In Vivo Evaluation of Acute and Subacute Toxicity of Jatropha curcas Seed Oil

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Abstract
Medicinal plants are regarded as safe because of their natural origin, nevertheless, they can contain toxic substances that can exert adverse effects. This study aims to evaluate acute and subacute toxicity of Jatropha curcas seed oil using modified Lorke method and 28 days repeated dosing of grouped rats with normal saline and -10, 300, 600 mg/kg body weight of the seed oil. At the end of the experimentation, haematological and biochemical analysis of blood samples and the histopathology of the liver and kidney of rats in each group were evaluated. The Lethal Dose (LD) of J. curcas seed oil was lower than 5000mg/kg b.wt. Haematological and biochemical analysis showed a dose-dependent decrease in the Hemoglobin, Packed Cell Volume and Red Blood Cells, an increase in the level of Total White Blood Count and Platelet Count, Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, urea and creatinine in the treated groups, while the histological evaluation revealed distortion in liver and kidney cytoarchitecture of rats administered with 600 mg/kg b.wt of the seed oil. The oil was found less toxic at the acute phase but there was toxicity manifestation in subacute phase causing adverse effects on haematological, biochemical parameters and the tissues of the kidney and liver. The study suggests that the seed oil can be used, but caution should be exercised when using it at high doses for prolonged periods.

Key Words: In vivo, Jatropha curcas, seed oil, toxicity

INTRODUCTION
Medicinal plants have consistently been accessible as a source of medication throughout the history of mankind. These plants are important part of traditional medicine for treating many disorders before the development of standard chemical and synthetic medications (Abdul et al., 2018). Traditional medicines are a significant component of cultures in the majority of underdeveloped nations. These treatments are frequently the only options accessible and have high levels of efficacy, social acceptance and economic viability (Million et al., 2019). In Nigeria, using plant products to cure or manage various illnesses is still a common practice (Emejulu et al., 2017). In spite of the myriad of ethno medicinal uses of therapeutic plants, many of these plants can contain potentially dangerous substances (Ciappin et al. 2017). Jatropha curcas (J. curcas) L. is an evergreen or deciduous multipurpose perennial tree that grows to a height of 3-6metre or more under suitable environmental conditions (Fabio et al., 2017). According to Asep et al. (2017), the genus name Jatropha is derived from the Greek terms iatros (physician) and trophe (nutrition) which is directly related to the plant’s medical properties. It is commonly referred to as the purging nut, physic nut or Barbados nut. In Nigeria, it is locally called Cnidazugu by the Hausas, Oolulu/uru by the Igbos, Lapalapa by the Yorubas, Kasha by the Nupe, Magalanga by the Kanubaris, and Muayi by the Gbagis. (Daniyan et al., 2011, Agbogidi et al., 2013, Roger, 2013). It is a member of the Euphorbiaceous or Spurge family, same family as cassava and rubber trees (Temesgen, 2016). Although it has its origins in Central America, it is now widespread around the world, even in Africa. It can adapt to dry conditions and poor soil (Azuibike et al., 2015). It is frequently employed in traditional medical procedures in Latin America, Asia, and Africa to treat a wide range of illnesses (Dada et al., 2014). In ethnomedicine, the oil that is derived from the seeds is employed as an abortifacient and purgative, the leaves are used to cure fever, oral infections, guinea worm sores, rheumatism, and jaundice (Olaipuje et al. 2011). It is also used to treat and manage a number of illnesses, including gout, eczema, rheumatism, jaundice, and inflammation of the joints, gonorrhea and burn (Komakech and Omujal, 2017).
MATERIALS AND METHODS

Collection and Authentication of Plant Materials

Fresh seeds of *J. curcas* were collected within Minna metropolis. Few seeds along side with leaves were taken to the Herbarium Department of National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja for authentication. The seeds were verified by Mr. Lateef Akeem and given the following voucher number: NIPRD/H/7195. The voucher specimen was deposited in the department.

Preparation of Plant Material

Fresh *J. curcas* seeds collected were thoroughly rinsed with water, allowed to air dry for three weeks. After being removed from the seed shell, the kernels were air-dried for an additional two weeks. The dried kernels were ground in an electric blender which resulted in a thick, gooey mixture that showed the seed had oil in it. Prior to soxhlet extraction, the ground kernels were in pristine polythene bags.

Soxhlet extraction

This was carried out according to the method described by Warra and Abubakar (2015). Grinded kernel sample was put into a porous thimble placed in a Soxhlet extractor, using n-hexane (with boiling point of 40-60°C) as extracting solvent for 6 hours repeatedly until required quantity was obtained. The excess solvent from the extracted oil was removed by evaporation using water bath at 55°C.

Toxicology of *Jatropha curcas* seed oil

Wistar rats weighing between 160 and 210g were used for this study. Ethical approval was gotten from Directorate of Research, Innovation and Development, Federal University of Technology, Minna, Niger State, Nigeria. The animals were kept in the Animal House of the Department of Biochemistry, Federal University of Technology, Minna. The animals were housed in cages in a ventilated room under room temperature of 25 ± 2°C. The animals were given 14 days to acclimatize to the environment before the experiment and were supplied with regular food pellets and water. The floor was covered in sawdust to reduce the possibility of uncomfortable contact with a solid surface. The test specimens were given orally.

Acute Toxicity

Using a modified method of Lorke as conducted by Mahe *et al*. (2017), three groups of three rats each were used in the first phase. Oral doses of the seed oil of -10, 100, and 1000mg/kg b.wt were given to the rats in each group. In the second phase, three rats received -1600, 2900, and 5000mg/kg b.wt doses of the oil, the rats were monitored for 24 hours. The Lethal Dose (LD₅₀) value was calculated using the formula below:

\[
LD_{50} = \frac{\text{Minimum dose without death} \times \text{Maximum dose with animal death}}{2}
\]

Repeated 28-day Subacute Toxicity

The subacute toxicity investigation was carried out largely in accordance with the method of Muhammad *et al*. (2015). Four cages were designated Group I, Group II, Group III, and Group IV, sixteen rats were randomly and equally placed in the four cages. Daily doses of seed oil -10, 300, 600mg/kg b.wt and normal saline were administered to the rats in Group I, II, III, IV designated cages respectively for a period of 28 days. Throughout the 28-day study period, the body weight of every rat in each cage was monitored weekly. At the end of experimentation period, the rats (one at a time) were euthanized in a jar containing cotton wool soaked in chloroform. Following jugular vein puncture of the euthanized rats, blood samples were swiftly drawn into EDTA and plain tubes for haematological and biochemical analysis, respectively. The liver and kidney from these animals were removed after dissection, blotted clean of blood, and stored in 10% formalin.

Haematological and Biochemical Evaluation

Haematological parameters, TWBC, PCV, RBC, Hb and PLC were estimated using an Auto-haematology analyzer (Abacus, Junior 30 Diatron, Hungary). The biochemical parameters, ALT, AST, ALP, urea, and creatinine were measured using readymade test kit manufactured by Agappe Diagnostics, Switzerland.

Histopathological Evaluation

The liver and kidney were processed as described by Asare *et al*. (2012) briefly, the organs were fixed in paraffin and afterwards sectioned into 5-M thick slices with a rotary microtome (Leitz microtome, Wetzlar, Germany), stained with hematoxylin and eosin, and then viewed under x40 light microscopy.

Data Analysis

Data obtained were analyzed using one-way ANOVA and Duncan’s test was performed to test the significance difference between means values using Statistical Package for Social Sciences (SPSS).
RESULTS

Table 1: Acute toxicity testing of *J. curcas* seed oil

The result of oral acute toxicity of *J. curcas* seed oil is shown in Table 1. There was no death in all the treated groups at the first phase and second phase. The LD$_{50}$ was <5000mg/kg b.wt.

<table>
<thead>
<tr>
<th>Dose (mg/kg b.wt)</th>
<th>Number of Animals</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>1000</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>1600</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>2900</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td>5000</td>
<td>3</td>
<td>0/3</td>
</tr>
<tr>
<td><strong>LD$_{50}$ &lt;5000mg/kg b.wt</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effect of oral administration of *Jatropha curcas* seed oil on bodyweight

The effect of the oral administration of *J. curcas* seed oil on body weight presented in Table 2 showed the initial body weights and weight changes of the rats treated with different doses of the seed oil and normal saline (Control). There was a significant decrease at P<0.05 in the body weight of the rats in group II and III, while the control group showed significant increase in body weight at P<0.05. The control group had the highest body weight gain while, the lowest body weight decline was recorded against the 600mg/kg b.wt treated group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>191.52±26.61$^a$</td>
<td>192.82±32.75$^a$</td>
<td>199.44±27.56$^a$</td>
<td>208.64±20.35$^a$</td>
<td>213.74±18.97$^a$</td>
</tr>
<tr>
<td>II</td>
<td>167.60±7.56 $^a$</td>
<td>157.26±9.34 $^b$</td>
<td>146.86±9.86 $^c$</td>
<td>137.51±8.23 $^d$</td>
<td>130.67±20.17 $^d$</td>
</tr>
<tr>
<td>III</td>
<td>170.18±11.03$^b$</td>
<td>165.60±12.48$^b$</td>
<td>152.68±10.98$^c$</td>
<td>141.73±11.81$^b$</td>
<td>134.72±9.94$^b$</td>
</tr>
<tr>
<td>IV</td>
<td>163.54±27.87$^b$</td>
<td>178.37±34.73$^c$</td>
<td>183.81±39.46$^c$</td>
<td>191.06±40.87$^d$</td>
<td>203.92±86.20$^c$</td>
</tr>
</tbody>
</table>

*Values with the same alphabet as superscript in a row have no significant difference at P<0.05*

*Group/Parameter: I:10mg/kg, II:300mg/kg, III:600mg/kg, IV: normal saline*

Effect of oral administration of *J. curcas* seed oil on haematological parameters

The result of the effect of *J. curcas* seed oil on haematological parameters is shown on Table 3. There was a dose dependent decrease at P<0.05 in the level of Hb, PCV and RBC among the treated group of which the lowest value was recorded against rats treated with 600mg/kg b.wt of the seed oil. Meanwhile, the level of, TWBC and PLC increased at P<0.05 in the treated groups relative to the control with the highest value also recorded against the 600mg/kg b.wt treated rats.

<table>
<thead>
<tr>
<th>G/P</th>
<th>Hb(g/dL)</th>
<th>PCV(%)</th>
<th>RBC($10^{12}$/L)</th>
<th>PLC($10^6$/L)</th>
<th>TWBC($10^{12}$/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18.57±0.82$^c$</td>
<td>40.00±1.00$^c$</td>
<td>11.80±0.51$^c$</td>
<td>120.50±2.50$^b$</td>
<td>9.16±0.17$^a$</td>
</tr>
<tr>
<td>II</td>
<td>13.62±1.16$^b$</td>
<td>35.50±1.80$^b$</td>
<td>8.20±0.77$^b$</td>
<td>122.00±1.00$^b$</td>
<td>9.69±0.49$^a$</td>
</tr>
<tr>
<td>III</td>
<td>10.15±0.77$^a$</td>
<td>30.50±1.50$^a$</td>
<td>5.74±0.71$^a$</td>
<td>129.50±1.50$^a$</td>
<td>13.43±1.13$^b$</td>
</tr>
<tr>
<td>IV</td>
<td>22.10±1.80$^d$</td>
<td>46.50±1.50$^d$</td>
<td>9.62±0.62$^b$</td>
<td>112.50±2.50$^a$</td>
<td>10.30±0.40$^a$</td>
</tr>
</tbody>
</table>

*Values with the same superscript in a column have no significant difference at P<0.05*

*Key: G/P: Group/Parameter, I:10mg/kg, II:300mg/kg, III:600mg/kg, IV: normal saline, Hb-Haemoglobin, PCV- Packed Cell Volume, RBCs- Red Blood Cells, PLC- Platelet Count, TWBC- Total White Blood Cells Count*
Effect of oral administration of *J. curcas* seed oil on biochemical parameters

Table 4 represents the effect of oral administration of *J. curcas* seed oil on biochemical parameters of the control and treated groups. From the results, the levels of the parameters were significantly higher at $P<0.05$ in the group of rats treated with 600mg/kg b.wt relative to other treated groups.

<table>
<thead>
<tr>
<th>G/P</th>
<th>AST (U/Min)</th>
<th>ALT (U/Min)</th>
<th>ALP (U/Min)</th>
<th>UREA (mg/dL)</th>
<th>CREATININE (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.71±0.29</td>
<td>12.02±0.88</td>
<td>49.61±0.81</td>
<td>4.48±0.64</td>
<td>4.13±0.15</td>
</tr>
<tr>
<td>II</td>
<td>10.34±0.96</td>
<td>13.40±0.99</td>
<td>45.72±0.60</td>
<td>47.63±1.65</td>
<td>5.62±0.63</td>
</tr>
<tr>
<td>III</td>
<td>13.45±0.61</td>
<td>25.80±1.39</td>
<td>53.71±1.78</td>
<td>51.93±1.60</td>
<td>8.15±0.23</td>
</tr>
<tr>
<td>IV</td>
<td>9.09±0.30</td>
<td>10.12±0.30</td>
<td>54.88±0.59</td>
<td>41.52±1.52</td>
<td>5.01±0.20</td>
</tr>
</tbody>
</table>

Values with the same superscript in a column have no significant difference at $P<0.05$

Key: G/P: Group/Parameter, I:10mg/kg, II:300mg/kg, III:600mg/kg, IV: normal saline

AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, ALP- Alkaline phosphatase

Histopathological features of kidney and liver section of rats treated with *J. curcas* seed oil

Plate IA: Photomicrograph of Kidney section of rat administered normal saline (Control) showing preserved architecture composed of normal glomeruli and tubules (H&E X40)
Plate II: Photomicrograph of Kidney section of rat administered 10mg/kg b.wt of *J. curcas* seed oil showing preserved architecture composed of normal glomeruli and tubules (H&E X40).
Plate III: Photomicrograph of Kidney section of rat administered 300mg/kg b.wt showing preserved architecture composed of normal glomeruli and tubules (H&E X40).
Plate IV: Photomicrograph of Kidney section of rat administered 600mg/kg b.wt showing moderate degenerative changes of glomeruli and tubular dilatation (H&E X40)
Plate V: Photomicrograph of Liver section of rat administered with normal saline (Control) showing largely preserved architecture with viable hepatocytes (H&E X40)
Plate VI: Photomicrograph of Liver section of rat administered with 10mg/kg b.wt of *J. curcas* seed oil showing largely preserved architecture with viable hepatocytes (H&E X40)
Plate VII: Photomicrograph of Liver section of rat administered with 300mg/kg b.wt of *J. curcas* seed oil showing largely preserved architecture (H&E X40).
Plate VIII: Photomicrograph of Liver section of rat administered with 600mg/kg b.wt of *J. curcas* seed oil showing mild sign of sinusoidal dilatation and oedema (H&E X40).

**DISCUSSION**

The *J. curcas* seed oil’s lethal dose (LD$_{50}$) was $<$5000mg/kg b.wt, which means the oil was not poisonous at its highest concentration of 5000mg/kg b.wt. According to classification, extracts or chemical compounds with an LD$_{50}$ of between 1000 and 5000mg/kg b.wt are regarded as practically low toxic (Hadi et al., 2019).
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When assessing an animal’s health, body weight is seen to be a crucial indicator, because it represents a number of organic changes in the animal (Manal et al., 2015). In this study, the body weight of the rats treated with 300 and 600mg/kg b.wt decreased (P<0.05) relative to control, whereas the body weight of the low dose group was comparable with the control. The decline in body weight in the treated group II and III in this study is an indication the seed oil had a negative impact on the body of the treated rats which also resulted in the decrease in body weight. The reduction in body weight in the J. curcas seed oil treated groups II and III was in line with the findings of AbdelSafy et al. (2011) and Barros et al. (2015). They reported a decline in the weight of rabbits receiving diets containing 5%, 7.5% and 10% J. curcas seed meal and broiler feed with 25, 50 and 100g of J. curcas seed meal respectively. In a similar study by Sawadogo et al. (2018), there was a decrease in the body weight of mice treated with higher doses of J. curcas leaves extract. Phytic acid and trypsin inhibitors reported by Shukla et al. (2015) to be present in J. curcas might be responsible for the decline in body weight because the consequences of their activities include reduction in bioavailability of many essential minerals like iron, calcium and magnesium and endogenous loss of important amino acids.

Blood comprises of red blood cells, white blood cells, platelets and plasma (Saganuwan, 2019). PCV is the percentage of cells in the blood. RBCs accounts for nearly all the cells because of its major physiological role of transporting of gases (O₂, CO₂) from the lung to the tissues and to maintain systemic acid/base equilibria, haemoglobin is the iron containing substance that transports the O₂ (Kuhn et al., 2017). WBCs plays the major role of defence against invasion. They trigger an inflammatory response typically induced by exogenous molecules from pathogens or endogenous molecules activated by tissue stress or damage as such WBCs and PLTs resident in damaged tissue aggregate at sites of injury as key mediators to prevent further injury and repair existing damage (Foy et al., 2022). The alteration in the blood parameters in animals is useful in predicting toxicity in animal. In this study, there was a significant decrease (P<0.05) RBCs, Hb and PCV accompanied by increase in PLC and TWBC in the group treated with 600mg/kg relative to the control. Similarly, there was decrease in the level of Hb and PCV accompanied with decrease in PLC in the group dosed 300mg/kg b.wt, however the level of RBC and TWBC was not significantly different (P<0.05) when compared to the control. For the low dose group (10mg/kg b.wt), all the parameters were significantly different (P<0.05) with exception of TWBC which was comparable with the control. Decreased RBCs production may result from damage of red blood cells, defined as haemolysis which results primarily from bone marrow diseases or from other causes such as renal failure, drugs and toxins (Kuhn et al., 2017, Cotter, 2022). The reduction in the RBCs in this study suggests toxic substances contained in the seed oil affected the cells which also resulted in the decline in the PCV and Hb. In addition, RBC production is regulated by erythropoietin which is released as needed by the kidney, hence the nephric impairment that will be discussed later also contributed to the decline in RBCs. The increase in the TWBC and platelets in this study suggest responds of the cells to the detrimental effect of the seed oil. Gadir et al. (2003) documented a similar report after Nubian goat kids were allotted 0.25g/kgb.wt of J. curcas seed per day. This corroborates the finding of Shukla and Singh. (2013) after dosing of goats with 1ml/kg body weight of seed oil for 28 days. Reduction in Hb, PCV and Total Erythrocyte Count (TEC) was also reported by Sreeelakshmi et al. (2017) following feeding of broiler chicken with deoiled seed cake. The change in haematological parameters may be caused by curcin’s hemolytic action, which not only interferes with normal protein synthesis by changing the affinity and reaction passing through the ribosome subunits but also damages the gastrointestinal tract, causing maldisgestion and malabsorption of nutrients needed for erythropoiesis (Shukla et al., 2015).

The kidney is the body’s organ which helps in the maintenance of homeostasis. It plays a key role in excretion of waste products of metabolism, drugs and chemicals (Nwankpa et al., 2018). Creatinine and urea are non-protein nitrogenous metabolites that are cleared from the body by the kidney following glomerular filtration. When the kidney is impaired, it results in accumulation of these metabolites (Arsad et al., 2013, Muhammad and Awatif, 2018). The liver is another important organ for the excretion and deposition of endogenous and exogenous substances. Hepatic cells participate in a variety of metabolic activities and contain a group of enzymes (ALT, AST and ALP), their activities are most basic biochemical markers to assess liver injury. High concentration of these enzymes indicates tissue damage and altered membrane permeability (AbdelAziz et al., 2014, Mahe et al., 2017).

In this study, there was a dose dependent increase in the level of urea, creatinine, AST and ALT among the treated group. The variation in the level of ALP was an exception. When compared to the control, the level of all the parameters was significantly different (P<0.05).
in the low dose group though the level of AST and creatinine was lower in this group. The level of creatinine was not significantly different (P<0.05) in the 300mg/kg b.wt treated group relative to the control, the level of all other parameters were higher except for ALP. There was a significant increase (P<0.05) in level of all the parameters in the group treated with 600mg/kg b.wt with the exception of ALP which was comparable to the control. The rise in activity of serum AST and ALT enzymes are corroborated by the disorientation of the cytoarchitecture (Plate VII) and cellular integrity of the hepatocytes and being cytoplasmic in nature, these enzymes are released into systemic blood circulation after cellular damage of hepatocytes which in turn increases the leakage of the liver specific enzymes. In this study, the high level of AST, ALT, urea and creatinine in the high dose group suggests hepatic and nephric impairment. The nephric impairment observed in this study as a result of increase in urea and creatinine corresponds with the findings Awasathy et al.(2010) after short term exposure of Wistar rats to 25% and 50% J.curtcas seed protein supplemented diet. This agrees with the findings of Shukla and Singh (2013) following administration of 1mL/kg bodyweight of seed oil in goats. In another study by Kumar et al. (2014), a marked rise in AST, ALT, urea and creatinine was reported following oral dosing of rats with 4seeds/rats and 1mL seed oil/rat. Several processes including the synthesis of phospholipids and proteins, enzyme activities, DNA synthesis, protein phosphorylation, cell differentiation, and gene expression have been shown to be affected by phorbol esters found in the seed and seed oil of J. curcas (Shukla and Singh, 2013). Assessment of liver and kidney functions using experimental animals involves measurement of biochemical makers and in addition, assessment of liver and kidney tissue designs (Mahe et al., 2017). Histological evaluation tallied closely with the biochemical analysis on liver and kidney. In this study, the kidney sections of group I and group II rats administered J. curcas seed oil revealed normal cytoarchitecture showing the normal renal tubules and glomeruli (Plate II and III) similar to the control group (Plate I). For group III, moderate degenerative changes of glomeruli and tubular dilatation with distorted Bowman’s capsules were the prominent features (Plate IV). Similarly, the assessment of control group liver showed normal histological structure of the tissue section (Plate V). Liver sections of rats administered J. curcas seed oil in group I and II also showed normal cytoarchitecture (Plate VI and VII) whereas the histoarchitecture of liver of rats in group III revealed mild sign of sinusoidal dilation and oedema (Plate VIII). The pathological change observed in this work suggests that the seed oil interferred with the function and integrity of the kidney and liver of rats treated with high dose of J. curcas seed oil. The observed changes in the histoarchitecture of the kidney and liver are similar to those documented in a previous report by Kumar et al. (2014) after oral dosing of rats with 1mL/kg b.wt of J. curcas seed oil. Similar disorientation of liver and tissue sections were reported by Azuibike et al. (2015) following acute intoxication of mice with 100mg/mL of crude aqueous extract of J. curcas leaves. Phorbol esters exert damage on tissues by the release of proteases, cytokines and activation of NADPH oxidase (Azuibike et al., 2015). They may have acted singly or in combination with other toxic principle earlier mentioned causing the degenerative changes observed in this study.

CONCLUSION

The toxicity level of J. curcas seed oil was tested in this study and found to be low with a LD_{50} of less than 5000mg/kg b.wt. However, it was observed that prolonged use of the oil at high doses affected body weight, blood parameters, enzyme activities, cyto-architecture of the liver and kidney indicating potential harm to health. Therefore, while the seed oil can be used, it is crucial to exercise caution while using it at high doses for an extended period.

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