INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Oral Acute Toxicity (LD_{50}) Study of Methanol Extracts of *Alstonia Boonei* Root Bark in Mice

Onwusonye J. C.
Department of Biochemistry, Federal Polytechnic Nekede, Owerri Imo State, Nigeria
Uwakwe A. A.
Department of Biochemistry, University of Portharcourt, Rivers State, Nigeria
Iwuanyanwu P.
Department of Biochemistry, University of Portharcourt, Rivers State, Nigeria
Muritala I. K.
Department of Biochemistry, Federal Polytechnic Nekede, Owerri Imo State, Nigeria

Abstract:
This study was conducted to evaluate the oral acute toxicity (LD50) of methanol extract of root bark of Alstonia boonei, a medicinal plant used locally by the people of South East Nigeria to treat conditions like Malaria, Insomnia, Diarrhea and rheumatic pains. The study was conducted in two phases. In the first phase, three groups of mice (4 per group) were given respective oral doses of 10mg, 100mg and 1000mg/kg body weight of the extract and observed, in 24hours, 72hours and up to four weeks. In the second phase, another three groups of mice (4 per group) were administered with increased doses of the extract 1600mg, 2900mg and 5000mg/kg weight). These were monitored as in the phase one study. It was observed that when the extract was administered up to a dose of 5000mg/kg body weight, no death was recorded among all the animals under investigation. Histological (H & E x400) examination of liver sections of animals showed relatively normal histological features (normal sinusoid with intact hepatic cytoarchitecture). It is thus concluded that administration of the extract to mice is safe at any dose less than or equal to 5000mg/kg body weight.

Keywords*: Acute toxicity, lethal dose, Alstonia boonei*

1. Introduction

Acute toxicity (Lethal toxicity) is the ability of a chemical to cause ill effect "relatively soon" after one oral administration or a 4-hour 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 2weeks) 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 100grams (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 eg 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). These tables differ both in numerical ratings and terms used to describe each class.

Alstonia boonei is a widespread genus of evergreen trees and shrubs from the dog bane family (Apocynaceae). It is commonly known as Cheesewood and grows up to 45m tall and 1.2m in diameter. The Stem bark has been reported to exhibit diuretic, spasmolytic and hypotensive properties (Oliver 1986). The plant has also demonstrated remarkable activity against conditions like fever, insomnia, chronic diarrhea and rheumatic pains (Olajide et al 2000). A decoction of the stem and root bark is drunk by the Ikeduru people of Imo State, South-East Nigeria as a strong remedy for malaria.

Most users of a substance will need to know its level of toxicity/safety to humans. Since toxicity data derived from animals, including LD_{50} figures could be applied by expert judgment to assess relevance to humans, there arises a need for research of this nature to access the level of toxicity of Alstonia boonei in mice.

2. Materials and Methods

2.1. Sample Collection

Fresh leaves and root bark of Alstonia boonei were collected from Umuri-Amaimo in Ikeduru area of Imo State. These were identified by a taxonomist in the department of Biotechnology, Federal University of Technology Owerri.

2.2. Sample Preparation and Extraction

The fresh root bark of A. boonei was sorted out to get rid of dead matter and other unwanted materials. They were subsequently cleaned, cut into pieces and air-dried under shed for two weeks. After drying, the samples were milled to powder using a mechanical blender. 500g of the ground material was soaked in 1500ml of 95% methanol and allowed to stand for 72hours. This was subsequently filtered using Whatman no. 48 filter paper. The liquid extract (filtrate) was concentrated by rotary evaporation at temperature of 45-50^{O}C.

2.3. Determination of Acute Toxicity (LD_{50})

The method used to determine acute toxicity (LD_{50}) 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 the methanol extract of A. boonei at respective oral doses of 10mg, 100mg and 1000mg per kilogram body weight. The animals were observed for signs of toxicity and possible deaths for 24hours, 72hours, and two weeks and for four weeks.

In the second phase, another three groups of four mice each were administered respective oral doses of 1500mg, 2900mg and 5000mg per kg body weight of the extract. The mice were equally observed for toxicity signs and possible deaths for 24hours, 72hours, two weeks and four weeks. Possible number of deaths was recorded and LD_{50} value was determined.

2.4. 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 5µm thick and stained with Haematoxylin and Eosin (H & E) for detailed observation.

3. Results and Discussion

Observation made in this study on the oral acute toxicity of methanol extract of Alstonia boonei in mice has shown that on the administration of the extract up to a dose of 5000mg/kg, no mortality was recorded in any of the test animal groups throughout the follow-up period. From our earlier studies (in press), the plant has been observed to contain various amounts of pharmacologically active compounds like Alkaloids and Saponins. Alkaloids have been shown to exhibit some pharmacological effects and are used as medications, recreational drugs, or in entheogenic rituals eg. The local anesthetics and stimulant cocaine, the stimulant Caffeine, the analgesic morphine or the antimalarial drug quinine (Tailang and Sharma, 2009).

Saponin enhances nutrients absorptions and thus aids in digestion of foods in animals. According to Hodger and Sterner (2005), any compound with oral LD_{50} of 5000mg/kg or more in rat should be considered as practically harmless. Also examination of liver sections taken from the test animals showed normal sinusoids and intact hepatic cytoarchitecture (H & E x400) as shown in figures1-3. Thus the oral administration of methanol extract of Alstonia boonei in mice is safe up to a dose of 5000mg/kg body weight.

4. Conclusion

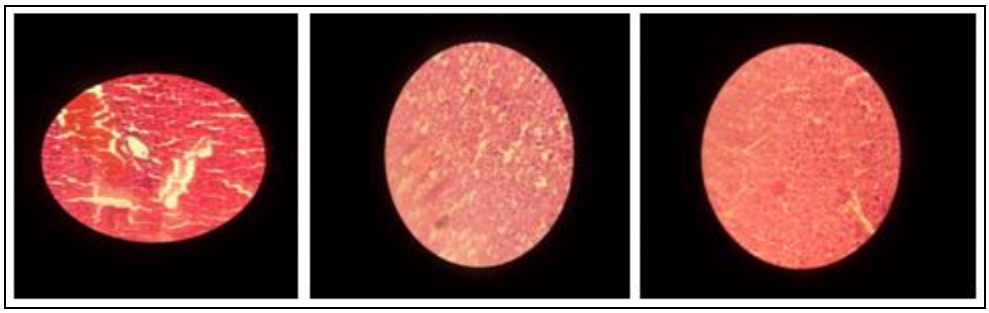
From the observation made in this study, it is hereby concluded that the administration of Alstonia boonei extract in mice is safe at a dose less than or equal to 5000mg/kg. However doses that are extremely high may not be advisable.

Extract Dose (mg/kg body weight)	Mortality
10	$\frac{0}{4}$
100	$\frac{0}{4}$
1000	$\frac{0}{4}$

Table 1: Record of Mortality in phase 1
Number of deaths per group = 0, Number of mice per group = 4

Extract Dose (mg/kg body weight)	Mortality
1600	0/4
2900	0/4
5000	0/4

Table 2: Record of Mortality in Phase 2
Number of deaths per group = 0, Number of mice per group = 4



Figures 1-3: Photomicrographs of the liver sections of the mice (H&E x400)

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