Acute Toxicity and Hepatocurative Effect of Aqueous Leaf Extract of *Jatropha curcas* in Rats

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**Authors’ contributions**

This work was carried out in collaboration between all authors. 'M. A. Dangambo and H. T. Kabara,' designed the study and performed the lab work. 'M. A. Dangambo and A. J. Alhassan performed the statistical analysis and wrote the first draft of the manuscript. M. S. Sule, M. K. Atiku, A. M. Wudil and J. A. Mashi' managed the analyses of the study. All authors read and approved the final manuscript.

**ABSTRACT**

Acute toxicity and effect of aqueous leaf extract of *Jatropha curcas* (ALEOJC) on carbon tetrachloride (CCl₄) induced hepatotoxicity in albino rats was evaluated. The rats (60) were divided into five groups designated as; I, II, III, IV and V of which groups I and II has 15 rats each while, group III, IV and V have 10 rats each. Groups II to V were induced with hepatotoxicity using 150mg/kg body weight CCl₄ intramuscularly. Serum liver marker enzymes were assayed 48hrs after induction of liver injury followed by 2nd and 4th weeks of treatment with ALEOJC and Livolin. Oral LD₅₀ of ALEOJC was found to be 2,792.85 mg/kg. Serum levels of alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline Phosphatase (ALP) and bilirubin level were significantly (P< 0.05) increased in group II (untreated rats) compared to group I (normal control). Following treatment with ALEOJC (10 mg/kg (Group III) and 1000mg/kg (Group IV)) orally, once daily for 4 weeks, a significant hepatocurative effect was observed as evident by decreased level of the serum liver marker enzymes, total bilirubin and direct bilirubin and increase in serum albumin. The hepatocurative effect of ALEOJC is comparable to the standard drug livolin (Group V receiving livolin at 10mg/kg/day orally for 4 weeks). This finding suggested that ALEOJC may possess hepatocurative effect against CCl₄ –


induced liver damage in rats. The curative effects may be associated with the phytochemical content of the plant.

**Keywords:** CCl4; Jatropha curcas; liver toxicity; marker enzymes.

### 1. INTRODUCTION

Liver is the largest organ in human body weighing 1.5kg, lies below the diaphragm in the thoracic region of the abdomen. The liver is the key organ of metabolism, secretion and excretion which is continuously and widely exposed to xenobiotics, environmental pollutants and chemotherapeutic agents because of its strategic location in the body [1, 2 and 3].

Liver disease is a broad term that covers all the potential problems that may cause liver to fail to perform its designated functions. Usually, more than 75% or three quarters of liver tissue needs to be affected before decrease in function occurs [4]. Liver disease is any condition that causes liver inflammation or tissue damage [5]. High incidence of liver disease causes death among the adult population globally [6] because of the absence of reliable drugs for the treatment of liver diseases in modern medicine [7].

Traditional medicine is considered as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses [8]. Natural medicinal products are increasingly gaining popularity and used worldwide as complementary alternative therapies [9], based on the fact that the raw materials are available naturally and in abundance with an estimated record of $10^{62-63}$ potentially beneficial substances [10]. Among such therapeutic preparations are plant-derived phytomedicines, nutraceuticals and cosmeceuticals [10].

*Jatropha Curcas* is a species of flowering plant in the spurge family, *Euphorbiaceae* that is native to the American tropics, most likely Mexico and Central America [11]. Common names include Barbados Nut, Purging Nut, Physic Nut, or *Jatropha curcas* Linnaeus [12]. It is known as bi ni da zugu/ bi ta da zugu or binda zugu in Hausa [13]. All parts of *J. curcas* have been used in traditional human medicine and for veterinary purposes [14]. Some *Jatropha* species are being sold as raw drugs and aqueous or alcoholic extracts or as tinctures for various applications [15]. *Jatropha Curcas* is a source of several secondary metabolites of medicinal importance. The leaf, fruits, latex and bark contain glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins that exhibit wide ranging medicinal properties [16]. The study is to establish the LD$_{50}$ and hepatocurative effect of *Jatropha carcus* in CCl$_4$ hepatotoxicity rats.

#### 1.1 Experimental Design

The experimental animals (60 rats) weighing 120-140g were purchased from Ahmadu Bello University, Zaria, Nigeria. The animals were kept in cages at an ambient temperature of 25±2°C light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially standard diet. They had free access to water. They were divided into five groups of which group I and II has 15 rats each while, group III, IV and V have 10 rats each. Liver toxicity was induced in group II – V using CCl$_4$ according to Alhassan et al (2009) [17].
Group I: negative control/normal rats.
Group II: No extract Administered
Group III: treated orally with aqueous leaf extract of *Jatropha curcas* at doses of 10mg/Kg
Group IV: treated orally with aqueous leaf extract of *Jatropha curcas* at doses of 1000mg/Kg
Group V: treated orally with livolin at doses of 10mg/Kg

Five animals were removed from group I and II 48 hours after CCl₄ treatment and sacrifice for blood sample to confirm induction of lipid peroxidation and liver damage. Group III and IV are treated orally with the respective doses of aqueous leaf extract of *Jatropha curcas* and group V with livolin for four weeks and five are removed from each group at interval of two weeks.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh leaf of *J. curcas* was collected from Bayero University, Kano-State, Nigeria in the month of July, 2011. Leaf was air dried under shade and powdered. The powder was weighed and soaked in distilled water for 48 hours. The mixture was filtered using whatman No.1 filter paper; the residue was dried and reweighed. The concentration of aqueous leaf extract (filtrate) was determined as the difference in weight/final volume of the solution.

2.2 Methods

- LD₅₀ were determined using the method of Lorke (1983) [18]. In the initial phase, nine rats were divided into three groups of three rats each. They were treated with aqueous leaf extract of *Jatropha curcas* at doses of 10, 100 and 1000mg/Kg orally. The treated rats were monitored for 24 h for mortality and general behaviour. In phase II, five (5) rats were used and grouped into 5 of one rat each. They were treated with aqueous leaf extract of *Jatropha curcas* at doses of 1400, 1800, 2200, 2600 and 3000mg/Kg orally. They were monitored again for 24 hrs. The geographic mean of the least dose that killed rats and the highest dose that did not kill rats was taken as the median lethal dose. i.e.

  \[
  \text{LD}_{50} = \sqrt{\text{min. Conc. Full death} \times \text{max. Conc. no death}}
  \]

- Serum Alanine aminotransferase and Aspartate Aminotransferase was estimated using the method of Reitman and Frankel (1957)[19], Alkaline Phosphatase by Rec (1972)[20], Albumin by the method of Grant (1987)[21], Bilirubin by Sherlock (1951)[22].

2.3 Statistical Analysis

The data was statistically analysed using One-way Analysis of Variance (ANOVA) with P value <0.05 considered extremely significant, a component of GraphPad Instat3 Software (2000) version 3.05 by GraphPad Inc.

3. RESULTS
Table 1 show the phase I and II results for LD$_{50}$ determination of ALEOJC. The LD$_{50}$ was found to be 2,792.85 mg/Kg. Table 2 shows the serum level of liver indices (AST, ALT, ALP, ALB, DB and TB) for group of rats 48 hours after treatment with 150mg/Kg CCl$_4$ (Group II) and compared to that of normal rats (Group I) non-CCl$_4$ treated which is to confirm inducement of hepatotoxicity by the CCl$_4$. Liver indices for groups of hepatotoxicity rats treated with ALEOJC and livolin for two and four weeks are respectively shown in Table 3 and 4. One-way Analysis of Variance (ANOVA) give a P value < 0.0001 (P<0.05) hence considered extremely significant, therefore variation among column means is significantly greater than expected by chance.

**Table 1. Shows the phase I and II results for LD$_{50}$ determination of aqueous leaf extract of *Jatropha curcas* (ALEOJC)**

<table>
<thead>
<tr>
<th>Doses (mg/Kg)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0/3</td>
</tr>
<tr>
<td>100</td>
<td>0/3</td>
</tr>
<tr>
<td>1000</td>
<td>0/3</td>
</tr>
<tr>
<td>Phase II</td>
<td></td>
</tr>
<tr>
<td>1400</td>
<td>0/1</td>
</tr>
<tr>
<td>1800</td>
<td>0/1</td>
</tr>
<tr>
<td>2200</td>
<td>0/1</td>
</tr>
<tr>
<td>2600</td>
<td>0/1</td>
</tr>
<tr>
<td>3000</td>
<td>1/1</td>
</tr>
</tbody>
</table>

LD$_{50}$ (oral) = $\sqrt{\text{min. conc. full death} \times \text{max. conc. no death}}$

LD$_{50}$ (oral) = $\sqrt{3000 \times 2600}$

LD$_{50}$ (oral) = 2,792.85 mg/Kg.

**Table 2. Serum Activity of AST, ALT and ALP in U/l and Serum Levels of ALB in g/dl, Total and Direct Bilirubin in µmol/l for Groups of Rats 48 Hours after Treatment with 150mg/Kg CCL$_4$**

<table>
<thead>
<tr>
<th>Group</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>ALB</th>
<th>DB</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>24.11±1.95</td>
<td>22.80±0.32</td>
<td>114.93±0.84</td>
<td>9.59±0.19</td>
<td>21.84±0.80</td>
<td>12.65±0.46</td>
</tr>
<tr>
<td>II</td>
<td>70.71±0.58</td>
<td>53.32±0.68</td>
<td>395.62±1.19</td>
<td>8.18±0.28</td>
<td>29.20±0.53</td>
<td>19.37±0.29</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD, n=5. Values bearing superscripts in the same column are significantly different.Key: Group I: Normal Control, Group II: CCL$_4$ Treated Rats. ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase and ALP: Alkaline Phosphatase, DB: Direct Bilirubin, TB: Total Bilirubin
Table 3. Serum Activity of AST, ALT and ALP in U/l and Serum Levels of ALB in g/dl, Total and Direct Bilirubin in µmol/l for Groups of Rats Two Weeks after Treatment with Livolin and Different Concentration of ALEOJC

<table>
<thead>
<tr>
<th>Group</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>ALB</th>
<th>DB</th>
<th>TB</th>
</tr>
</thead>
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<td>8.18 ±0.28</td>
<td>29.20 ±0.53</td>
<td>19.37 ±0.29</td>
</tr>
<tr>
<td>III</td>
<td>67.50 ±0.87</td>
<td>39.81 ±1.24</td>
<td>293.08 ±2.44</td>
<td>5.85 ±0.16</td>
<td>24.12 ±0.81</td>
<td>16.60 ±0.66</td>
</tr>
<tr>
<td>IV</td>
<td>47.82 ±1.64</td>
<td>31.06 ±1.12</td>
<td>190.10 ±0.99</td>
<td>6.11 ±0.32</td>
<td>21.76 ±0.98</td>
<td>14.60 ±0.37</td>
</tr>
<tr>
<td>V</td>
<td>78.57 ±2.98</td>
<td>44.16 ±1.53</td>
<td>279.08 ±5.64</td>
<td>5.80 ±0.05</td>
<td>25.65 ±0.73</td>
<td>28.37 ±0.98</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD, n= 5. Values bearing superscripts in the same column are significantly different. Key: Group I: Normal Control, Group II: CCl₄ (Untreated), Group III: CCl₄ + 10mg/kg of ALEOJC, Group IV: CCl₄ + 1000mg/kg of ALEOJC, Group V: CCl₄ + 10mg/kg of Livolin. ALEOJC: Aqueous Leaf Extract of Jatropha Curcas. ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase and ALP: Alkaline Phosphatase, ALB: Albumin, DB: Direct Bilirubin, TB: Total Bilirubin

Table 4. Serum Activity of AST, ALT and ALP in U/l and Serum Levels of ALB in g/dl, Total and Direct Bilirubin in µmol/l for Groups of Rats Four Weeks after Treatment with Livolin and Different Concentration of ALEOJC

<table>
<thead>
<tr>
<th>Group</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>ALB</th>
<th>DB</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25.80 ±0.73</td>
<td>23.89 ±0.91</td>
<td>115.57 ±2.87</td>
<td>9.40 ±0.30</td>
<td>21.08 ±0.41</td>
<td>12.55 ±0.48</td>
</tr>
<tr>
<td>II</td>
<td>99.02 ±2.71</td>
<td>69.03 ±14.47</td>
<td>529.37 ±6.13</td>
<td>5.70 ±0.16</td>
<td>30.87 ±1.52</td>
<td>29.83 ±0.47</td>
</tr>
<tr>
<td>III</td>
<td>37.31 ±0.93</td>
<td>28.82 ±0.73</td>
<td>151.94 ±6.26</td>
<td>8.01 ±0.20</td>
<td>23.79 ±1.78</td>
<td>14.19 ±0.62</td>
</tr>
<tr>
<td>IV</td>
<td>23.96 ±0.93</td>
<td>23.12 ±0.46</td>
<td>122.54 ±2.62</td>
<td>9.14 ±0.31</td>
<td>21.15 ±0.39</td>
<td>12.05 ±1.12</td>
</tr>
<tr>
<td>V</td>
<td>81.45 ±3.07</td>
<td>76.86 ±2.79</td>
<td>462.10 ±5.60</td>
<td>7.95 ±0.16</td>
<td>28.04 ±1.94</td>
<td>22.74 ±0.83</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD, n= 5. Values bearing superscripts in the same column are significantly different. Key: Group I: Normal Control, Group II: CCL₄ (Untreated), Group III: CCL₄ + 10mg/kg of ALEOJC, Group IV: CCL₄ + 1000mg/kg of ALEOJC, Group V: CCL₄ + 10mg/kg of Livolin. ALEOJC: Aqueous Leaf Extract of Jatropha Curcas. ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase and ALP: Alkaline Phosphatase, ALB: Albumin, DB: Direct Bilirubin, TB: Total Bilirubin
4. DISCUSSION

Acute toxicity study reveals oral administration of 3000 mg/Kg (body weight) of the extract produces death in the rats within 24 hours. The LD_{50} of the aqueous leaf extract of *J. curcas* was found to be 2,792.85 mg/Kg. This agrees with the work of Shanti *et al* (2010) [23] who reported that, the oral LD_{50} of methanol extract of *J. curcas* leaf extract of 2500 mg/Kg. The higher LD_{50} value by this research compared to Shanti *et al* (2010) [23] could be due to nature of solvent used that differ in polarity hence the degree of solubility of constituents of the *J. curcas*. The plant extract may be considered as Slightly Toxic according to Hodges and Sterner (2005) [24] scale for toxicity classes.

This research was designed to evaluate the effects of aqueous leaf extracts of *Jatropha curcas* (ALEOJC) on carbon tetrachloride induced albino rat model. The extract at dose of 10 and 1000 mg/Kg body weight (especially four weeks after administration) reduced the serum activity of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP); Serum concentrations of direct and total bilirubin levels in the CCl₄ induced hepato-toxicity rats. The values of serum albumin for groups of rats treated with the extract (Group III and IV) are comparatively higher than that of hepato-toxicity group (Table 4). The reduction in the level of these enzymes and metabolites in the serum of rats is suggestive of the hepatocurative potential of the extract. The mechanism by which the ALEOJC induces its hepatocurative activity might be due to its phytochemical content (flavonoids, alkaloids, tannin, saponins and cardiac glycosides). An additional and important factor in the hepatocurative activity of any drug/plant is the ability of its constituents to inhibit the aromatase activity of cytochrome P₄₅₀, thereby favouring liver regeneration [25,26]. Livolin at 10mg/Kg (body weight) aggravate damage to the hepatocytes as evidenced by biochemical studies. The most likely reason is that, livolin may only be useful as hepatocurative drug in patients that are on low protein and fats diet, a condition normally recommended by clinicians.

5. CONCLUSION

These results suggest that aqueous leaf extract of *Jatropha curcas* possess hepatocurative effect against CCl₄-induced liver damage in rats and it should be used with caution.

ETHICAL APPROVAL

All experiments were carried out with strict compliance to the “principle of laboratory animal care” (NIH Publication No. 85-23) [27] and ethical guidelines for investigation of experimental pain in conscious animals [28].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


