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Phytochemical analysis and toxicological evaluation of the methanolic extract of *Jatropha tanjorensis* leaf

Patrick O. Igbinaduwa*, Cyril O. Usifoh and Comfort C. Ugwu

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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Abstract
The work evaluated the behavioral and toxicological effects and its consequences on hematological parameters and the phytochemical analysis of *Jatropha tanjorensis* leaf. Acute and sub-acute toxicity studies were done on Wistar albino mice and rats for 14 days and 28 days respectively. Hematological parameters of the rats were also determined. During acute toxicity, there were no adverse effect found in the general behavior and mortality at any dose level given (1000-8000mg/kg b.wt.). Sub-acute toxicity did not cause any changes in the body and organ weight except a statistical decrease in the spleen weight. Hematological investigation revealed an increase in the packed cell volume, red blood cells and hemoglobin. Phytochemical analysis revealed the presence of tannins, Flavonoid, Alkaloids, Anthraquinone, Saponin and Cardiac glycosides. This work thus justifies the ethnomedicinal use of the plant in the treatment of anaemia and its safety profile.

Keywords: Toxicological, Ethno toxicity, Hematological and phytochemical

INTRODUCTION

Plant chemotherapy which essentially relies on an application of the principle of pharmacology and medicinal chemistry to the plant system has developed an important area of the pharmaceutical industry (Ross and Brain, 1977). Increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patient make wise decision before using them (Hara *et al.*, 1998). Most medicinal plants have not been thoroughly evaluated for their toxicity profile, although it is generally agreed that medicinal plants and their products are relatively safer than their synthetic counterpart drugs because medicinal plant constituents mimic more closely the natural constitution of the human somatic system. However, this should not be used as a blanket assumption about the safety of medicinal plant and their products, and thus a thorough and detailed pharmacological and toxicological assessment of these plants and their approval for therapeutic purposes is very necessary because seemingly innocuous plants may turn out to be toxic (Gamaniel, 2000). To the best of our knowledge, there is no reported literature on the toxicology of the plant.

Jatropha tanjorensis (J.L. Ellis & Saroja) is a common weed of field crops, in rainforest zones of West Africa including Nigeria (Iwalewa and Agbani, 2005). It is

* Corresponding author. *E-mail address*: pigbinaduwa@yahoo.com Tel: +234 (0) 7063806665
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commonly called "Hospital too far", "Catholic vegetable", "Iyana-ipaja", "Lapalapa". Its primary use is for fencing while its secondary uses are as a source of edible leafy vegetables and medicine. It is useful in herbal medicine, prepared locally in most parts of Southern Nigeria by collecting the leaves and squeezing out the juice (Prabakaran and Sujatha, 1999).

J. tanjorensis leaf exhibit low antioxidant and very low haemagglutination titre value, the latter indicating low toxicity on red blood cells. The leaf extract has hypoglycemic properties and is taken as a remedy against diabetes (Olayiwola *et al.*, 2004). It is popular as a natural remedy against malaria infection and hypertension in southern Nigeria where they drink the squeezed out juice, however there is dearth in scientific validation of these claims. Research has shown that fresh *Jatropha tanjorensis* leaves contain a high water and low protein content. The trace elements, zinc, iron and selenium are in concentrations comparable to those found in food regarded as good dietary sources of these minerals, hence, a good dietary source of these elements. The leaf extract also possesses antimicrobial properties and inhibit the growth of *S. aureus* and *E. coli* (Oboh and Masodje, 2009).

Jatropha tanjorensis leaf has antianaemic effect (blood replenishing potentials). The leaf was found to contain some important biogenic principles that are important for rapid haemopoiesis in the bone marrow (Omoregie and Osagie, 2007). *J. tanjorensis* leaf is also a potent Anti-HIV agent (effective against HIV-1 vector). Result obtained from this research, *J tanjorensis* showed the most significant degree of antiviral activity yielding 4 potent anti-HIV fractions designated as JTD10, JTD11, JTD12 and JTD13 (Esimone *et al.*).

EXPERIMENTAL

The leaf of *Jatropha tanjorensis* was freshly collected from a home garden in University of Benin, Benin City, Edo State, Nigeria on the 15th of September, 2010. The fresh leaf was identified by the plant curator in the Department of Pharmacognosy Herbarium, University of Benin, Benin City, Edo state, Nigeria. The leaves were air- dried at room temperature for four days then further dried in an oven at 40^{0}C for 6 hours. The crispy leaves were ground into powder and filtered using a sieve aperture of 1.0mm. The fine powder was preserved in moisture-free, airtight container and used for phytochemical tests and toxicological evaluation.

Phytochemical studies. Standard Phytochemical screening techniques were employed to detect the presence of some secondary metabolites (Harborne, 1992).

Toxicological Evaluation

Preparation of extract. 950g of the powdered leaf sample was macerated in 6 liters of methanol (Analytical grade) for 72hours. The suspension was filtered and the resulting filtrate was evaporated to dryness over a water bath. The sticky extract of weight 105g was tested in mice and rats to determine its therapeutic risk.

Acute toxicity study. Forty-eight healthy albino mice of either sex weighing between 20-30g were bought from animal poultry in Ibadan and acclimatized in the animal house of the department of pharmacology, university of Benin, for 3 weeks. The mice were divided into 6 groups (I-VI) of 8 animals per group and were fed with standard rat pelleted diet (Ewu Feed) and had free access to water ad libitum. Colored marker pen was used to distinctly label each animal for easy identification. *Jatropha tanjorensis* extract was administered orally as a single dose through gastric gavange at doses of 1000, 2000, 4000, 6000 and 8000 mg/kg body weight and control group (Group I) received 10ml/kg of the vehicle alone (30% ethanol in

0.9% normal saline). The animals were observed continuously for 72hours for any signs of behavioral changes, toxicity and mortality and further observed for 14 days.

Twenty-four healthy Wistar albino rats of either sex weighing between 110-200g were also bought from animal poultry in Ibadan and acclimatized in the animal house of the department of pharmacology, university of Benin, for 4 weeks. The rats were divided into 3 groups (I-III) of 8 animals per group and were fed with standard rat pelleted diet (Ewu Feed) and had free access to water ad libitum. Colored marker pen was used to distinctly label each animal for easy identification. *Jatropha tanjorensis* extract was administered orally as a single dose through gastric gavages at doses of 6000 and 8000mg/kg body weight and control group (Group I) received 10ml/kg of the vehicle alone (30% ethanol in 0.9% normal saline). The animals were observed continuously for 72hours for any signs of behavioral changes, toxicity and mortality and further observed for 14days (Basu and Anvukkarasu, 2006).

Sub-acute toxicity study. Wistar albino rats of either sex weighing between 155-225g were divided into 3 groups (Group I-III) of 7 animals per group and were housed under the same condition as described above. *Jatropha tanjorensis* extract was administered for 28 days at doses of 500 and 2000mg/kg body weight and control group (Group I) received 5ml/kg of the vehicle alone (30% ethanol in 0.9% normal saline). Toxic manifestation and mortality were monitored daily and body weight changes were recorded every 7 days till the end of the study.

Clinical test parameters. At the 28th day, animals were fasted for 12hours and their weight taken, and then sacrificed with chloroform anesthesia, and 5ml of the blood sample collected from the abdominal aorta was kept in EDTA (ethylene diamine tetra acetic acid) tube for immediate analysis of hematological parameters.

The hematological parameters evaluated include Hemoglobin (HB), Red Blood Cell (RBC), White blood cells (WBC) and Packed Cell Volume (PCV), mean Corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet (PLT), Lymphocyte (LYMP), mixed (MXD) and neutrophils (NEU) were performed using an automatic multichannel blood cell counter (Systemx kx 21 Hematology Analyzer) (Oduola *et al*., 2007) at the Federal Medical centre Asaba, Delta state, Nigeria.

Statistical analysis. The values are expressed as mean ± standard deviation (SD). Statistical analysis of variance was done by ANOVA (one-way Analysis of Variance) followed by Dunnett Multiple Comparison Test. The significant difference between the groups are considered at $p < 0.05$ level.

RESULTS

Phytochemical analysis. The result of the photochemical evaluation is shown in Table1. It revealed the presence of alkaloids, tannins, cardiac glycoside, flavonoid and anthraquinone.

Acute toxicity study. There was no mortality or any signs of behavioral changes or toxicity observed after oral administration of *J. tanjorensis* up to the dose level of 8000mg/kg body weight in both mice and rats.

Subacute toxicity study.

Effect of J.tanjorensis on body and organ weights. The body (at 28th day) and organ weight changes of control and *J.tanjorensis* treated rats are presented in Table2.

Table 1: Phytochemical composition of *Jatropha tanjorensis* leaf .

Test	Class of compound	Result
Molisch	soluble carbohydrate	+
Fehling's	reducing sugar	+
Dragendorff's	Alkaloid	+
Wagner's	Alkaloid	+
Mayer's	Alkaloid	+
Hager's	Alkaloid	+
Borntrager's	Anthraquinone	+
Iron (III) chloride	Tannin	+
Lieberman-Burchard's	Cardiac glycoside (steroidal nucleus)	+
Salkowski's test	Aglycone and steroidal ring of cardiac glycoside	+
Sodium picrate	cyanogenetic glycoside	-
Froth	Saponin	+
Lead acetate	Flavonoid	+
Ferric chloride	Flavonoid	+

Table 2: Effect of methanolic extract of *J. tanjorensis* on Body and Organ weight changes in control and treated rats of subacute toxicity studies.

	Day/ organ	Group I (control)	Group II (500 mg/kg)	Group III (2000 mg/kg)
Body weight (g)	Day 0	188.33+22.95	199.17±17.44	185±00±17.32
	Day 28	195.00+24.50	213.33±23-38	204.17±19.60
Organ weight (g)	Liver	6.83±0.78	7.79±1.15	6.57±0.73
	Heart	0.77±0.08	0.79±0.08	0.7±0.09
	Kidney	0.58±0.09	0.70±0.14	0.56±0.08
	Spleen	0.77±0.09	0.92±0.18	0.71±0.05*****

Values are expressed as mean ± SD for six albino Wistar rats.
Comparisons were made between Group I with group II and III and between group II and group III.
The symbol ***** also represent statistical significance P<0.05

Table 3: The effect of *Jatropha tanjorensis* on the haematological parameters.

Parameters	Group I (control)	Group II (500 mg/kg)	Group III (2000 mg/kg)
WBC (10^3cell/mm^3)	11.4±3.72	11.27±5.03	10.07±3.83
RBC (10^6cell/mm^3)	7.70±0.66	6.24±1.99	8.20±0.36*****
Hb (g/dl)	13.38±1.45	11.35±3.09	14.13±0.54*****
PCV (%)	40.90±5.96	42.10±11.90	51.57±2.40*****
MCV (FL)	64.67±3.52	68.12±4.70	63.57±2.26
MCH (Pg)	17.40±0.70	18.48±1.36	17.42±0.61
MCHC (g/dl)	26.83±0.38	27.15±0.10	27.42±0.37
PLT (10^3cells /mm^3)	893.50±102.82	813.17±228.86	864.67±108.55
LYMP (%)	73.12±9.72	76.77±9.37	73.68±12.48
MXD (%)	5.80±2.30	5.35±1.48	4.98±1.70
NEU (%)	21.17±8.66	17.88±8.89	21.33±11.61

Values are expressed as mean ± SD for six rats.
Comparisons were made between Group I with Group II and Group III and between Group II and Group III.
The symbol ***** also represent the statistical significance at $p < 0.05$

There was no significant difference in the body and organ weight gain except the spleen which is considered significant between the control and *J. tanjorensis* treated groups. Moreover, no lethality and signs of observable toxicity was recorded for any dose up to the maximum of 2000mg/kg during the 28days period of treatment.

Hematological parameters.

The effect of *J. tanjorensis* on the hematological parameters is represented in Table 3. The red blood cell was found to be significantly increased ($p < 0.05$) in Group III treated albino rats when compared with Group II and Group I. The Hemoglobin and Packed Cell Volume were significantly different. The plant increases the RBC, PCV and haemoglobin level of the test animal.

DISCUSSION

The preliminary phytochemical test on the powdered leaf of *Jatropha tanjorensis* indicated the presence of Saponin, Tannins, Flavonoid, Anthraquinone, Alkaloids and Cardiac glycosides. These bioactive constituents may be responsible for the observed therapeutic effect of the plant.

Herbal medicines have received greater attention as an alternative to clinical therapy and the demand of these remedies has currently increased. Experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products and also to establish the active components of the herbal products. Observations of behavioral and hematological parameters have been employed in toxicological studies.

In acute toxicity study, there was no mortality observed up to the maximum dose level of 8000mg/kg body weight of *Jatropha tanjorensis* leaf administered orally, recommended for testing acute toxicity.

In subacute toxicity study, changes in body weight have been used as an indicator of adverse effect of *Jatropha tanjorensis* and chemicals. Since there were no changes in the average body weight at all doses ($p > 0.05$) of the treated rats when compared with the control group and between treatment groups (Table 2), it suggest that at the oral doses administered, *Jatropha tanjorensis* has no adverse effect.

With the exception of a slight decrease in the weight of the spleen, there were no significant changes in the average weight of the organs at all doses ($p > 0.05$) of the treated rats when compared with the control group and between treatment groups (Table 3), it suggest that at the oral doses administered, *Jatropha tanjorensis* may not be toxic. The significant decrease ($p < 0.05$) in the spleen weight is from 0.92 ± 0.18 to 0.71 ± 0.05 in Group II and Group III respectively. It has earlier been said that the enlargement of spleen could occur as a result of destruction of the red blood cells and platelet as well. It is of interest to note that higher concentration of *Jatropha tanjorensis* significantly reduced spleen weight, an indication that the plant is cytoprotective (Omoregie and Osagie, 2007).

With the exception of a transient increase in the Red Blood Cell (RBC), Hemoglobin (Hb) and Packed Cell Volume (PCV) ($p < 0.05$), there were no significant alteration in other hematological parameters. This increase in RBC, Hb and PCV value from 6.24 ± 1.99, 11.35 ± 3.09 and 42.10 ± 11.90 respectively to 8.20 ± 0.36, 14.13 ± 0.54 and 51.57 ± 2.43 between Group II and Group III respectively may be an indication of improvement in bone marrow function (Omoregie and Osagie, 2007).

Conclusion

Jatropha tanjorensis leaf can be considered safe, as it did not cause either any mortality or adverse changes with general behavior of albino rats and mice in acute toxicity study (up to the dose of 8000mg/kg b.wt.), and also there were no observable detrimental effects caused by *Jatropha tanjorensis* (up to 2000mg/kg b.wt.) in subacute toxicity study in rat model. The plant contains Phytochemical constituents such as tannins, flavonoids, alkaloids Saponin, cardiac glycosides and Anthraquinone which may be responsible for its ethnomedicinal effects. Further, the above

results substantiate the beneficial and extensive utilization of the plant in tradition medicine.

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