Acute Toxicity Studies of Erythrophleum suaveolens in Albino Mice (Mus musculus)

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Abstract: The activity of crude hydro-extract of stem bark of Erythrophleum suaveolens against Albino Mice was investigated. Extracted doses (50-300mg/kg) of the crude extract were administered. No mortality was recorded at low doses (50-100mg/kg) in comparison with high doses with progressive increase in mortality rate 150mg/kg = 20%, 175mg/kg = 20%, 200mg/kg = 40%, 250mg/kg = 60%, 280mg/kg = 80% and 300mg/kg = 100% respectively. Affected animals also exhibited physical signs of toxicity restlessness between 2-3 minutes, and increase in respiratory rate between 2-5 minutes, continued restlessness, convulsion and finally death. Probit analysis of mortality revealed an LD₅₀ = 223.8±0.05smg/kg. Post mortem examination of dead animals revealed evidence of poisoning as observed from clotted blood in the coronary artery. Investigation confirms the toxic effect of E. suaveolens, thus provides an area to explore in the use of the indigenous plant for medicinal and animal grazing purposes.

Keywords: Erythrophleum suaveolens, acute toxicity, Mus musculus. Ordeal plant, LD₅₀

1. Introduction

Medicinal plant research has been and continues to be considered as a fruitful approach for the search of new drugs especially as the consequences of loss of biodiversity in same context has posed great challenges to the third world [1] -[4]. E. suaveolens is a perennial tree of about 30m in height, slightly buttressed, often low-branching and producing a dense spreading crown. It is referred to by various names by natives [5], [6]. These include Obo/erun (Yoruba), iniy (Igbo), baska (Hausa), Kor (Tiv), lakpa(Nupe), ijien (Isekiiri), idip (Ibibio), akpa (Efik), Ovinyin (Benin), aba (Akan-Asante, Ghana), digpende (Bassari-Togo), tel (Koranko-Sierra Leone) etc [7], [8]. It is often referred to in English as sassy, sasswood, redwater tree and ordeal tree [9], [10]. Studies have shown that E. suaveolens spp. Are extremely toxic to livestock especially sheep and cow. In Savannah regions, the cattle herders are very careful not to allow their animal to graze along the routes where the trees of these species are known to grow [11]. Elevation of activities of acid and alkaline phosphotase in the intestine and hepatopancreas, haemolymph and total protein level were observed in tissues of fresh water snail in an investigation of the activity of saponin from ethanolic extract of E. suaveolens crude, pure, aqueous and lipid fraction extracts of air-lined leaves of E. suaveolens is not recommended for use on the fingerlings of the clarid catfish despite its anaesthetic effect, [7], [12]. It is also reported to be used as poison or repellant against rodent, insects and some aquatic animals and also in tanning hides and as dye [7]-[8]. The search of herbal preparations, that do not produce any adverse effects in the non-target organisms, and which are easily biodegradable, remains a challenge to research issue for scientists [13].

2. Statement of Problem

One of the greatest argument against traditional medicine today, apart from improvise diagnosis and unhygienic methods amongst many others is the lack of scientific proof, its efficiency and huge problem of toxicity. It is imperative to address the following questions as regarding the various ethno botanical surveys available in Africa:

1. Why was E.suaveolens chosen as ordeal plant?
2. Could the mode of administration be responsible for its toxic effect?
3. Was there any addition made to its preparation that led to its toxicity?

However, acute toxicity studies using lower animals like the Albino Mice which are more resistant to toxicants than Rats may address the laid challenges.

2.1 Aims and Objective

The objective of this study is to investigate the toxic effect of cold water extract of the stem-back of Erythrophleum suaveolens using Albino Mice as indicator by way of:

- Determination of signs, symptoms and gross behavior of test animals from poisoning
- Determination of the minimum concentration of extract that would cause toxicity and mortality in test animals.
3. Materials and Methods

A simple procedure for a preliminary assessment of a drug is the determination of the lethal dose in mice or rats. The minimum number of animals used per test is five, because the smaller the number, the higher the margin especially calculating the medium dose LD50 of the test compound [14]. Mice or Rats are mostly used in preliminary toxicity tests. The routes of administration commonly used include oral, subcutaneous, intraperitoneal and intramuscular [15].

Stem-back of *Erythrophleum suaveolens* was collected within the premises of the Federal School of Forestry, Jos Plateau State, Nigeria. Identification and authentication were done by Mr Okonkwo a Taxonomist with the Federal School and Professor S.W Hussseni of the Department of Botany, University of Jos, Nigeria.

The bark was dried under the shade, in the pharmacology research Laboratory of the University of Jos, Nigeria. Sample was pulverized using wooden Mortar and Pestle according to the method of Ibrahim et al. (1984); Audu et al. (2001). The pulverized sample was stored at room temperature until required.

3.1 Extraction of Plant Material

100g of powdered stem-bark of the plant was weighed out in 1000ml capacity Pyrex glass beaker. This was dissolved in 200ml of distilled water according to the method of Audu et al. (2001). The mixture was allowed to stand for 24hours at ambient room temperature. Mixture was stirred with a glass rod and then filtered through Whatman number one filter paper, using suction pump. The filtrate was concentrated in a water bath at a temperature of 80 + - 1.0 °C until a reddish, sticky extract was obtained. This gave a yield of 6.125g of the extract from 100g powdered sample. The recovered extract was stored in the Refrigerator at -4 °C.

3.2 Crude Extract Preparation

1g of crude water extract was weighed and dissolved in 10ml of distilled water to give a stock concentration solution of 1x10^-3g/ml. Other concentration used for the test were prepared by diluting 1ml of stock solution in 9ml of distilled water (1:9) to give 1x10^-2g/ml. Various concentrations were obtained through serial dilutions of the series as appropriate throughout the experiment.

3.3 Albino Mice

Young Albino Mice (*M. musculus*) with an average body weight 10g – 35g were obtained from the Animal House Unit of the University of Jos, Nigeria in cages and then acclimatized to laboratory condition for 5 days prior to experiment. These were fed with standard mash and water ad libitum.

3.4 Toxicity Tests

The animals were individually weighed and marked for identification accordingly, using a digital electronic balance - acculab model 2001, capacity 2000g/100z. They were subsequently divided into 9 groups of 5 animals each. Crude extract was orally administered to the animals, according to body weight of each animal viz:

- Groups 1 – 8 (experimental): Administered with 50 – 300mg/kg body weight of extract.
- Group 9 (untreated/control): Without plant extract. Administered with distilled water

Dead animals in each group were counted and the percentage mortality calculated. The percentage mortality in each case was transformed into probit according to the method of Ibrahim et al. (1984); Audu et al. (2001). The relationship between the concentration of the observed and the expected probits were computed and graphically plotted according to the method of Wadlow (1981).

3.5 LD50 Determination

Values of LD16, LD50, LD85 and LD99 were determined by converting the percentage mortality figures of all mortality data (0% - 100%) to probit according to the method of Litchfield and Wilcoxon [16]. The relationship between the concentration of the observed and the expected probits were computed and graphically plotted according to the method of Wadlow (1981).

4. Results

Toxicity Test results from oral administration of crude extract of stem-back of *Erythrophleum suaveolens* showed no mortality in Tables 1 and 2 (50mg/kg; 100mg/kg) body weight mice. Toxic effect of crude were observed in Tables 3 and 4 (150mg/kg; 175mg/kg) with 20% mortality in each case, while significant effect of *E. suaveolens* on mice is recorded in Tables 5, 6, 7 and 8 (200mg/kg; 250mg/kg; 280/mg/kg and 300mg/kg) at 40%, 60%, 80% and 100% mortality rate respectively. Table 9 showed zero lethal effect, as the group was administered with only distilled water. Table 10 illustrates the dependence of mortality rate on the dose of crude extract, showing percentage mortality and probit for groups 1- 8. During the 14 days of observation, the animals that died, did so within 72 hours of crude administration (groups 3 – 6 = 2 days), with exceptions in (groups 7 = 10–12 hours; and 8 = within 1 hour)

4.1 Visible Signs of Poisoning

Administration of various doses of crude extract of *E. suaveolens* led to the manifestations of some physical signs of toxicity on the mice. These manifested as initial discomfort in form of stretching, restlessness and increase in respiratory rate between 2 and 5 minutes of administration. The animals that recovered did so after 30 minutes with a drastic decrease in effect of toxicity after 60minutes and full recovery after 126 minutes, while those that died showed prior manifestation of prostration, loss of locomotory coordination that was followed by brief convulsion. It was also observed that some of the animals that survived were such that vomited the extract. It was discovered that haemorrhage occurred in only the heart and the lungs of the dead animals.
### Table 1: Frequency of effect of administration of 50mg/kg mice body weight crude extract

<table>
<thead>
<tr>
<th>Weight of Animal</th>
<th>Vol. of Extract Administered</th>
<th>Mean Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>13g</td>
<td>0.06ml</td>
<td>0</td>
</tr>
<tr>
<td>12g</td>
<td>0.06ml</td>
<td>0</td>
</tr>
<tr>
<td>12g</td>
<td>0.075ml</td>
<td>0</td>
</tr>
<tr>
<td>11g</td>
<td>0.09ml</td>
<td>0</td>
</tr>
<tr>
<td>11g</td>
<td>0.055ml</td>
<td>0</td>
</tr>
</tbody>
</table>

Total number dead 0/5 0 (%)

### Table 2: Frequency of effect of administration of 100mg/kg mice body weight crude extract

<table>
<thead>
<tr>
<th>Weight of Animal</th>
<th>Vol. of Extract Administered</th>
<th>Mean Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>13g</td>
<td>0.13ml</td>
<td>0</td>
</tr>
<tr>
<td>12g</td>
<td>0.12ml</td>
<td>0</td>
</tr>
<tr>
<td>12g</td>
<td>0.12ml</td>
<td>0</td>
</tr>
<tr>
<td>11g</td>
<td>0.11ml</td>
<td>0</td>
</tr>
<tr>
<td>11g</td>
<td>0.11ml</td>
<td>0</td>
</tr>
</tbody>
</table>

Total number dead 0/5 0 (%)

### Table 3: Frequency of effect of administration of 150mg/kg mice body weight crude extract

<table>
<thead>
<tr>
<th>Weight of Animal</th>
<th>Vol. of Extract Administered</th>
<th>Mean Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>17g</td>
<td>0.25ml</td>
<td>0</td>
</tr>
<tr>
<td>18g</td>
<td>0.27ml</td>
<td>1</td>
</tr>
<tr>
<td>20g</td>
<td>0.3ml</td>
<td>0</td>
</tr>
<tr>
<td>19g</td>
<td>0.28ml</td>
<td>0</td>
</tr>
<tr>
<td>19g</td>
<td>0.28ml</td>
<td>0</td>
</tr>
</tbody>
</table>

Total number dead 1/5 20 (%)

### Table 4: Frequency of effect of administration of 175mg/kg mice body weight crude extract

<table>
<thead>
<tr>
<th>Weight of Animal</th>
<th>Vol. of Extract Administered</th>
<th>Mean Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>28g</td>
<td>0.49ml</td>
<td>1</td>
</tr>
<tr>
<td>23g</td>
<td>0.4ml</td>
<td>0</td>
</tr>
<tr>
<td>26g</td>
<td>0.45ml</td>
<td>0</td>
</tr>
<tr>
<td>20g</td>
<td>0.35ml</td>
<td>0</td>
</tr>
<tr>
<td>18g</td>
<td>0.31ml</td>
<td>0</td>
</tr>
</tbody>
</table>

Total number dead 1/5 20 (%)

### Table 5: Frequency of effect of administration of 200mg/kg mice body weight crude extract

<table>
<thead>
<tr>
<th>Weight of Animal</th>
<th>Vol. of Extract Administered</th>
<th>Mean Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>15g</td>
<td>0.3ml</td>
<td>0</td>
</tr>
<tr>
<td>15g</td>
<td>0.3ml</td>
<td>1</td>
</tr>
<tr>
<td>18g</td>
<td>0.36ml</td>
<td>1</td>
</tr>
<tr>
<td>13g</td>
<td>0.26ml</td>
<td>0</td>
</tr>
<tr>
<td>17g</td>
<td>0.34ml</td>
<td>0</td>
</tr>
</tbody>
</table>

Total number dead 2/5 40 (%)

### Table 6: Frequency of effect of administration of 250 mg/kg mice body weight crude extract

<table>
<thead>
<tr>
<th>Weight of Animal</th>
<th>Vol. of Extract Administered</th>
<th>Mean Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>35g</td>
<td>0.87ml</td>
<td>0</td>
</tr>
<tr>
<td>35g</td>
<td>0.87ml</td>
<td>1</td>
</tr>
<tr>
<td>30g</td>
<td>0.75ml</td>
<td>1</td>
</tr>
<tr>
<td>30g</td>
<td>0.75ml</td>
<td>1</td>
</tr>
<tr>
<td>30g</td>
<td>0.75ml</td>
<td>0</td>
</tr>
</tbody>
</table>

Total number dead 1/5 60(%)

### Table 7: Frequency of effect of administration of 280mg/kg mice body weight crude extract

<table>
<thead>
<tr>
<th>Weight of Animal</th>
<th>Vol. of Extract Administered</th>
<th>Mean Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>15g</td>
<td>0.42ml</td>
<td>0</td>
</tr>
<tr>
<td>19g</td>
<td>0.53ml</td>
<td>1</td>
</tr>
<tr>
<td>17g</td>
<td>0.47ml</td>
<td>1</td>
</tr>
<tr>
<td>15g</td>
<td>0.42ml</td>
<td>1</td>
</tr>
<tr>
<td>10g</td>
<td>0.28ml</td>
<td>1</td>
</tr>
</tbody>
</table>

Total number dead 4/5 80(%)
5. Discussion

The physical manifestations of toxicity of *E. suaveolens* within just 2 – 3 minutes of administration at various concentrations on *Mus musculus* is an indication that it could penetrate blood brain barriers within a short time. Loss of locomotory coordination in the mice may be due to the interference of crude extract with the skeletal muscle, especially the neuromuscular transmission by blocking the neurotransmitter acetylcholine. Singh and [17] reported that AchE is responsible for the termination of cholinergic impulses by the hydrolysis of acetylcholine released during synaptic transmission; inhibition of Acetylcholinesterase thus permits accumulation of the synapses when concentration raises several folds in comparison to the normal levels, leading first to paralysis and then eventually death. Acetylcholinesterase (AchE) is a key enzyme in the nervous system of animals, which occurred in the outer basal lamina of nerve synapses, neuromuscular junction and in certain other tissues [18]. Muscle fatigue may result from impaired excitation – contraction mechanisms, neurotransmission failure or both [19]. The blockade might not be permanent because some of the animals recovered after 2 hours of administration of extract due to some other factors.

Results from toxicity test also showed that mortality rate was dependent on dose of extract, thus mortality rate increased with increased dosage i.e. in table 10, 300mg/kg dose gave 100% mortality. Acetylcholinesterase activity index has been widely used to indicate exposure of both vertebrates and invertebrate species to organo-phosphorous and carbamate pesticides [20].

Emesis was capable of reducing toxicity, as the animals that vomited the extract survived. No record of mortality in groups 1-2, as crude extract was not up to threshold to cause toxicity.

100g of marsh food was all consumed by the animals in the control group (9); same amount was also consumed in the treated groups, especially groups 1 and 2 where no deaths were recorded. There were few remnants of food in other group. Similarly 100ml of water was consumed daily by the control group, while about 60ml by the treated groups; hence extract of *E. suaveolens* did not affect appetite in the animals. This claim is supported by the survival of the animals in both groups. Water intake was however low most probably due to the toxicity of *E. suaveolens*.

Probit/ log dose plot of lethal concentration values in Table 11 and figure 1 (LD_{10}, LD_{50}, LD_{80}, and LD_{90}) gave LD50 value of 223.8±0.05mg/kg body weight. This falls within the very toxic range as per Hodge and Sterner (1947) categorization.

The determination of LD_{50} gives an insight into safety margin of the drug, and safety margin is defined by Loomis (1978) as the dosage range between the dose producing a lethal effect and the dose producing the desirable effect.

5.1 Conclusion

Indications that the toxic effect of *E. suaveolens* was also manifested through initial discomfort in form of stretching, restlessness increase in respiratory rate, prostration and loss of locomotory coordination that was followed by brief convulsion as it inhibits acetylcholinesterase activity indicates that some organ may have been affected. The haemorrhage that occurred in the heart and lungs of the dead animals suggests the cardio-toxic nature of the crude extract of *E. suaveolens* to mice (*Mus musculus*). Hence, mortality incurred by people who were administered crude extract of same plant as an ordeal plant in those days, could be due to cardiac failure. The foregoing manifestations confirmed the toxic nature of the Stem-bark extract of *Erythrophleum suaveolens* on animals and therefore justify its use as ordeal plant in the past.

5.2 Recommendation

Phytochemical analysis, characterization and isolation of the plant extract should be carried out so as to determine its toxic constituent, in order to fish out the exact constituent(s) responsible for the toxic effects.

5.3 Future scope of the study

Histopathological evaluation of the acute and chronic toxicity effects of the extract on vital organs and tissues of treated animals should be considered. Pharmacological investigation on the effects of crude extract of *E. suaveolens* on the GIT of Rabbit and Guinea Pig should be carried out.

5.4 Conflicting interest

No conflict of interest.

5.6 Acknowledgement

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