Evaluation of Hepatotoxic effects of Leaves Extract of Cassia italica (Mill.) Lam. ex F.W. Ander (Leguminosae) in Albino Rats

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ABSTRACT: The hepatotoxic effect of aqueous leaves extract of Cassia italica on some liver function parameters was investigated in albino rats. Five groups of the rats were administered the following graded doses of the extract orally: 0, 300, 600, 1500 and 3000 mg/kg bw, for groups 1, 2, 3, 4 and 5 respectively, once daily for 28 days. The serum ALAT, ASAT and ALP levels were found to increase significantly (p<0.05) in all the groups when compared to the control; whereas the serum Albumin levels decreased significantly (p<0.05) in all the groups when compared with the control. There was also a significant increase in Total Bilirubin level (p<0.05) in the groups administered with 3000 and 1500 mg/kg bw, but the reverse was the case in the groups administered with 600 and 300 mg/kg bw of the extract, which showed significant decrease (p<0.05) when compared with the control. These results suggest that, the aqueous leaves extract of Cassia italica has adverse effects on the functional capacities of rat liver.

DOI: https://dx.doi.org/10.4314/jasem.v22i9.28

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Dates: Received: 30 August 2018; Revised: 11 September 2018; Accepted: 30 September 2018

Keywords: Hepatotoxicity, extract, Cassia italic, rats

Educational plants and herbs contain substances known to modern and ancient civilizations for their healing properties. A number of plants have been used in traditional medicine for many years; some do seem to work, although there may not be sufficient scientific data to confirm their efficacy (Sofowara, 1982). The medicinal value of these plants lies in some chemical substances they contain, that produce definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Elsayed et al., 1992).

Herbal knowledge is gathered through trial and error over a period of time. Traditional herbalists detect effective substances by trying them out on themselves or on patients and therefore, toxicity and side effects may be encountered during treatment (Bandiera et al., 1999). Traditional medicines include practices, such as herbal medicine, ayurvedic medicine, unani medicine, acupuncture, as well as other medical knowledge and practices (not orthodox) all over the globe. Inappropriate use of traditional medicines or practices can have negative or dangerous effects and so, further research is needed to ascertain the efficacy and safety of the medicinal plants used in traditional medicine system (WHO, 2003).

MATERIALS AND METHODS

Experimental Animals: The study was carried out with adult albino rats, weighing 180 - 220g of both sexes. The animals were purchased from animal house of the Department of Biological Sciences of Usmanu Danfodiyo University, Sokoto. The animals were provided with standard diet (Vital Feed) and fresh clean water ad libitum. They were allowed to acclimatize to the experimental conditions in cages for one week prior to the commencement of the experiment.

Collection of the Plant Sample: The mature leaves of Cassia italica were collected from the farmland area.
within the main campus of Usmanu Danfodiyo University, Sokoto. The plant was identified by a taxonomist in the herbarium of Botany Unit, Biological Sciences Department, Usmanu Danfodiyo University, Sokoto. Where a voucher specimen was kept for record purposes.

Preparation of Aqueous Leaves Extract: The leaves of the plant were shed dried at room temperature and then pulverized to coarse powder. 400g of the powdered sample were dissolved in about 4 litres of water and left for 24 hours at room temperature. The mixture was filtered using muslin cloth after 24 hours to obtain the filtrate. The filtrate was evaporated in a drying cabinet at 45°C to obtain a concentrated extract. The concentrated extract was reconstituted in distilled water to obtain a known concentration of the extract. The animals were weighed prior to the identification. The animals were weighed prior to the administration of the extract. One animal serves as the control and the four others were administered 3000mg/kg body weight of the extract, orally in a single dose using intubation canula. Each animal was observed at the interval of one hour for the first four hours after dosing for any symptom of toxicity and subsequently for next 48 hours. The number of survivors was noted after 48 hours. The result was recorded on the basis of mortality and expressed as LD50. The animals were reweighed to determine change in weight over the 48 hours period.

Acute Toxicity Studies (Determination of LD50): The acute toxicity test was carried out based on OECD guideline (OECD, 2001). Five rats were randomly selected and marked to permit individual identification. The animals were weighed prior to the administration of the extract. One animal serves as the control and the four others were administered 3000mg/kg body weight of the extract, orally in a single dose using intubation canula. Each animal was observed at the interval of one hour for the first four hours after dosing for any symptom of toxicity and subsequently for next 48 hours. The number of survivors was noted after 48 hours. The result was recorded on the basis of mortality and expressed as LD50. The animals were reweighed to determine change in weight over the 48 hours period.

Sub-chronic Toxicity Test: Repeated toxicity study was carried out according to OECD guideline (OECD, 2001). The animals were divided into five groups of five rats each. Group 1 served as control, receiving only 1 ml of distilled water daily. Groups 2, 3, 4 and 5 were respectively administered 300, 600, 1500, and 3000 mg/kg body weight of the extract, representing 10, 20, 50 and 100% of the LD50, respectively. The extract was administered orally, daily for 28 days. The body weights of the animals were evaluated a day before the start of administration and then weekly for four weeks.

Collection of Blood Samples: On the 29th day, the animals were sacrificed after overnight fast and the blood samples were collected into labeled centrifuge tubes and allowed to stand for 10 minutes before being subjected to centrifugation at 4000rpm for 15 min. The serum was separated from the whole blood using Pasteur pipette and transferred to labeled dry clean serum tubes and then kept refrigerated.

Liver Function Tests: Total Protein was determined by Biuret Method (Hiller, 1926), based on reaction of Cupric ions in an alkaline medium, which interacts with protein peptide bonds resulting in the formation of a blue-violet complex (the so-called biuret reaction). Albumin was determined by Bromocresol Green method, measured based on its quantitative binding to the indicator 3,3,5,5-tetramethoxy-m cresol sulphonephthalein (bromocresol green, BCG). Aspartate Aminotransferase (AST) was determined by monitoring the concentration of oxaloacetate hydrazone formed from 2,4-dinitrophenylhydrazine (Reitman and Frankel, 1957). Alanine Aminotransferase (ALT) was determined by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine (Reitman and Frankel, 1957). Alkaline Phosphatase (ALP) was measured based on p-nitrophenyl phosphate, which is hydrolyzed to p-nitrophenol and inorganic phosphate by alkaline phosphatase at pH 10.4 (Kind and King, 1954).

Statistical Analysis: The results were analyzed using Analysis of Variance (ANOVA) with INstat3 software (SanDiago, USA). Values were expressed as mean ± standard deviation (SD). Differences in mean (±SD) were considered significant at p<0.05.

RESULTS AND DISCUSSION
The results of the studies were presented in tables 1-3. All the rats survived after 48 hours of administration of 3000mg/kg body weight of the extract (Table 1). The result revealed no observable gross negative effects such as salivation, diarrhea, coma or convulsion in the rats administered the various doses of the extract. There were no changes on the skin colour or eyes, hair removal was not noticed either. All the rats had normal tail (flexible), granular excrement and would isolate themselves at the corners of the cage after dosing. There was no observable depressive effect on the central nervous system and mortality was not recorded. It could be concluded, therefore, that the LD50 for the extract is greater than 3000 mg/kg body weight.

There was a significant (p<0.05) decrease in the body weight of all the groups treated with the extract when compared with their initial weights before treatment. The rats in the control group gained weight when compared with their initial weights (Table 2). This suggests that the extract had adversely affected the weights of the treated rats.
There was a significant (P<0.05) increase in ALAT in the rats administered 3000 mg/kg bw and 1500 mg/kg bw when compared with the control (Table 3). However, a significant (P<0.05) decrease in ALAT was observed in the rats administered 600 mg/kg bw and 300 mg/kg bw of the extract, suggesting that there was no adverse effect on the liver at these concentrations. The dose-dependent elevations observed in the serum enzymes in the groups administered 3000 mg/kg bw and 1500 mg/kg bw indicates possible hepatic cell damage (Sigma Diagnostic, 1985). There were significant (P<0.05) increases in ASAT and ALP when compared with the control in all the treated groups (Table 3). Increase in the activity of these enzymes in the plasma is often seen following liver damage and it is attributable to the loss of the enzyme from damaged hepatocytes rather than increased production (Tilkian, 1979). This also suggests that other non-specific tissue damage could have occurred as these enzymes have a wider tissue distribution beyond the liver (Boyd, 1988). ALAT and ASAT are liver specific enzyme markers of necrotic injury and cholestasis (Lott and Wolt, 1986). Total bilirubin significantly (P<0.05) increased in animals administered 3000 mg/kg bw and 1500 mg/kg bw respectively, but significantly (P<0.05) decreased in animals administered 600 mg/kg bw and 300 mg/kg bw of the extract (Table 3). The significant increase in total bilirubin in animals administered 3000 mg/kg bw and 1500 mg/kg bw suggests that the extract had adversely affected the excretory function of the liver. However, the decrease in bilirubin levels in animals administered 600 mg/kg bw and 300 mg/kg bw of the extract may be attributed to the depressant effect of the extract (Odutola, 1992).

Albumin significantly (P<0.05) decreased in all the treated groups when compared with the control (Table 3). Significant decrease in albumin in all the treated groups suggests that both the excretory and synthetic functions of the liver were altered as these are markers of liver excretory and synthetic functions (Weiss et al., 1983).

Table 1: The effect of administration of acute dose (3000 mg/kg bw) of aqueous leaves extract of *Cassia italica* on the behavior of albino rats.

<table>
<thead>
<tr>
<th>Animals</th>
<th>0 - 5min</th>
<th>6 - 30min</th>
<th>31 - 59min</th>
<th>1 – 48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat -1</td>
<td>Restlessness</td>
<td>Mouth scratch</td>
<td>Calm</td>
<td>Normal</td>
</tr>
<tr>
<td>Rat -2</td>
<td>Restlessness</td>
<td>Mouth scratch</td>
<td>Weak</td>
<td>Normal</td>
</tr>
<tr>
<td>Rat -3</td>
<td>Restlessness</td>
<td>Mouth scratch</td>
<td>Weak</td>
<td>Normal</td>
</tr>
<tr>
<td>Rat -4</td>
<td>Restlessness</td>
<td>Mouth scratch</td>
<td>Weak</td>
<td>Normal</td>
</tr>
<tr>
<td>Rat -5</td>
<td>Restlessness</td>
<td>Mouth scratch</td>
<td>Sleep</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± standard deviation (n=5).

Table 2: The effect of four weeks administration of graded doses of aqueous leaves extract of *Cassia italica* on total weight of albino rats.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Initial weight</th>
<th>Week1</th>
<th>Week2</th>
<th>Week3</th>
<th>Week4</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>153.4 ± 19.45</td>
<td>137.2 ± 19.40</td>
<td>139.9 ± 22.35</td>
<td>159.2 ± 22.60</td>
<td>155.2 ± 23.83</td>
</tr>
<tr>
<td>100</td>
<td>153.2 ± 2.39</td>
<td>152.0 ± 15.52</td>
<td>153.4 ± 16.33</td>
<td>148.6 ± 15.37</td>
<td>135.4 ± 13.83</td>
</tr>
<tr>
<td>1500</td>
<td>179.2 ± 3.42</td>
<td>171.2 ± 7.92</td>
<td>169.4 ± 5.94</td>
<td>165.2 ± 0.86</td>
<td>165.4 ± 7.23</td>
</tr>
<tr>
<td>300</td>
<td>163.0 ± 1.58</td>
<td>153.4 ± 2.88</td>
<td>156.8 ± 6.58</td>
<td>154.6 ± 2.67</td>
<td>157 ± 8.27</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation (n=5).

Table 3: The effect of administration of graded doses of aqueous leaves extract of *Cassia italica* on liver function indices of albino rats.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>ALAT (U/L)</th>
<th>ASAT (U/L)</th>
<th>ALP (U/L)</th>
<th>Albumin (g/dl)</th>
<th>Total bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>50.22 ± 2.83</td>
<td>119.61 ± 1.42</td>
<td>138.64 ± 2.71</td>
<td>3.46 ± 0.31</td>
<td>1.04 ± 0.07</td>
</tr>
<tr>
<td>3000</td>
<td>72.92 ± 2.92</td>
<td>201.80 ± 2.67</td>
<td>197.5 ± 1.00</td>
<td>3.49 ± 0.45</td>
<td>1.07 ± 0.29</td>
</tr>
<tr>
<td>1500</td>
<td>59.12 ± 1.14</td>
<td>185.95 ± 5.12</td>
<td>178.13 ± 2.41</td>
<td>3.39 ± 0.43</td>
<td>1.02 ± 0.05</td>
</tr>
<tr>
<td>600</td>
<td>52.82 ± 3.32</td>
<td>168.12 ± 1.18</td>
<td>152.18 ± 2.56</td>
<td>2.64 ± 0.38</td>
<td>0.73 ± 0.04</td>
</tr>
<tr>
<td>300</td>
<td>44.52 ± 4.16</td>
<td>153.73 ± 3.54</td>
<td>133.77 ± 1.68</td>
<td>2.03 ± 0.43</td>
<td>0.67 ± 0.08</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation (n=5). * = Significantly different from control (P<0.05). ALAT = Alanine Aminotransferase. ASAT = Aspartate Aminotransferase. ALP = Alkaline Phosphatase.

Conclusion: In the light of the foregoing, it is evident that the aqueous leaves extract of *Cassia italica* had adverse effects on the liver function in the treated rats, which were however, dose dependent. The liver function was impaired at higher doses while at therapeutic dose the leaves extracts of *Cassia italica* could be used to manage constipation, oedema and skin infections with an acceptable margin of safety.

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