ACUTE AND SHORT TERM TOXICITY STUDIES ON THE AQUEOUS EXTRACT OF ANCHOMANES DIFFORMIS

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ABSTRACT

Anchomanes difformis (Blume) Engler (Araceae) is widely used in the West African sub-region for the treatment of various diseases such as diabetes, ulcers, tuberculosis, malaria and as a diuretic. The aqueous extract of the rhizome of Anchomanes difformis was evaluated for its oral acute and short term (28 days) toxicity in rats. For acute toxicity studies, the extract was administered orally in graded doses of 0.5 - 10 g/kg to the rats and mice. For subacute toxicity studies, different doses of A. difformis (0.5, 1.0 and 2.5 g/kg) were administered orally to the rats once daily for 28 days. Animals were sacrificed and toxicity was evaluated using morphological (body and organ weight changes), haematological, biochemical and histopathologic parameters. No mortality was observed up to 10 g/kg of the extract in acute toxicity study. Daily administration of as high as 2.5 g/kg dose of the extract did not result in any mortality or changes in absolute or relative organ weights of the various groups. However, there was a dose-dependent increase in the body weight of the extract treated animals compared to control. A significant decrease (p<0.05) in platelet count was observed in the treated animals at all doses, while the highest dose of the extract caused a significant reduction (p<0.05) in the haemoglobin and haematocrit levels. All other blood parameters remained within normal limits. Biochemical analysis showed no significant differences in the parameters examined, except for an increase in AST at the highest dose of the extract. This same dose also caused major pathologic lesions in the various organs examined. In conclusion, acute and subacute administration of Anchomanes difformis aqueous extract did not produce toxic effects in rats. However, the decreased platelet count coupled with the increase in serum level of AST as well as the lesions in different organs may indicate a possible toxic effect at very high doses.

Key words: Anchomanes difformis; subacute toxicity; histopathology, haematology

INTRODUCTION

Anchomanes difformis (Blume) Engler (Araceae), also known as forest anchomanes in English (Morton, 1961) is a self-supporting rhizomatous herb with a long stem that can reach two meters in height. The stem is green in colour with a diffuse white colour at the base close to the soil level (Dalziel, 1937). The plant grows in tropical African forests in moist and shady places. The tuber called “Isu igo”, in
the South West of Nigeria, is used to prepare a decoction used in cough treatment and in ulcer. The peeled tuber soaked in water is used in treating cases of dysentery while the tuber powder given with honey is used as diuretic and purgative to treat diabetes, oral and anal lesions, tuberculosis and malaria (Gills, 1992; Adjanohoun et al., 1988, 1989; Kerharo and Adam, 1974).

Laboratory studies have confirmed that the rhizomes of *Anchomanes difformis* possess significant analgesic, anti-inflammatory and antipyretic (Akah and Njike, 1990), antiulcer (Okpo et al., 2012), trypanocidal (Atawodi et al., 2003), antimicrobial (Chukwura and Ajali, 2000; Oyetayo, 2007; Adeleke and Adetunji, 2010) effects. Adegoke et al. (1968) had reported the presence of strong alkaloids and saponins in *Anchomanes difformis*, while Oyetayo (2007) found tannins in addition to alkaloids and saponins.

The lyophilized rhizome of *A. difformis* had been found to be very toxic to guinea pigs but not to mice (Tchiakpe et al., 1996) while the crude stem extract exhibited high contact toxicity to adult beetles, *Callosobruchus masculatus*, thereby providing good protection to stored grains (Akinkurolere et al., 2006).

Based on the known wide usage of this plant for treatment of various ailments, in traditional medicine, it is necessary to evaluate its toxicity potential in order to caution or encourage its use. Although much work has been done on the pharmacological properties and chemical compositions of this plant, only limited data are available in the literature on its toxicity to warrant its safe use. The present study was therefore undertaken to evaluate the acute and short term toxic effects of the aqueous extract of the *Anchomanes difformis*.

**MATERIALS AND METHODS**

**Plant collection and extraction**

Fresh rhizomes of *Anchomanes difformis* were collected in the month of October from Ifon in Owan Local Government Area, Edo State, Nigeria. The plant material was identified and authenticated by Mr. S. Olufemi of the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria where a voucher specimen (No. FHI 109585) has been deposited for future reference.

**Plant preparation**

The rhizomes were washed, peeled and sliced thinly to aid drying. The sliced rhizomes were oven-dried for one week, at 60°C after which they were blended into powdery form. Extraction was performed by macerating 500g of the sample in 2.5 litres of water/methanol mixture (3:1) for 48 hours. The resultant mixture was filtered and concentrated into a semi-solid form using a rotary evaporator. Stock solution of the extract (500mg/ml) was prepared from which other concentrations were made as required.

**Animals**

Sprague-Dawley rats (150±20g) and albino mice of both sexes were obtained from the Laboratory Animal House of the Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. The animals maintained under standard diet (Bendel Feeds Ltd. Ewu, Edo State, Nigeria) and water *ad libitum*, were acclimatized for two weeks and fasted overnight, with free access to water prior to experiments. Animals were handled according to standard protocols as outlined in “Principles of Laboratory Animal Care” (National Institute of Health Guide for Care and Use of Laboratory Animals, Pub No. 85 – 23, revised 1985).
Acute toxicity Study

A total of 30 rats and 30 mice were randomly allotted to the different control and test groups with 5 animals in each group. Five doses of the aqueous extract (0.5, 1, 2, 5 and 10mg/kg body weight) were administered by oral intubation and the control group received distilled water (5ml/kg).

Another batch of 30 mice was divided into six groups of 5 animals each, with one group serving as the control. The mice were subjected to the same conditions as above. The extract was administered intraperitoneally. Five groups of animals received 0.5, 1, 2, 4 and 8mg/kg body weight of extract, respectively, while the control group received distilled water.

The general symptoms of toxicity and mortality were recorded for 24 hours and a further 2 weeks for any signs of delayed toxicity. The median lethal dose (LD$_{50}$) was determined using the method of Litchfield and Wilcoxon (1949).

Sub-acute toxicity Study

Rats were allotted to four groups of 7 rats each. Three of the groups were given, by oral intubation, 0.5, 1 and 2.5g/kg body weight of the extract, respectively, while the control group received distilled water only, through the oral route. The animals were maintained under standard laboratory conditions including 12h light /dark cycles and had free access to food (Bendel Feeds, Plc Ltd, Ewu, Nigeria) and tap water during the study period of 28 days.

The body weight of each rat was recorded at weekly intervals and behavioral changes and any external general symptoms of toxicity were noted.

On the 28$^{th}$ day, blood was collected, under light ether anaesthesia, via the posterior iliac artery for haematological and biochemical assays. Haematological parameters like red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), haematocrit (HCT), platelet count (PLT), lymphocytes (LYM) and neutrophils (NEUT) were determined by standard laboratory procedures (Dacie and Lewis, 1991; Ghai, 1995; John, 1972). Biochemical parameters, including serum alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) were also determined (King, 1965a and b; Schumann et al., 2002; Doumas et al., 1981; Doumas et al., 1971). Animals were then sacrificed by excess ether inhalation and the liver, kidney, heart and spleen were isolated. Relative wet weights of the respective organs were calculated based on the body weight of the animal. Each organ was examined macroscopically for colour changes or any obvious lesions and sections were fixed in 10% buffered formalin for histopathological studies.

Statistical analysis

Data are presented as mean ± SEM (standard error of the mean) and n represents the number of rats used for a particular experiment. Comparisons were made between treated and control groups using one-way analysis of variance (ANOVA) followed by Dunnett’s test and significance of difference was accepted at p<0.05.

RESULTS

Acute toxicity study

In the acute toxicity test, the extract given by the oral route showed no visible signs of toxicity. No deaths were recorded up to the highest dose of 10mg/kg in both rats and mice. The animals kept under observation for two weeks showed no obvious toxic symptoms; neither food nor water intake was found to be reduced during this period. Administration of the extract via the intraperitoneal route caused
sedation, increased respiratory rate and writhing in the animals, but these effects resolved within one hour. However, no deaths were recorded at the lowest dose of the extract and the LD$_{50}$ was estimated as 1.78g/kg.

**Subacute toxicity study**

There was progressive increase in body weight from day 1 to day 28 in all the groups. The highest changes in the body weights were observed in the extract treated groups with 2.5mg/kg dose showing up to 15% increase in body weight on the 28$^{th}$ day, which was significantly different from control value. The weight change was observed to be the least with the lowest dose of extract (Figure 1). The relative weights of the liver, kidney, heart and spleen of the extract-treated rats were not altered compared to the control group (Table 1).

![Figure 1: Effect of A. difformis on body weight changes in rats](image)

Values are expressed as mean ± S.E.M. ($n = 6$-$7$ animals/group), *p<0.05 vs. control.

**Table 1: Effect of A. difformis on absolute (g) and relative organ weights of rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (g/kg)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>-</td>
<td>6.37±0.24 (4.07±0.37)</td>
<td>1.36±0.08 (0.83±0.07)</td>
<td>0.59±0.04 (0.35±0.02)</td>
<td>0.73±0.05 (0.44±0.02)</td>
</tr>
<tr>
<td><strong>A. difformis</strong></td>
<td>0.5</td>
<td>7.05±0.04 (3.95±0.27)</td>
<td>1.29±0.05 (0.72±0.03)</td>
<td>0.58±0.03 (0.33±0.02)</td>
<td>0.84±0.06 (0.47±0.03)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7.29±0.75 (3.65±0.24)</td>
<td>1.37±0.06 (0.71±0.06)</td>
<td>0.64±0.03 (0.33±0.01)</td>
<td>1.07±0.07 (0.54±0.01)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>7.40±0.62 (3.79±0.16)</td>
<td>1.40±0.09 (0.73±0.05)</td>
<td>0.61±0.06 (0.31±0.03)</td>
<td>0.85±0.08 (0.44±0.04)</td>
</tr>
</tbody>
</table>
Values are mean ± SEM, (n= 6-7 animals). Figures in parentheses indicate relative weight (as % of body weight).

The effect of the extract on haematological indices is shown on Table 2. All doses of the extract significantly (p<0.05) decreased the haematocrit level while only the highest dose significantly reduced haemoglobin levels and platelet counts when compared to the control. All the other parameters remained within normal limits.

### Table 2: Effect of *A. difformis* on haematological indices

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>WBC (x 10^3/μL)</th>
<th>RBC (x10^3/μL)</th>
<th>HGB (g/dL)</th>
<th>HCT (%)</th>
<th>PLT (x 10^3/μL)</th>
<th>LYM (%)</th>
<th>NEUT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>10.45±1.58</td>
<td>7.09±0.32</td>
<td>13.09±0.44</td>
<td>42.50±1.17</td>
<td>747.13±36.99</td>
<td>76.04±2.06</td>
</tr>
<tr>
<td><em>A. difformis</em> 0.5</td>
<td>9.75±1.51</td>
<td>6.46±0.37</td>
<td>11.99±0.37</td>
<td>37.95±1.93*</td>
<td>777.00±51.04</td>
<td>70.60±5.49</td>
<td>23.30±3.45</td>
</tr>
<tr>
<td>1</td>
<td>7.78±2.15</td>
<td>6.75±0.22</td>
<td>12.02±0.36</td>
<td>38.47±1.39*</td>
<td>779.83±71.27</td>
<td>77.88±1.88</td>
<td>19.72±2.08</td>
</tr>
<tr>
<td>2.5</td>
<td>6.80±2.11</td>
<td>5.77±0.38</td>
<td>10.50±0.68*</td>
<td>33.05±1.97*</td>
<td>645.17±81.68*</td>
<td>72.98±4.07</td>
<td>22.85±3.45</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *p<0.05, significantly different from control group; (n= 6-7 animals).

The effect of *A. difformis* on biochemical parameters is presented in Table 3. While the ALP levels were significantly reduced in the 2 lower doses of the extract, AST was found to be significantly increased (p<0.05) in the 2.5g/kg group when compared to the control rats. All the other parameters (ALT, total and conjugated bilirubin, total protein and albumin) in all extract groups remained within the normal limits.

### Table 3: Effect of *A. difformis* on biochemical parameters

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>ALP (UI/L)</th>
<th>AST (UI/L)</th>
<th>ALT (UI/L)</th>
<th>TB (mg/dl)</th>
<th>CB (mg/dl)</th>
<th>TP (mg/dl)</th>
<th>ALB (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>83.67±6.21</td>
<td>129.68±8.94</td>
<td>38.50±1.59</td>
<td>0.35±0.11</td>
<td>0.22±0.01</td>
<td>5.97±0.24</td>
</tr>
<tr>
<td><em>A. difformis</em> 0.5</td>
<td>65.57±5.48**</td>
<td>126.29±9.58</td>
<td>38.14±3.06</td>
<td>0.21±0.01</td>
<td>0.19±0.09</td>
<td>5.79±0.24</td>
<td>3.97±0.17</td>
</tr>
<tr>
<td>1</td>
<td>63.67±3.50**</td>
<td>130.50±7.97</td>
<td>44.83±4.97</td>
<td>0.32±0.08</td>
<td>0.12±0.02</td>
<td>6.32±0.20</td>
<td>3.67±0.16</td>
</tr>
<tr>
<td>2.5</td>
<td>87.00±14.45</td>
<td>159.17±7.94*</td>
<td>49.67±5.60</td>
<td>0.43±0.11</td>
<td>0.12±0.02</td>
<td>5.92±0.33</td>
<td>3.73±0.13</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *p<0.05, **p<0.001, significantly different from control group; (n= 6-7 animals).

Histopathologic analysis

Histopathological examination of the various organs (Fig. 2), showed that control rats given distilled water during the 28 days study period maintained normal livers with central vein and radiating hepatocytes (Fig. 2A). The heart showed intact pericardium and myocardium (Fig. 2E) and the renal tubules were unaffected (Fig. 2C). Figure 2B shows the liver section of rat treated with 2.5g/kg of the extract. There was congestion and dilatation, mild infiltrates of chronic inflammatory cells and fibrosis of the liver. Figures 2D and 2F show sections of the kidney and heart, respectively, of rat treated with the extract. There was focal cortical tubular necrosis, mild interstitial infiltrates of chronic inflammatory cells and oedema while the heart presented with moderate transmural oedema and mild infiltrates of chronic inflammatory cells.
Figure 2: Histopathological sections of the liver (A), kidney (C) and heart (E) of control rats given distilled water for 28 days showing normal architecture. Administration of aqueous extract of *A. difformis*(2.5g/kg/day) for 28 days shows the liver with periportal congestion and dilatation, mild infiltrates of chronic inflammatory cells and fibrosis (B); kidney with focal cortical tubular necrosis, mild interstitial infiltrates of chronic inflammatory cells and oedema (D); and heart with moderate transmural oedema and mild infiltrates of chronic inflammatory cells (F) [H&E x40]

DISCUSSION

In the acute toxicity study, no mortality was observed up to a maximum oral dose of 10000 mg/kg body weight, neither were there any signs of delayed toxicity, so the oral LD$_{50}$ could not be determined. According to OECD-423 guidelines for acute oral toxicity, an LD$_{50}$ dose of 2,000 mg/kg is categorized as unclassified (OECD, 2000). Though the extract is not used by the parenteral route, the high LD$_{50}$ of 1780mg/kg suggests that the extract is non toxic.

The consistent increase in body weight shown by the extract, throughout the 28 day period of study across all the treated groups is suggestive of growth response and may be as a result of food and water intake stimulated by the extract in the animals. Change in body weight of animals is useful in assessing response to therapy with drugs (Winder *et al.*, 1969)

and to indicate the adverse effects (Teo et al., 2002).
The absolute and relative organ (liver, kidney, heart, spleen) weights remained normal in the extract-treated groups which suggests that A. difformis is not toxic to these vital organs. However, the histopathological findings indicate otherwise as the normal architecture of the various organs were found to have been significantly altered at the highest dose of the extract. Absolute and relative organ weights are known indices often used in toxicological evaluations (Michael et al., 2007) although they are not necessarily indicative of the absence of lesions. Absolute organ weight is also known to be a relative sensitive indicator of nephrotoxicity for known nephrotoxicants (Kluwe, 1981).

The significant decrease in platelet counts by the extract at all the tested doses suggests that it can precipitate thrombocytopenia, which is the presence of low level platelets in the circulatory system. This might also mean that it possesses anticoagulant properties. The significant reduction in haematocrit and haemoglobin levels in the 2.5g/kg/day group is an indication of possible lysis of blood cells and/or inhibition of blood cell synthesis by the active metabolites of A. difformis. It thus means that the use of the extract at such high doses may result in anaemia, a reduction in the number of erythrocytes, haemoglobin or both in the circulating blood, resulting from excessive red blood cell destruction, loss or decreased production (Strauss, 1998).

Alanine amino transferase and serum alkaline phosphatase directly reflect a major permeability of cell rupture (Witter and Bohmwald, 1986; Benjamin, 1978). While ALT is a hepatospecific enzyme found in the cytoplasm in rats (Benjamin, 1978; Ringler and Dabich, 1979), ALP is widely used to indicate hepatobiliary obstruction (Zimmerman, 1984). The significantly reduced level of ALP without alterations in other parameters, at the lower doses of the extract, is suggestive of a possible hepatoprotective effect at these doses.

Aspartate transferase (AST) is an enzyme that is present in high quantities in the cytoplasm and mitochondria of the liver, also in the heart, skeletal muscle, kidneys and brain (Benjamin, 1978; Ringler and Dabich, 1979). This enzyme exhibited significantly higher values in the A. difformis (2.5g/kg) treated rats compared to control. This could indicate some degree of tissue lesion in some of the above mentioned organs. Histopathological assessment of selected organs (heart, liver, kidney, spleen) from rats treated with this dose of extract showed chronic inflammation of the heart, liver and kidney as well as necrosis of the renal tubules, suggesting detrimental changes and morphological disturbances caused due to the administration of the extract for 28 days. It is possible that the toxicity of this enzyme in this group of rats may have resulted in the organ damage observed.

The results did not reveal any significant toxic effects at the lower doses (0.5 and 1g/kg body weight/day). There were no visible colour changes or irregularities in the architecture of the various organs. However, the highest dose of 2.5g/kg/day exhibited serious lesions in all the organs studied hence it is necessary to exercise caution in the use of the extract at such high doses. However, the doses used in this study are far higher than those used for earlier pharmacological studies (Akah and Njike, 1990; Okpo et al., 2012). Therefore such doses may be safe for daily administration without causing any serious side effects.

In conclusion, A. difformis can be considered safe as it did not cause lethality or any obvious toxic manifestations in the acute toxicity study up to a dose of 10000mg/kg body weight. There were also
no observable deleterious effects caused by the extract up to 1000mg/kg body weight in the sub-acute toxicity study. However, use of large quantities of the extract over a long period may not be advisable and caution is advised for individuals with bleeding disorders considering its propensity to cause a reduction in platelets at high doses, as observed from this study.

REFERENCES


