Acute And Subacute Toxicity Of Aqueous Extract Of Abrus Precatorius Seed In Wister Rats

R Sunday, O Ilesanmi, E Obuotor

Abstract

In this study, acute toxicity of aqueous extract of Abrus precatorius seed was carried out in mice and Wister rats by oral (p.o.) and intraperitoneal route (i.p.) while subacute toxicity was evaluated in Wister rats (i.p.). The LD₅₀ of the extract in mice was greater than 5000 mg/kg (p.o.) and 0.71 mg/kg (i.p.) while in rats it was 316.20 mg/kg (p.o.) and 0.35 mg/kg (i.p.). In subacute toxicity studies, the extract elicited a significant (p<0.05) decrease in body weight, red blood cell count, lymphocyte count, feed and water intake, and significant (p<0.05) increase in white blood cell count and eosinophil count. Biochemical investigation showed a significant (p<0.05) change in serum and liver cholesterol, alkaline phosphate, alanine transaminase, aspartate transaminase and albumin. The study concluded that the aqueous extract of A. precatorius seed could possess moderate toxicity and adequate caution should be exercised in its use in ethnomedicine.

INTRODUCTION

A. precatorius plant is a slender, annual plant that has a small, high climbing tropical vine that twines around trees, shrubs and hedges (Frohne and Pfander, 1983). It grows in tropical climates such as West Africa, India, Sri Lanka, Thailand, the Philippine Islands, South China and North America. The seeds are commonly known as Rosary pea, crab's eye (Fosberget al., 1979) and Ojuologbo in southwestern Nigeria. The seeds are used as anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, emollient, febrifuge, laxative, purgative, refrigerant, sedative, vermifuge and are also considered as abortifacient (Nath et al., 1992). Various African tribes use powdered seeds as oral contraceptives. In China the herb of A.precatorius is used as a folk-medicine for the treatment of bronchitis, laryngitis and hepatitis because of their platelet inhibiting activity (Kuo et al., 1995)

The present study was carried out to evaluate the potential toxicity of aqueous extract of A. precatorius seed due to its wide spread use in ethnomedicine.

MATERIALS AND METHODS

The animal experiments were performed according to the approved guidelines of the Obafemi Awolowo University research ethics committee.

PLANT COLLECTION AND EXTRACTION

Dry seeds of A. precatorius plant, were obtained from a local market in Ile-Ife (Osun state) and were authenticated by Mr. G. Ibhanesebhor of Ife-Herbarium, Department of Botany, Obafemi Awolowo University, Nigeria. A voucher specimen (No. 16282) was deposited at Ile-Herbarium. 1.0 kg of dried seeds of A. precatorius were ground into powder soaked in distilled water overnight and then filtered using muslin cloth and cotton wool in funnel. The filtrate was then concentrated in vacuo to give a residue (yield, 27.7% w/w).

ANIMALS

Thirty-six albino mice of both sexes weighing between 20-24g and fifty-six Wister rats weighing between 150-190g were obtained from Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. They were kept in well ventilated polypropylene cages with steel grid floors and were given standard feed (produced by Ola Dokun, Ibadan, Nigeria) and water ad libitum. They were allowed to acclimatize with the environment at ambient temperature under natural day light/night conditions for two weeks before the start of the experiment.

CHEMICALS

Assay kits for the estimation of serum alanine aminotransferase, aspartate aminotransferase, albumin,
cholesterol and bilirubin were purchased from Rando Laboratories Limited, U.K. All other chemicals were of analytical grade.

**ACUTE TOXICITY TESTING**

Acute toxicity studies were carried out using the method of Lorke, 1983. In the first phase, nine mice randomly divided into three groups of three mice each were given 10, 100 and 1000 mg extract/kg body weight p.o (via a cannula). The mice were observed for signs of adverse effects which include but not limited to paw-licking, salivation, stretching, rubbing of nose on the floor and wall of cage, change in body weight and death for 24 h and then weighed daily for 14 days. The surviving animals were sacrificed under chloroform anesthesia, autopsied and examined macroscopically for any pathological changes. The procedure was repeated using another set of nine mice, based on the results of the phase one study higher doses (1600, 2900 and 5000 mg extract/kg body weight p.o) was administered in the second phase. The number of deaths in each group within 24 h was recorded and the final LD$_{50}$ values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred). The procedure for first phase above was repeated in mice using i.p and in the second phase lower doses (0.25, 0.50 and 1.0 mg extract/kg body weight i.p) were administered. This method was repeated using rats through oral route (lower doses 150, 200 and 500 mg extract/kg body weight p.o were administered in the second phase) and intraperitoneal route (lower doses 0.25, 0.50 and 1 mg extract/kg body weight i.p. were administered in the second phase).

**SUBACUTE TOXICITY TESTING**

Twenty Wister rats of either sex were divided into four groups of five rats each. Group one, which served as the control received distilled water (10 ml distilled water/kg body weight (i.p.), while rats in groups two, three and four were given 0.05, 0.10 and 0.20 mg extract/kg body weight (i.p.) respectively daily for 14 days. All the rats had food and water ad libitum for 14 days and they were observed daily for general symptoms of toxicity. The weight of feed and volume of water consumed by rats in each group were measured daily. Rats in all the groups were weighed twice every week during the period of treatment and on the last day of study. Doses of the extract administered were adjusted accordingly. On the 15$^{th}$ day of the experiment, the rats that survived (one at a time) were euthanized in an air tight glass chamber saturated with chloroform, they were opened up surgically and blood samples were collected by cardiac puncture. One portion was collected into potassium + ethylenediaminetetraacetic acid (K + EDTA) bottles for estimation of Packed Cell Volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC) and differential white blood cell count (lymphocyte, monocyte, eosinophil, basophil and neutrophil). Another portion was dispensed into plain vials, allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, stored at -4°C and used for evaluation of biochemical assays.

**HAEMATOLOGY**

Packed Cell Volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC) and differential white blood cell count (lymphocyte, monocyte, eosinophil, basophil and neutrophil) were determined using the method of Barham and Trinder, 1972.

**BIOCHEMICAL ANALYSIS**

Rat serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) were determined by the enzymatic colorimetric methods of Reitman and Frankel, 1957 and Schmidt and Schmidt, 1963, alkaline phosphatase (ALP) was determined by the enzymatic colorimetric methods of Rec, 1972 and Englehardt, 1970, total bilirubin and direct bilirubin were determined by the enzymatic colorimetric methods Jendrassik, 1938 and Sherlock, 1951, total albumin was determined by the enzymatic colorimetric methods of Grant et al., 1987 and Doumas et al., 1971 and total cholesterol levels was determined by the enzymatic colorimetric methods of Abbel et al., 1952, Richmond, 1973, Roeschlau et al., 1974 and Trinder, 1969 using commercially available kits (Randox, U.K).

**STATISTICAL ANALYSIS**

All quantitative data were expressed as the mean ± standard error of mean (SEM). Statistical analysis was carried out using one way analysis of variance (ANOVA) and significant difference between means was assessed by Bonferroni t-test at 95% level of significance using Primer (version 3.01).

**RESULTS**

In the acute toxicity study, the median lethal dose (LD$_{50}$) of the extract was greater than 5000 mg/kg (p.o.) and 0.71 mg/kg (i.p.) in mice while in rats it was 316.2 mg/kg (p.o.) and 0.35 mg/kg (i.p.).
EFFECT OF THE EXTRACT ON DAILY FEED INTAKE
At 0.10 and 0.20 mg/kg aqueous extract of A. precatorius seed elicited a significant (P < 0.05) decrease in feed intake in week 1 and week 2 when compared to the control (Figure 1).

Figure 1
Figure 1:

EFFECT OF THE EXTRACT ON DAILY WATER INTAKE
At 0.05, 0.10 and 0.20 mg/kg, A. precatorius seed extract elicited a significant (P < 0.05) decrease in water intake in week 1 and week 2 when compared to the control (Figure 2).

Figure 2
Figure 2:

EFFECT OF THE EXTRACT ON BODY WEIGHT
At 0.20 mg/kg, the extract elicited no significant (P < 0.05) decrease in body weight in week 1 but there was a significant decrease in body weight of the rats in week 2 when compared to that of the control (Figure 3).

Figure 3
Figure 3:

EFFECT OF AQUEOUS SEED EXTRACT OF A. PRECATORIUS ON HAEMATOLOGICAL PARAMETERS
The group of rats treated with 0.05 mg/kg of extract exerted no significant (P < 0.05) change in haematological parameters when compared to that of the control. At 0.10 mg/kg there was a significant (P < 0.05) decrease in red blood cells and a significant increase in white blood cells when compared to that of the control while at 0.20 mg/kg there was a significant (P < 0.05) decrease in red blood cells, lymphocyte and eosinophil count, a significant increase in white blood cell when compared to that of the control (Table 1).
**Acute And Subacute Toxicity Of Aqueous Extract Of Abrus Precatorius Seed In Wister Rats**

**Figure 4**

Table 1: Effect of Seed Extract on Haematological Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>0.05 mg/kg</th>
<th>0.1 mg/kg</th>
<th>0.2 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>37.40 ± 2.23</td>
<td>34.10 ± 1.44</td>
<td>31.50 ± 3.20</td>
<td>36.17 ± 2.73</td>
</tr>
<tr>
<td>HAEM (%)</td>
<td>12.38 ± 0.37</td>
<td>12.98 ± 0.61</td>
<td>11.94 ± 1.13</td>
<td>9.71 ± 2.12</td>
</tr>
<tr>
<td>RBC</td>
<td>11.19 ± 1.65</td>
<td>8.01 ± 0.42</td>
<td>5.65 ± 1.44*</td>
<td>5.00 ± 2.11*</td>
</tr>
<tr>
<td>WBC</td>
<td>73.80 ± 9.64</td>
<td>70.80 ± 24.64</td>
<td>39.38 ± 23.2</td>
<td>38.33 ± 21.19</td>
</tr>
<tr>
<td>NEUT (%)</td>
<td>20.90 ± 8.28</td>
<td>19.50 ± 2.19</td>
<td>15.80 ± 1.72</td>
<td>18.3 ± 2.13*</td>
</tr>
<tr>
<td>EOS (%)</td>
<td>4.50 ± 1.02</td>
<td>9.30 ± 1.72</td>
<td>8.13 ± 1.84</td>
<td>16.83 ± 2.13</td>
</tr>
<tr>
<td>LMP (%)</td>
<td>67.10 ± 4.66</td>
<td>55.40 ± 5.71</td>
<td>46.63 ± 5.43</td>
<td>28.0 ± 3.51*</td>
</tr>
<tr>
<td>MNC (%)</td>
<td>12.50 ± 2.27</td>
<td>14.80 ± 2.74</td>
<td>14.75 ± 1.76</td>
<td>15.0 ± 2.02</td>
</tr>
<tr>
<td>BAS (%)</td>
<td>1.30 ± 0.25</td>
<td>1.20 ± 0.25</td>
<td>1.38 ± 0.31</td>
<td>1.83 ± 0.44</td>
</tr>
</tbody>
</table>

٭Significantly different from control at p < 0.05

Values are mean ± SEM; n = 5

Effect of aqueous seed extract of A. precatorius on biochemical parameters

>A. precatorius seed extract at 0.05 mg/kg elicited a significant (P < 0.05) increase in serum alanine transaminase (ALT) and alkaline phosphatase (ALP). At 0.10 mg/kg and 0.20 mg/kg there was a significant increase in aspartate transaminase (AST), ALT and ALP and a significant decrease in albumin when compared to that of the control (Table 2).

**Figure 5**

Table 2: Effect of Seed Extract on Biochemical Parameters in Serum of Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>0.05 mg/kg</th>
<th>0.1 mg/kg</th>
<th>0.2 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (40 µL/L)</td>
<td>50.00 ± 0.71</td>
<td>61.80 ± 0.58*</td>
<td>66.73 ± 1.03*</td>
<td>71.02 ± 1.05*</td>
</tr>
<tr>
<td>AST (40 µL/L)</td>
<td>53.60 ± 0.51</td>
<td>43.80 ± 1.59*</td>
<td>49.75 ± 1.11*</td>
<td>58.81 ± 1.30*</td>
</tr>
<tr>
<td>ALP (14 µL/L)</td>
<td>71.00 ± 18.28</td>
<td>167.20 ± 35.72</td>
<td>275.75 ± 13.55*</td>
<td>271.21 ± 10.02*</td>
</tr>
<tr>
<td>DLR (mg/dL)</td>
<td>1.20 ± 0.21</td>
<td>1.44 ± 0.36</td>
<td>1.54 ± 0.56</td>
<td>1.57 ± 0.62</td>
</tr>
<tr>
<td>TOT (mg/dL)</td>
<td>4.46 ± 1.18</td>
<td>2.65 ± 0.76</td>
<td>2.86 ± 0.35</td>
<td>2.90 ± 0.43</td>
</tr>
<tr>
<td>CHOL (mg/dL)</td>
<td>108.19 ± 13.89</td>
<td>118.21 ± 30.40</td>
<td>30.16 ± 8.89</td>
<td>48.25 ± 2.00</td>
</tr>
<tr>
<td>ALB (g/dL)</td>
<td>3.71 ± 0.48</td>
<td>2.53 ± 0.51</td>
<td>1.71 ± 0.33*</td>
<td>1.51 ± 0.51*</td>
</tr>
</tbody>
</table>

٭Significantly different from control at p < 0.05

Values are mean ± SEM; n = 5

**DISCUSSION**

In acute toxicity studies, the LD$_{50}$ of the seed extract in mice was greater than 5000 mg/kg (p.o.) and 0.71 mg/kg (i.p.) while in rats it was 316.2 mg/kg (p.o.) and 0.35 mg/kg (i.p.), this indicates that acutely, the aqueous extract of A. precatorius seed is highly toxic in rodent model through intraperitoneal route and the toxicity is very low through oral route. In subacute toxicity studies, there was a significant decrease in body weight. This decrease in body weight may be due to decrease in feed and water intake and toxic effect of the extract resulting in muscle wastage as reported by Morton and Griffiths, 1985. There was also a significant decrease in red blood cells (RBC) and lymphocytes and a significant increase in white blood cells and eosinophils. An increase in eosinophil may be due to adrenosteroid production (Pagana et al., 1997).

The extract also caused a dose dependent significant increase in serum ALP, ALT and AST and a significant decrease in serum albumin. Significant increases in these liver enzymes may signify that there was a leakage of these enzymes from the liver due to liver damage (Nyblom et al., 2004).

**CONCLUSION**

In conclusion, the present result showed that the aqueous extract of A. precatorius seed is moderately toxic in our rat model hence; adequate caution should be exercised in its use in traditional medicine practice.

**References**

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