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Acute Toxicity and Histopathological Effects of Crude Aqueous Extract of *Jatropha curcas* Leaves in Mice

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ABSTRACT

The poultice of *Jatropha curcas* leaves is used traditionally for the treatment of sores, bruises, muscular pains and jaundice in Nigeria. Even without toxicological assessment, medicinal plants are generally believed to be naturally safer than synthetic drugs. Therefore, the present study investigates the acute toxicity and histopathological effects of the crude aqueous leaf extract of *J. curcas* in mice. Thirty six Wistar mice of both sexes weighing between 25-35 g were used. Graded doses of the extract were administered once to groups of mice intraperitoneally to determine the median lethal dose [LD₅₀]. Adverse effects were examined within 24 h by using clinical signs of toxicity. After 48 h of extract administration, tissues of surviving mice were harvested for necropsy and histopathological examination. The extract yielded an intraperitoneal LD₅₀ of 141.1 mg kg⁻¹ b.wt. under our environmental conditions. The mice showed dose-dependent signs of toxicity ranging from tremors, vomiting, anorexia, weakness and diarrhea to death. No gross changes were observed at necropsy of excised organs, however, histopathological lesions in the heart, liver, kidney, spleen, lungs and stomach were observed. From these observations, it may be inferred that the extract of *J. curcas* leaves is very toxic and its use may be associated with tissue structural damage of vital organs.

Key words: *Jatropha curcas*, aqueous extract, LD₅₀, histopathology, mice

INTRODUCTION

Jatropha curcas [*J. curcas*] also known as Barbados nut or Physic nut, is a shrub with oily seeds or nut. The plant belongs to the Euphorbiaceae or Spurge family. Its origin is Central America, but it is now grown elsewhere in the world including Africa (Little *et al.*, 1974). It grows well in poor soil and arid environment (List and Horhammer, 1969). It has a wide variety of medicinal uses in traditional medicine such as in the treatment of malaria, cough, jaundice, neuralgia, paralysis, scabies, sexually transmitted diseases, stomach ache, dermatitis, rheumatism, hemorrhoidal disorder, snake bites, ringworms, tooth ache and sores (Prasad *et al.*, 2012; Morton and Thomas, 1981; Sofowora, 1993). The plant is used as a laxative, purgative, abortifacient, depurative, anodyne, styptic and vulnerary agent (List and Horhammer, 1969; Duke and Wain, 1981).

The anti-oxidant, anti-inflammatory, anti-cancer, anti-diarrheal, coagulative, anti-leukaemic and anti-microbial activities of the plant parts have been documented (Morton and Thomas, 1981;

Thomas, 1989; Mujumdar *et al.*, 2001; Oskoueian *et al.*, 2011). The latex of the plant is used for the production of cyclosporine, an immunosuppressant (Parawira, 2010). Apart from medicinal values, *J. curcas* is also used extensively for the production of biodiesel fuel (Singh and Mehta, 2005). The oil from the seeds serve as insecticides, pesticides and fungicides and the by-product is press cake, a good organic fertilizer (Agaceta *et al.*, 1981; Thomas, 1989). The seeds are also used in making soap and candles while the bark is used for fish poisons (Watt and Breyer-Brandwijk, 1962). The sap stains linen and can be used in making histological stains (Mitchell and Rook, 1979).

Scientific documentation on the toxicity of *J. curcas* leaves is scarce, but instead, there are many *in vivo* and *in vitro* toxicity reports on the seeds (Adam, 1974; Joubert *et al.*, 1984; Horiuchi *et al.*, 1987; Kulkarni *et al.*, 2005). Traditionally in Nigeria, the leaves are used for treatment in macerated, poultice, decoction and tincture forms. The poultice is commonly used to treat sores, bruises, muscular pain and jaundice (ASICUMPON., 2005). Plants used medicinally are generally assumed to be safe (George, 2011), however many have toxic potentials and low safety margin. Hence, the present study was conducted to determine the acute toxicity effects of intraperitoneal administration of *J. curcas* leaves in mice and to examine the histology of some visceral organs for pathological changes associated with toxicity.

MATERIALS AND METHODS

Plant material collection: Mature fresh leaves of *J. curcas* were obtained from several sites in the University of Nigeria, Nsukka premises. The plant was taxonomically identified and authenticated by a taxonomist at the International Centre for Ethnomedicine and Drug Development [InterCEDD] and a voucher specimen with number-1INTERCEDD/086 was deposited at the Herbarium section for future reference.

Preparation of the extract: The leaves were dried under the shade for about two weeks for constant weight to be attained. About 300 g of the dried leaves was ground into fine powder in a gasoline grinding machine. The powder was allowed to macerate in 360 mL of water which was homogenized by stirring. The mixture was sieved with a muslin cloth and the resultant filtrate was concentrated in an evaporator to achieve a desired concentration (100 mg mL^{-1}). It was later stored in a refrigerator (4°C) until needed.

Animals: Thirty-nine albino Wistar mice (25-35 g) obtained from Panacea Research Laboratory, Enugu, Nigeria were used for the experiment. The animals were kept under standard laboratory conditions of temperature ($25 \pm 2^{\circ}\text{C}$) and 12/12 h light/dark cycle. They received a commercial rodent diet (Standard Top[®] feed) and tap water *ad libitum*. They were used for the study after one week of acclimatization. Animal handling was in strict compliance with Institutional and International guidelines for care and use of Animals in Scientific Research (WMA and APS., 2002).

Acute toxicity testing: The acute toxicity testing [LD_{50} determination] was done using the variation method of Lorke (1983) with slight modification. The experiment was done in two stages. In stage 1, exploratory trials were performed in five groups of 3 mice each of both sexes. Group I (control) received no treatment while the rest of the groups [II-V] were given graded doses [10, 100, 1000 and 2000 mg kg^{-1} b.wt., respectively] of the extract (i.p.) to establish the smallest toxic dose that causes no mortality and the lowest toxic dose to cause 100% mortality; the obtained doses were

100 and 1000 mg kg⁻¹ b.wt., respectively. Hence within these limits, graded doses of 200, 400, 600 and 800 mg kg⁻¹ b.wt. were then derived for the Stage 2 experiment using 4 groups [VI-IX] of 6 mice each. Each animal was given a single intraperitoneal dose of the crude aqueous extract after a 24 h fast in the respective groups. After the drug administration, clinical observations were performed hourly for 24 h and mortality and/or clinical signs of toxicity were recorded.

Histopathological studies: At the end of the experimental period, the surviving animals were sacrificed and necropsied. Some visceral organs [liver, kidney, heart, spleen, lungs and stomach] were excised and fixed in 10% formal saline. The tissues were further processed with the Automatic Tissue Processor and sectioned at 5 µm using the Rotary Microtome (Heitz 150 Rotary Microtome, Cambridge model). Sections were stained according to Haematoxylin and Eosin (H and E) technique (Carleton *et al.*, 1967) for microscopical examination. The sections were examined using Swift Binocular Microscope with in-built lighting system. The sections were then photographed using a microscope-digital-camera with an Olympus photomicroscope.

RESULTS

Clinical signs: The clinical signs observed were tremors, vomiting, anorexia, weakness, diarrhea and death. The percentage mortality (number of deaths) observed within 24 h is as shown in Table 1.

Determination of intraperitoneal LD₅₀:

$$LD_{50} = \sqrt{a \times b}$$

where, a is lowest dose that killed the animals [200 mg kg⁻¹ b.wt.], b is highest dose that did not kill the animals [100 mg kg⁻¹ b.wt.], Therefore, the intraperitoneal LD₅₀ of *J. curcas* = 141.4 mg kg⁻¹ b.wt.

Gross and histopathological findings: The macroscopical features of most of the organs excised from surviving mice in both stage 1 and 2 did not reveal apparent changes. Microscopical examination of the control sections of all the studied organs showed normal histoarchitecture. However, dose-dependent histomorphological derangements of organs of the treated mice were observed (Fig. 1a-l). Features observed are as follows: heart: hemorrhage and presence of necrotic myocardial fibres with mild lymphocytic infiltration (Fig. 1b); liver: ruptured vessels, hemorrhage and inflammatory cellular infiltration (Fig. 1d); kidney: tubular erosion, necrosis, hemorrhage, ruptured vessels and focal lymphocytic infiltration (Fig. 1f); stomach: infiltration of inflammatory cells at periglandular areas and reduced parietal cell numbers (Fig. 1g); lungs: hemorrhage,

Table 1: Percentage mortality within 24 h in mice given *Jatropha curcas* leaf extract (i.p.) at different doses

Groups	Stage one					Stage two			
	I	II	III	IV	V	VI	VII	VIII	IX
Dose (mg kg ⁻¹)	0 (Control)	10	100	1000	2000	200	400	600	800
No. of animals	3	3	3	3	3	6	6	6	6
No. of death	0	0	0	3	3	3	6	6	6
Mortality (%)	0	0	0	100	100	50	100	100	100

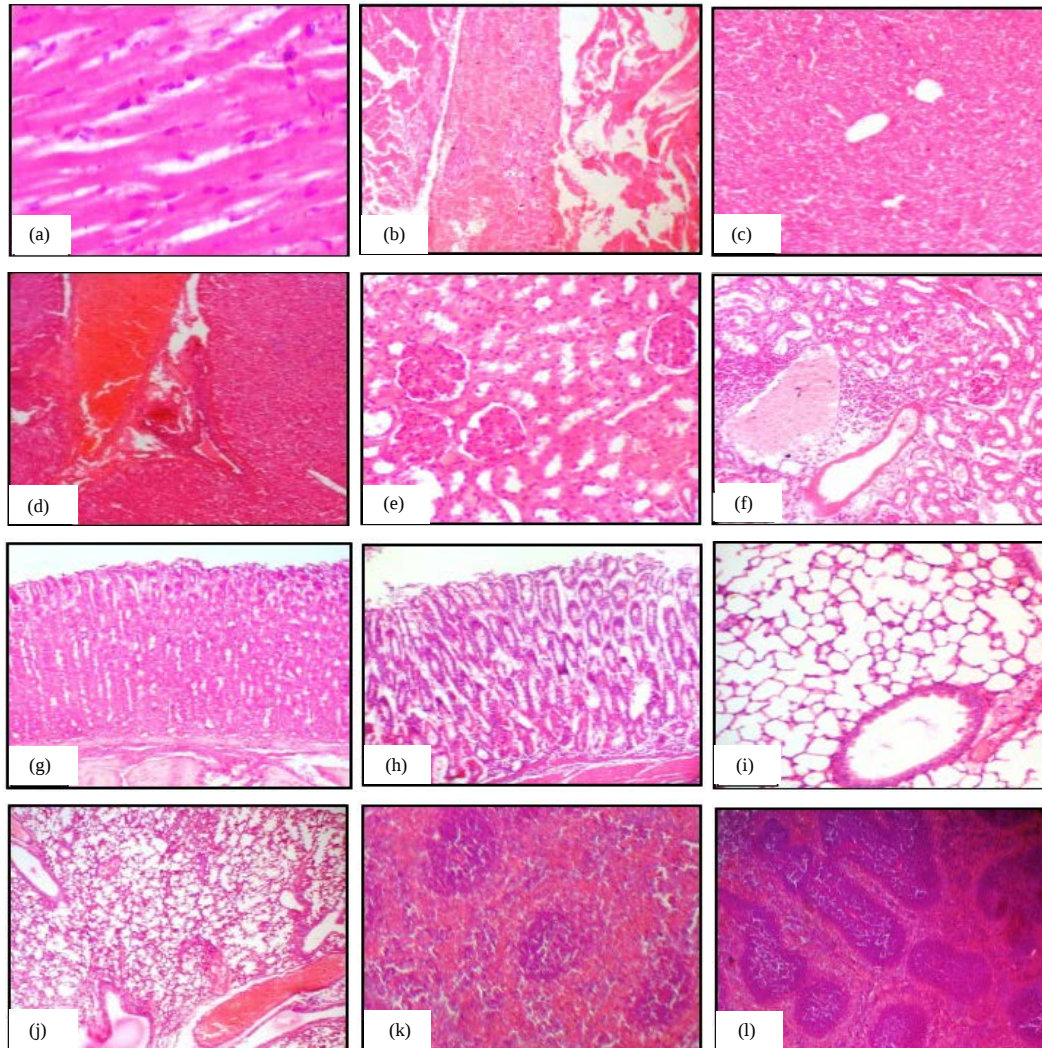


Fig. 1(a-l): Photomicrographs of (a, b) Heart sections, (c, d) Liver section, (e, f) Kidney section, (g, h) Stomach section, (i, j) Lungs section and (k, l) Spleen section in normal control and *J. curcas* extract treated mice respectively (H and E Stain: x400)

congestion of the vessels and interstitium, thickened alveolar septa, lymphocytic cellular infiltration at alveolar and perivascular regions (Fig. 1i) and spleen: enlarged and fused white pulps (Fig. 1l).

DISCUSSION

Search on available literature revealed paucity of information concerning the acute toxicity of *J. curcas* leaves despite the common use of the plant in some herbal remedies. From the results of the present study, the intraperitoneal [i.p.] doses of the crude extract of *J. curcas* produced dose-dependent organ toxicities including the heart, liver, kidney, lungs and spleen. The LD₅₀ obtained being 141.4 mg kg⁻¹ b.wt. [i.p.] suggests that the substance would be considered harmful to health according to Clark and Clark (1977). This is of great importance in view of the wide spread use of this plant and hence should be a matter of concern.

A previous report documented an oral LD₅₀ in rats of approximately 2793 mg kg⁻¹ and this was considered slightly toxic (Dangambo *et al.*, 2015). The route of administration (oral) in their study, besides the specie of experimental animal used, is the probable indicative factor for the higher value obtained. It is known that drugs/biological compounds when ingested orally undergo events that reduce the amount reaching the circulatory system for pharmacological effects (Brander *et al.*, 1991). The clinical signs of toxicity manifested in the present study may be linked to a single or combined effects of the toxic principle(s) present in the extract. Thus, these overall effects suggest a low safety margin of the aqueous extract of *J. curcas* leaves.

The reliability on LD₅₀ as the ultimate tool for the determination of toxicity is deterred as there is a wide variation in results between different species and even in the same specie under different experimental conditions (Dapar *et al.*, 2007). In addition, extrapolating LD₅₀ value obtained for a specie to other species presents considerable uncertainties and does not provide information on the particular system failure that led to death (Zbinden and Flury-Roversi, 1981). In the present study, therefore, the histopathological effects observed in the organs studied are direct or indirect responses to toxic principles in *J. curcas* leaf extract. Curcin, diterpenes, phenolic compounds, phytates and trypsin inhibitors are the toxic principles detected in *Jatropha curcas* (Shukla *et al.*, 2015).

The observed changes in the histoarchitecture of the organs are similar those documented in a previous report (Adam and Magzoub, 1975) which revealed hemorrhage in Nubian goats' visceral organs after an intoxication with *J. curcas* seeds. Several other studies have shown gross and histological changes of the heart, lungs, kidney, liver and spleen after *J. curcas* administration in a dose dependent manner (Adam, 1974; Abdel Gadir *et al.*, 2003; Abd-Elhamid, 2004; Rakshit *et al.*, 2008). In our work, the inflammatory cellular infiltration observed in tissues suggests prominent response of the tissues to injurious impacts (El-Banhawy *et al.*, 1993). The disorganization of the spleen histoarchitecture suggests hyperplasia following the administration of the test substance. Damaged blood vessels could be the plausible reason for the hemorrhage observed in the organs, or in case of the lungs, may also be due to the destruction of the alveolar cells (pneumocytes). More so, the lesions observed in the renal tissue portrays a good picture of the nephrotoxic characteristics of the plant. Some medicinal plants with nephrotoxic properties have been reported (Dapar *et al.*, 2007; Azubike *et al.*, 2009). The reason for the deaths recorded in our work may be associated with a combination of these structural tissue derangements observed.

The major toxins in the seeds and oils of *J. curcas* are phorbol esters. These toxins are also found to be present in the leaves, flowers, roots and stems of the plant. Phorbol esters are among the diterpenes present in *Jatropha* species and six types have been identified in *J. curcas* (Haas *et al.*, 2002). The intragastric LD₅₀ of phorbol esters isolated from *J. curcas* was observed to be 27.34 mg kg⁻¹ b.wt. in mice (considered highly toxic) and histopathological changes observed were fatty vacuoles in the liver hepatocytes, congested pulmonary alveolar capillaries, hemorrhage of the alveolus, abruption of cardiac muscle fibres, glomerular atrophy and hemorrhage of the spleen (Li *et al.*, 2010). These changes are consistent with the observed effects in our work and thus suggests that phorbol esters may have acted singly or in combination with other toxic principles in the plant material in eliciting the severe pathological findings. The exact mechanism for the observed effects was not elucidated in the present study, however, it has been documented that phorbol esters exert damage on tissues by the release of proteases, cytokines and activation of NADPH oxidase (Goel *et al.*, 2007).

CONCLUSION

The present study has demonstrated the acute toxicity of *J. curcas* leaves in mice. Cautious use of the plant in traditional remedies is hereby advised. More work is on-going to assess the sub-chronic effects of low doses of the extract and also isolation of the specific toxic principles in the plant material so as to maximize its therapeutic benefits.

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