

Original Research Article

# Oral acute toxicity study of methanol leaf extracts of *Croton Zambesicus* in mice

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## Abstract

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The present study was conducted to evaluate the oral acute toxicity profile of methanol leaf extract of *Croton zambesicus*, a medicinal plant used locally by the people of South East Nigeria to treat conditions like fever associated with Malaria. 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). Another group of four mice administered with normal saline at a dose of 5ml/kg served as control. These were monitored as in the phase one study. Results showed 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 and E x400) examination of liver sections of animals showed relatively normal histological features (normal sinusoids with intact hepatic cytoarchitecture). It is thus concluded that administration of the extract to mice is safe up to the dose of 5000mg/kg body weight.

**Keywords:** Acute toxicity, lethal dose, *C. zambesicus*

## INTRODUCTION

*Croton zambesicus* is a shrub or small tree that grows to about 16m high in fringing forests and savannah, the Gambia to south Nigeria and widely distributed elsewhere in tropical Africa. The tree has a scaly bark and silvery leaves rusty scaly below and has an attractive appearance (Figure 1). The leaves which are silvery have greenish colour on top and some of the leaves are orange in colour. It has a sweet smell and it is often planted in towns and villages. The wood is pale yellow, fine grained, hard and gives a good polish. The stems are used in parts of West Africa for hunt posts and in Yoruba houses for beams in default of other timbers. The bark slash emits an aromatic smell also. *Croton zambesicus* is extensively used in African traditional medicines (Watt and Breyer-Brandwijk, 1962) and other parts of the world. It contains alkaloids, terpenes, flavonoids, glycosides,

saponins, volatile oils such as sesquiterpenes, monoterpenes and diterpenes and other chemicals (Block *et al.*, 2006). It has been shown to be a free radical scavenger and to protect against lipid peroxidation. This ability has been reported to increase peripheral testosterone level in Swiss albino mice (Okokon *et al.*, 2005). The components are appropriate for detoxification and antioxidants (Okokon *et al.*, 2005).

Boyom *et al.*, (2002) studied the composition of essential oils from the leaves, stems and roots of *croton zambesicus* and found three types of oils to be similar in composition, with those from the leaves and stem rich in monoterpenes while that of root bark contains sesquiterpenes. The root and stem bark oils were found to be rich in oxygen containing compounds with spathulenol and linalool as major components.

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<sub>50</sub> is an abbreviation for “Lethal Dose 50%.” It is sometimes also referred to as the “Median Lethal Dose.” The median lethal dose 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<sub>50</sub> obtained at the end of a study is reported in relation to the route of administration of the test substance eg LD<sub>50</sub> (oral), LD<sub>50</sub> (dermal) etc. the most frequently performed lethal study is the oral LD<sub>50</sub>. Results obtained from oral studies are important for drugs, food and accidental domestic poisonings. Generally, the smaller the LD<sub>50</sub> value, the more toxic the substance is and vice versa. LD<sub>50</sub> 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 Dodge Scale” (Senin 2006). These tables differ both in numerical ratings and terms used to describe each class.

Despite the huge achievements on the successful isolation of some important phytochemicals, there is very little literature on safety/ toxicity profile of *Croton zambesicus* leaves. Thus, in this research work, an attempt has been made to determine the median lethal dose (LD50) value of the leaf extract as well as the effect of its acute dosing on the liver histology of albino mice.

## MATERIALS AND METHODS

### Collection and identification of plant

*Croton zambesicus* leaves were collected from a tree on the campus- Federal Polytechnic Nekede, Imo State. The leaves were identified by a taxonomist in the department of Biology, Federal University of Technology Owerri. The fresh leaves of *Croton zambesicus* were washed, and dried under shed for two weeks. These were ground to coarse powder using a mechanical blender. The methanol extract was prepared and used for this study.

### Preparation of plant extract

Five hundred grams of the ground leaf sample was weighed precisely and soaked in 1500ml of 95%

methanol in a beaker and covered with aluminium foil. The mixture was stirred intermittently and allowed to stand for 72hours. Filtration was carried out using filter paper and the filtrate was subsequently concentrated in a rotary evaporator at the temperature of 45-50°C. The extract obtained was packaged in an air tight container and stored in a refrigerator at 4°C until it was used for the study.

### Determination of Median Lethal Dose (LD<sub>50</sub>)

The method used to determine LD<sub>50</sub> 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 *C. zambesicus* 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<sub>50</sub> value was determined.

### Histological Procedure

Histological examination was done by fixing the liver carefully excised from each mouse 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 and E) for detailed observation.

## RESULTS

The results of this study are as shown in tables 1 and 2 and plates 1-3.

Table 1 shows the record of mortality when the extract was administered to the mice at the doses of 10mg/kg, 100mg/kg and 1000mg/kg.

It was observed that at these three extract doses no death was recorded.

Table 2 shows the record of mortality at extract doses of 1600mg/kg, 2900mg/kg and 5000mg/kg. Also no mortality was recorded at these three doses levels.

Plates 1-3 are photomicrograph results of liver histology for the mice treated with normal saline (control) as well as animals treated with extract at doses of 2900mg/kg and 5000mg/kg. All three photomicrographs indicated normal hepatic features.



**Figure 1.** Picture of *Croton zambesicus*.

**Table 1.** Record of Mortality in phase 1

Extract Dose ( mg/kg body weight)	No of Mice	No. of Deaths
10	4	0
<b>100</b>	4	0
1000	4	0

**Table 2.** Record of Mortality in Phase 2

Extract Dose ( mg/kg body weight)	No of Mice	No. of Deaths
1600	4	0
<b>2900</b>	4	0
5000	4	0



**Plate 1.** Photomicrograph of liver of mice treated with *normal saline* (5ml/kg) showing well preserved hepatocytes. (H&E x400)



**Plate 2.** Photomicrograph of liver of mice treated with *C. zambesicus* extract (2900mg/kg) showing well preserved hepatocytes. (H&E x400)



**Plate 3.** Photomicrograph of liver of mice treated with *C. zambesicus* extract (5000mg/kg) showing well preserved hepatocytes. (H&E x400)

## DISCUSSION AND CONCLUSION

Observation made in this study on the oral acute toxicity of methanol leaf extract of *C. zambesicus* 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 animal groups throughout the follow-up period. 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 Hedger and Sterner (2005), any compound with oral LD<sub>50</sub> 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 Plates 1- 3. Thus the oral administration of methanol extract of *C.zambesicus* in mice is safe up to a dose of 5000mg/kg body weight.

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