, 2004).

Although plant based natural medicines are popularly acclaimed to be safe, scientists advocate for proper toxicological studies (Oyewole *et al*., 2007; Ozolua *et al*., 2006) in other to ensure safety in the use of natural medicines. Toxicity is the undesirable property of any drug or chemical capable of producing injurious or detrimental effects on a living organism. Whether or not these injuries occur depend on the amount of chemical absorbed (Gossel and Bricker, 1990; Betram, 1998). The toxic effect caused by a drug is similar in man and some other animals, a premise for use of animal models in toxicological studies (Range *et al*., 1995). Most toxic effects of drugs occur at a predictable time after administration. However, the target organ of toxicity is not necessarily the site of accumulation of the chemical (Curtis, 2001).

Jatropha tanjorensis belongs to the Family Euphorbiaceae, it is a common weed of field crops, bush re-growth, road sides and disturbed places in the higher rainfall forest zones of west Africa. It is commonly called hospital too far, catholic vegetable, iyana-ipaja, lapalapa (Iwalewa *et al*., 2005). The leaf is a commonly consumed vegetable in many parts of Southern Nigeria. It is also popular as a natural remedy against diabetes in this region (Olayiwola *et al*., 2004). 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).

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It is popular as a natural remedy against malaria infection and hypertension in some parts of Nigeria, however there is dearth in scientific validation of these claims.

In the present study, we investigated the toxicity profile of *J. tanjorensis* by examining the body and organ weights, hematology and histopathology of rabbits treated with the leaves of the plant.

MATERIALS AND METHODS

The plant material used in this study was harvested in the month of March, 2007 from the vicinity of a home garden in BDPA, Ugbowo, Benin City, Nigeria. The plant was identified as *J. tanjorensis* by Professor MacDonald Idu of the Botany Department, University of Benin, Benin City, Nigeria. Voucher specimen was deposited in the herbarium of Botany Department, University of Benin, Benin City, Nigeria.

The harvested leaves were air-dried for 2 days then further dried in an oven at 40°C for 24 h. before grinding. The ground leaves were preserved in moisture-free, airtight laboratory containers for further use. Forty rabbits (20 male and 20 female) weighing between 1.3 and 1.5 kg were bought from Aduwawa Market in Benin City and acclimatized in the Animal House, for 2 weeks. The rabbits were divided by sex into 4 groups of 5 animals per cage. They were provided with commercial feed-mash and water *ad libitum*. Colored marker pen was used to distinctly label each animal for easy identification.

Jatropha tanjorensis powder was mixed with commercial feed and administered to the rabbit treatment groups viz., 5:95, 10:90 and 25:75% of plant to feed mash representing groups B, C and D, respectively. While the control (group) was administered feed-mash only (0:100).

Physical characteristics such as agility, appetite and eye color were closely observed through out the period. The average weekly weight of rabbit in each group was recorded. After the 5th week period, blood samples were collected through the marginal ear vein of each rabbit with small needles and 5 mL of the blood sample was kept in EDTA (ethylenediaminetetra acetic acid) bottles for analysis. The hematological examination was performed using an automatic multichannel blood cell counter (Systemx Kx 21 Haematology Analyser). The parameters included hematocrit value (HCT), red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HG), mean corpuscular volume (MCV) mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LYMPH), neutrophils (NEUT), mixed (MXD), red cell distribution width (RDW) and platelet cell distribution width (PDW).

The animals were then sacrificed by cervical dislocation and immediately dissected in order to collect the relevant organs, which were fixed in formaldehyde before weighing. The organs were dehydrated in different stages of alcohol concentration and cleared in toluene. The tissues were then impregnated in molten paraffin and embedded. Sectioning was done at 3 μ and stained using eosin and haematoxylin. The samples were finally cleared in xylene and mounted in paraffin wax.

The mean, standard deviation and standard error and the level of significance for the differences between means were calculated by using SPSS 10. The level of significance was at $p = 0.05$

RESULTS AND DISCUSSION

Table 1 reports on physical observations. Only the female groups B, C and D had moderate appetite for the feed preparation when compared with the control and other treatment groups.

Table 2 shows there was no significant difference in average body weight between the treatment groups and the control and between the treatments.

Table 3 shows that HGB, HCT and PLT values in female Group D were significantly higher, 11.83±0.69, 36.03±1.87 and 394.00±103.41 than those of the control, 5.47±3.37, 16.13±10.41 and 94.33±47.90, respectively.

Table 1: Effect of 5 weeks consumption of *J. tanjorensis* on some physical parameters in rabbits

Groups	Agility	Eye colour	Appetite	Conc. (g) Feed-mash	Conc. (g) (*J.tanjorensis*)
Male					
A (Control)	Normal	Normal	+ + +	200	0
B	Normal	Normal	+ + +	190	10
C	Normal	Normal	+ + +	180	20
D	Normal	Normal	+ + +	150	50
Female					
A (Control)	Normal	Normal	+ + +	200	0
B	Normal	Normal	+ +	190	10
C	Normal	Normal	+ +	180	20
D	Normal	Normal	+ +	150	50

+++ : High appetite, ++ : Moderate appetite

Table 2: Effect of intake of *J. tanjorensis* leaves on the average body weights of rabbits

	Weight (g)				
Groups	Week 1	Week 2	Week 3	Week 4	Week 5
Male					
A	1.43±0.35^a	1.57±0.15^a	1.73±0.21^a	1.70± 0.22^a	1.80±2.00^a
B	1.70±0.17^a	1.60±0.10^a	1.67±0.25^a	1.63±0.12^a	1.70±0.10^a
C	1.26±0.25^a	1.37±0.25^a	1.50±0.20^a	1.53±0.25^a	1.60±0.20^a
D	1.33±0.42^a	1.10±0.46^a	1.23±0.45^a	1.00±0.92^a	1.07±0.97^a
Female					
A	1.53±0.15^a	1.50±0.10^a	1.67±0.58^a	1.60±0.00^a	1.13±0.98^a
B	1.63±2.23^a	1.43±0.12^a	1.57±0.15^a	1.53±0.21^a	1.57±0.15^a
C	1.33±0.35^a	1.23±0.15^a	0.93±0.81^a	0.97±0.84^a	1.67±0.23^a
D	1.43±0.38^a	1.50±0.26^a	1.60±0.35^a	1.60±0.26^a	1.57±0.15^a

Values and expressed as Mean±SD with similar superscript within a row are not significantly different, p>0.05

Table 4 reveals that apart from the female spleen, significant differences were recorded in all other isolated organs between the control and treated rabbits. Spleen weight of the male control (0.23±0.15) was significantly lower than Groups B, C and D (0.5±0.15, 0.43±0.57 and 0.53±0.11, respectively). However, the treatments were not significantly different for the same organ.

Table 5 reveals that most the organs showed very mild histopathologic changes compared with the control. Severe triaditis was however observed in the liver of female treatment group compared with that of the male treatment group.

The observations of behavioral, hematological and histological parameters have been employed in toxicological studies (Basu and Arivukkarasu, 2006). Decrease in body weight could either be due to the effect of a plant material on the internal organs or to general discomfort resulting in lowered feeding in treated animals (Brodie *et al*., 1970).

In the present study, the agility, the treatments did not appear to have any observable influence on the agility, eye colour and appetites of the rabbits and there was no significant difference (p>0.05) in the average body weight of treatment groups compared with the control group after 5 weeks (Table 1, 2).

The significantly higher HGB, HCT and PLT values in the female Group D of 11.83±0.69, 36.03±1.87 and 394.00±103.41, respectively compared with 5.47±3.37, 16.13±10.41 and 94.33±47.90 (Table 3) for the control may be an indication of improvement in the bone marrow function. This is in line with the views of Oduola *et al*. (2007).

Although significant differences were recorded in the weights of most of the isolated organs, no regular pattern could however be established in both sexes (Table 4). Whereas the weight of the right kidney in the 25% treatment male group was significantly lower (4.23±0.32) than the control (5.92±0.36), the reverse was recorded for the female rabbits as similar treatment recorded a significantly higher value (4.23±0.32) than the control (3.70±0.17).

Table 3: Effect of *Jatropha tanjorensis* leaves on hematological parameters of rabbits

	Concentration (%)			
	0	5	10	25
Parameters	Group A (Control)	Group B	Group C	Group D
Male				
WBC ($\times10^3\mu L$)	2.90±1.11^a	6.60±2.17^a	6.03±1.00^a	4.89±1.94^a
RBC ($\times10^6\mu L$)	4.87±0.40^a	2.58±1.41^a	5.13±0.31^a	3.20±1.79^a
HGB (g dL^{-1})	12.43±0.47^a	6.07±3.07^a	5.80±0.72^a	6.80±3.67^a
HCT (%)	38.57±1.36^a	18.87±9.50^a	32.60±2.80^a	20.96±11.42^a
MCV (fl)	65.06±2.35^a	44.60±22.32^a	67.23±3.07^a	44.27±22.20^a
MCH (pg)	21.00±0.75^a	14.33±7.19^a	22.93±1.42^a	14.40±7.24^a
MCHC (%)	32.20±0.12^a	21.40±10.71^a	2.64±1.52^a	21.73±10.87^a
LYMP (%)	67.40±4.99^a	35.85±19.39^a	56.77±12.20^a	26.87±15.68^a
NEUT (%)	16.90±8.73^a	28.43±15.96^a	2.66±1.53^a	36.80±19.89^a
PLT (µL)	329.33±163.8^a	128.33±70.24^a	274.33±194.63^a	333.89±192.77^a
MXD (%)	2.83±0.69^a	2.40±1.21^a	3.87±0.62^a	3.00±1.59^a
RDW (%)	13.17±0.32^a	9.30±4.65^a	2.44±1.41^a	9.33±4.70^a
PDW (fl)	7.10±0.86^a	4.78±2.43^a	4.70±2.35^a	4.53±2.35^a
Female				
WBC$\times10^3$(µL)	2.90±2.28^a	6.60±0.44^a	6.03±0.81^a	4.87±1.03^a
RBC $\times10^6$ (µL)	2.58±1.64^a	5.13±0.15^a	4.84±0.38^a	5.35±0.29^a
HGB (g dL^{-1})	5.47±3.37^a	11.03±0.92^{ab}	10.60±0.67^{ab}	11.83±0.69^b
HCT (%)	16.13±10.41^a	32.17±2.07^{ab}	31.67±2.14^{ab}	36.03±1.87^b
MCV (fl)	41.33±20.68^a	62.67±3.64^a	65.60±2.03^a	67.40±0.51^a
MCH (pg)	14.43±7.24^a	21.33±1.67^a	21.93±0.44^a	22.13±0.52^a
MCHC(%)	23.36±11.77^a	34.23±0.98^a	33.50±0.56^a	32.83±0.55^a
LYMP (%)	32.16±16.23^a	38.10±8.49^a	46.67±5.52^a	62.87±5.20^a
NEUT (%)	31.93±16.08^a	58.43±8.73^a	49.60±5.35^a	34.83±4.81^a
PLT (µL)	94.33±47.90^a	228.00±93.24^{ab}	245.33±57.03^{ab}	394.00±103.41^b
MXD (%)	2.47±1.25^a	3.47±0.50^a	3.73±0.22^a	2.30±0.42^a
RDW (%)	11.00±5.73^a	14.63±1.04^a	14.50±0.96^a	13.90±0.61^a
PDW (fl)	3.90±2.03^a	8.57±0.82^b	7.17±0.33^{ab}	13.90±0.17^{ab}

n = 5 replicates, Mean±SE with similar superscript within a row are not significantly different at $p>0.05$, Mean±SE with different Superscript within a row are significantly different at $p<0.05$ HT: Hematocrit value RBC: Red blood cell count, WBC: White blood cell count, HGB: Hemoglobin level, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, LYMPH: Lymphocytes, neutrophils, MXD: Mixed, RDW: Red cell distribution width and PDW: Platelet cell distribution width

Table 4: Organ weights in rabbits given feed containing varying concentrations of *J. tanjorensis* leaves

	Concentration (%)			
Organs	A	B	C	D
Male				
Heart	4.62±0.68^b	3.60±0.10^a	3.61±0.35^a	3.70±0.40^a
Liver	42.87±0.32^d	24.48±0.11^a	30.93±0.36^b	34.97±0.35^c
Spleen	0.23±0.152^a	0.50±0.15^b	0.43±0.57^b	0.53±0.11^b
Left kidney	6.50±0.01^c	3.28±0.16^a	2.80±0.66^a	4.23±0.32^b
Right kidney	5.92±0.36^c	3.73±0.12^b	3.20±0.35^a	4.23±0.32^d
Lungs	7.63±5.77^b	8.35±0.31^c	4.40±0.35^a	9.90±0.36^a
Female				
Heart	4.03±0.15^b	4.37±0.47^b	3.23±0.25^a	3.37±0.31^a
Liver	37.53±1.31^c	27.83±1.51^a	32.57±1.42^b	38.83±0.25^c
Spleen	0.67±0.12^a	0.61±0.35^a	0.67±0.50^a	1.13±0.32^a
Left kidney	3.90±0.36^{ab}	4.43±0.67^b	3.63±0.15^a	4.67±0.21^b
Right kidney	3.70±0.17^a	3.83±0.21^a	4.20±0.26^{ab}	4.63±0.38^b
Lungs	5.37±0.32^b	5.33±0.31^b	4.33±0.25^a	8.37±0.47^c

n = 5 replicates Mean±SE with different superscript within a row are significantly different, $p<0.05$. Mean±SE with similar superscript within a row are not significantly different, $p>0.05$

Table 5: Incidence of histopathologic findings in rabbits given feed containing *J. tanjorensis* leaves for 5 weeks

Organs	Findings	Grade	Control	Treatment
Male				
Heart	Infarction with areas of necrosis	+	0	1
Liver	Necrosis with congested vessels and mild triaditis	+	1	3
Small Intestine	Necrosis of villi	+	0	2
Kidney	Necrosis	+	0	3
Lungs	Thickness of alveoli	+	0	3
Female				
Heart	Infarction	+	0	1
Liver	Necrosis, congested vessels and severe triaditis	++	1	3
Small Intestine	Necrosis of villi	+	0	2
Kidney	Infarction	+	1	1
Lungs	Necrosis and thickness of the alveoli walls	+	0	2

+= Moderate, ++= Severe, n = 5 replicates

The histopathology revealed that the heart, liver and kidney showed areas of mild necrosis (Table 5). Although there was severe triaditis in the liver of the female treatment group compared with that of the male, such isolated event cannot sufficiently be interpreted as meaningful toxicological effect.

There was no noticeable neurological sign in the 25% group in the present study suggesting that the possibility of the induction of neurotoxic effects of *J. tanjorensis* is quite low even with the ingestion of up to 25% of the plant.

In conclusion, this study reveals that the toxicity profile of *J. tanjorensis* is low since no observable adverse effect (NOAE) was recorded in this study. However, specific dose preparation and administration using syringe (oral, intramuscular or intravenous) may yield more precise results compared with the challenge of differential appetite observed in the present study.

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