Oral acute toxicity (LD$_{50}$) study of methanol extract of *Annona senegalensis* leaf in albino mice

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Oral acute toxicity (LD$_{50}$) of methanol extract of leaves of the medicinal plant *Annona senegalensis* was studied using mice as animal models. The study was conducted in two phases. In the first phase, three groups of mice (4 per group) were administered with respective oral doses of 10 mg, 100 mg and 1000 mg/kg body weight of the extract. These were monitored in 24 h, 72 h, and up to a follow up period of 4 weeks. In the second phase, the dosage of the extract was increased to 1600 mg, 2900 mg and 5000 mg/kg body weight respectively for another three groups of mice (4 per group). These were equally observed as in phase 1 for toxicity signs and possible deaths. It was observed that throughout the 4 weeks follow up period, no mortality was observed in any of the test animal group up to the highest dose of the extract tested. Examination of liver section of the animals (H & E x400) showed relatively normal histological features. It is thus concluded that administration of *A. senegalensis* in mice is safe up to a dose of 5000 mg/kg body weight.

Key words: Acute toxicity, lethal dose, *A. senegalensis*.

INTRODUCTION

*Annona senegalensis* is a species of flowering plant in the family Annonaceae. It is commonly known as “African Custard Apple” “Wild Custard Apple” and “Wild Sour Sop”. The primary use of *A. senegalensis* is for food but it has applications in numerous aspects of human endeavour and every part of the plant has unique properties and uses (Idowu et al., 2010). The plant is commonly used in Nigeria folk medicine as a remedy for malaria. The roots are used in the treatment of conditions like dizziness, indigestion, chest colds and venereal diseases (Fabricant and Farnsworth, 2001). The flowers, leaves and fruits are also edible and culinary.

Acute toxicity (Lethal toxicity) is the ability of a chemical to cause ill effect "relatively soon" after one oral administration or a 4 h exposure of a chemical in air (Senin, 2006). According to Senin (2006), “relatively soon” is usually defined as a period of minutes, hours (24) or days (up to about 2 weeks) but rarely longer. LD$_{50}$ is an abbreviation for “Lethal Dose 50%.” It is sometimes also referred to as the "Median Lethal Dose." The LD$_{50}$ for a particular substance is essentially the amount that can be expected to cause death in half (i.e. 50%) of a group of some particular animal species, usually rats or mice, when entering the animals’ body by a particular route. It is usually expressed as the amount of chemical administered (eg. Milligrams) per 100 g (for small animals) or per kilogram (for bigger subjects) of the body weight of the test animal (Gadanya et al., 2011). LD$_{50}$ obtained at the end of a study is reported in relation to the route of administration of the test substance e.g. LD$_{50}$ (oral), LD$_{50}$ (dermal) etc. The most frequently performed lethal study is the oral LD$_{50}$. Results obtained from oral studies are important for drugs, food and accidental domestic poisonings. Generally, the smaller the LD$_{50}$ value, the more toxic the substance is and vice versa. LD$_{50}$ values can be compared to other values using a toxicity scale. Confusion sometimes occurs owing to the fact that there are many different toxicity scales in use. The two most common scales used are the “Hodge and Sterner scale” and “Gosselin”, “Smith and Hodge Scale” (Senin 2006).
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Table 1. Record of mortality in phase 1.

<table>
<thead>
<tr>
<th>Extract Dose (mg/kg body weight)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

Number of deaths per group = 0, Number of mice per group = 4.

Table 2. Record of mortality in phase 2.

<table>
<thead>
<tr>
<th>Extract dose (mg/kg body weight)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1600</td>
<td>0</td>
</tr>
<tr>
<td>2900</td>
<td>0</td>
</tr>
<tr>
<td>5000</td>
<td>0</td>
</tr>
</tbody>
</table>

Number of deaths per group = 0, Number of mice per group = 4.

These tables differ both in numerical ratings and terms used to describe each class.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *A. senegalensis* were collected at Ihiagwa in Owerri West area of Imo State, Nigeria. Identification was done by a taxonomist in the department of Biotechnology Federal University of Technology Owerri.

Sample preparation and extraction

The fresh leaves of *A. senegalensis* were air dried under shed for one week and later milled to powder using a mechanical blender. 500 mg of the ground sample was soaked in 1500 ml of 95% methanol and allowed to stand for 72 h. This was subsequently filtered using Whatman No. 48 filter paper. The filtrate obtained was concentrated by rotary evaporation at a temperature of 45 - 50°C.

Determination of acute toxicity (LD50)

The method used to determine acute toxicity was that described by Lorke(1983). The study was conducted in two phases. In the first phase, three groups of four mice each were administered with the extract at respective oral doses of 10mg, 100mg, and 1000mg per kg body weight. The mice were observed for signs of toxicity and possible deaths for 24 h, 72 h, 2 weeks and for 4 weeks. In the second phase, another three groups of 4 mice each were administered respective doses of 1500, 2900 and 5000mg per kg body weight of the extract. They were equally monitored as in phase one for toxicity signs and deaths. From data obtained, LD50 was determined.

Histological procedure

Histological examination was done by fixing the organs (liver) in 4% formaldehyde. They were subsequently processed and embedded in Paraffin wax. Tissue blocks were sectioned 5 µm thick and stained with Haematoxylin and Eosin (H & E) for detailed observation.

RESULTS AND DISCUSSION

From the result of the acute toxicity study of methanol extract of *A. senegalensis* in mice, no mortality was recorded in any of the test groups in 24 h, 72 h and up to the four weeks follow up period for both phases of the study (Tables 1 and 2). In our earlier study, we have observed the presence of various phytochemicals e.g.
Figures 1a, b and c. Photomicrographs of the liver sections of the mice (H&E x400).

Alkaloids, Saponins, Glycosides, Flavonoids etc., occurring in various amounts in the plant. These phytochemicals elicit a wide array of pharmacological actions e.g. Saponins enhance nutrient absorption and promote digestion. Alkaloids are used as medications, recreational drugs or in entheogenic rituals e.g. The local anaesthetic and stimulant cocaine, the stimulant caffeine, the analgesic morphine and the anti-malarial drug quinine (Tailang and Sharma, 2009).

Any compound with oral LD₅₀ of 5000 mg/kg or more in rat should be considered as practically harmless (Hodge and Sterner, 2005). The photomicrograph of liver sections of the animal showed normal sinusoids, no histopathological lesion but intact hepatic cytoarchitecture (H & E x400) thus implying the safety level of the extract up to the highest dose studied (Figure 1).

Conclusion

From the observation made in this study, it could be concluded that the administration of A. senegalensis in mice is safe at any dose less than or equal to 5000 mg/kg. However, extremely high doses may not be advisable.

REFERENCES