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Acute Toxicity of Aqueous and Ethanolic Extracts of *Strophanthus hispidus* Stem Bark

M. Osibemhe[1*], B. O. Abdulrahman[1] and I. O. Onoagbe[2]

[1]Department of Biochemistry and Molecular Biology, Faculty of Science and Education, Federal University Dutsin-Ma, Katsina State, Nigeria.
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author IOO designed the study, wrote the protocol and supervised the work. Authors MO and BOA carried out all laboratories work and performed the statistical analysis. Author MO wrote the first draft of the manuscript, managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to determine the median lethal dose (LD_{50}) of the extracts (aqueous and ethanol) of *S. hispidus* stem bark. A modified Lorke's (1983) method was used in this study. Varying doses of the extracts were administered orally to male albino rats. Treatment-related signs of toxicity and mortality were monitored for 24 hrs and continued for 72 hrs. Treatment-related mortality was observed at the dose of 1000 mg/kg body weight and above in the ethanolic extract and 1600 mg/kg body weight in the aqueous extract. Signs of sedation, exophthalmos, decreased locomotion and appetite in the aqueous or ethanolic treated animals were observed in the early stages of experimentation. However, these signs were not sustained in surviving animals. The LD_{50} was 2154 and 2039 mg/kg body weight for aqueous and ethanolic extracts of *S. hispidus* stem bark respectively. The results showed that 1000 mg/kg body weight and above of *S. hispidus* stem bark may be slightly toxic. Therefore, moderate dosage is advised for purposes of medication.

**Corresponding author: E-mail: mosibemhe@fudutsinma.edu.ng;*

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1. INTRODUCTION

Plants produce a great diversity of substances that can have therapeutic significance for maintaining human health and improving the quality of human life, thus justifying their use in traditional medicine [1]. According to their traditional use, natural compounds are often assumed to be safe. However, several studies have reported that a great number of plant species used as food ingredients or in traditional medicine present mutagenic, carcinogenic or toxic properties [2-4]. Drug toxicity can occur on many different time-scales. Acute toxicity results from a single exposure to a drug, with adverse effects resulting in minutes to hours [5]. A fundamental goal of toxicology is to determine safe levels of exposure to potentially poisonous substances for human and the environment [5].

The scientific exploitation of herbs used ethno-medically for pain relief, wound healing and abolishing fevers has resulted in the identification of a wide range of compounds that have been developed as new therapies for several ailments including diabetes [6].

A few of these herbal products have scientific data on their toxic levels in literatures. Acute toxicity testing can provide a guide to selection of doses of herbal medicines, in order to avoid potential harmful effects when used. *Strophanthus hispidus* is an African shrub that belongs to the genus; *Strophanthus*, the family of Apocynaceae. Its popularity in folklore medicine for the treatment of diabetes, ulcer, gonorrhea as well as its bitter taste may not be unconnected with the presence of important phytochemicals such as saponins, alkaloids, phenols, flavonoids and tannins [7]. This work is therefore aimed at providing scientific records as to the amount of *S. hispidus* that may constitute potential risk in animal model.

2. MATERIALS AND METHODS

2.1 Animals

Male rats (Wistar strain) obtained from the Department of Anatomy, University of Benin, Benin City, Nigeria were used. The rats weighing between 100-232 g were maintained under standard animal house condition and were allowed free access to food (growers mash produced by Bendel Feed and flour Mill, Ewu Edo State) and water for two weeks to acclimatize to the new environment. All animals were handled with proper care and humanely treated according to our Institutional Animal Ethics Committee guidelines as well as the internationally accepted practices for use and care of laboratory animals as contained in US guidelines [8].

2.2 Medicinal Plant

The stems of *S. hispidus* were collected from Galadimawa, in Giwa Local Government, Kaduna State, Nigeria. They were identified by Mr. U. S. Gallah of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria where a specimen with voucher number (no: 2714) was deposited.

2.3 Preparation and Extraction of Plant Materials

A modified method of Onoagbe et al. [9] was used. The stems of *S. hispidus* were thoroughly washed with clean water and the barks were peeled off by incision. They were then dried under shade for two weeks and then pulverized into fine powder with the aid of a mechanical pulverizer. Measured quantities of the powdered sample were extracted separately in aqueous and 99% ethanol for 72 hrs followed by periodic stirring and they were kept in a refrigerator to avoid any microbial growth. The extracts were filtered using cheese-cloth and the filtrate re-filtered using Whatman No. 42 (125 mm) filter paper. The filtrates collected were lyophilized using a freeze-dryer and stored in an airtight container for further analysis.

2.4 Acute Toxicity Study

The method of Lorke, (1983) [10] for the determination of lethal dose (LD_{50}) with little modification was used in this experiment. A total of 26 male rats were used. The rats were randomly selected into two major groups of 13 rats each and were kept in standard cage. The groups were labeled 1 and 2. The experiment was carried out in two phases. In the phase one, rats in group 1 were again selected randomly into three sub-groups of three rats each and were administered orally with the aid of a gavage, doses of 10, 100, and 1000 mg/kg body weight

aqueous extract respectively. Similarly, the second group (2) which was also randomly selected into three sub-groups of three rats each was administered respective doses of ethanolic extract. Both groups were monitored frequently during working hours for signs of toxicity and mortality and subsequently for 24 hours. Observations were continued for 72 hours for any late sign of toxicity. In the second phase, four rats in group 1 were made into four groups of one rat each and were administered doses of 1000, 1600, 2900 and 5000 mg/kg body weight aqueous extract respectively. In a similar manner, four rats were also selected from group 2 into four groups of one rat each. They were administered ethanolic extract at the doses of 800, 1600, 2600 and 3600 mg/kg body weight respectively. Again, both groups were monitored for signs of toxicity and mortality as in the phase one. The second phase was used in the calculation of the median lethal dose (LD_{50}).

3. RESULTS

The results for the acute toxicity study of both extracts of *S. hispidus* stem bark are presented in Tables 1-2. 1000 mg/kg body weight and above in the ethanolic extract (Table 2) and 1600 mg/kg body weight and above in the aqueous extract (Table 1) resulted in mortality. The LD_{50} was beyond 2000 mg/kg for both extracts. (Tables 1-2).

4. DISCUSSION

Diabetes mellitus is a disease that is increasingly affecting millions of people all over the world. And this disease has no known cure in spite of its age long existence. Currently there are over 150 millions diabetics worldwide and this is likely to increase to 300 million or more by 2025 [11]. Research to provide scientific data for plants with acclaimed hypoglycemic and anti-diabetic properties are on the increase. This became more apparent following WHO (1994) [12] recommendations regarding the need to develop and evaluate better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta-cell destruction, promoting beta-cell regeneration or repair and interrupting pathways leading to the various

Table 1. LD_{50} determination on aqueous extract

Phase one		
Doses (mg/kg)	**Number of rats/Group**	**Ratio of dead rats**
10	3	0/3*
100	3	0/3
1000	3	0/3
Phase two		
Doses (mg/kg)	**Number of rats/Group**	**Ratio of dead rats**
1000	1	0/1
1600	1	0/1
2900	1	1/1
5000	1	1/1

* *Number of dead animals/ number of animals used, LD_{50}= $\sqrt{a \times b}$. (Where a= highest non-lethal dose, b= least lethal dose) within 24 hours of extract administration, LD_{50}= $\sqrt{1600 \times 2900}$ = $\sqrt{4640000}$ = 2154 mg/kg (oral)*

Table 2. LD_{50} determination on ethanolic extract

Phase one		
Doses (mg/kg)	**Number of rats/Group**	**Ratio of dead rats**
10	3	0/3*
100	3	0/3
1000	3	1/3
Phase two		
Doses (mg/kg)	**Number of rats/Group**	**Ratio of dead rats**
800	1	0/1
1600	1	0/1
2600	1	1/1
3600	1	1/1

* *Number of dead animals/ number of animals used, LD_{50}= $\sqrt{a \times b}$. (Where a= highest non-lethal dose, b= least lethal dose). Within 24 hours of extract administration, LD_{50}= $\sqrt{1600 \times 2600}$ = $\sqrt{4160000}$ = 2039 mg/kg (oral)*

complications of diabetes. The major contributory factors to this growing interest include these recommendations, the cost and side effects of most orthodox hypoglycaemic agents, low therapeutic index of synthetic compounds and growing incidences of drug resistance [13,14]. Ojiako and Igwe, [15] has reported the use of *S. hispidus* for the treatment of diabetes by the ethnic tribal people of Africa. The major hindrance to the use of traditional herbal preparations is the lack of scientific and clinical data in support of better understanding of the efficacy and safety of the drugs [16]. In screening drugs, determination of LD_{50} is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. The oral LD_{50} of aqueous and ethanolic extracts of *S. hispidus* stem bark were found to be 2154 and 2039 mg/kg body weight (Tables 1 and 2) respectively in the present study. Treatment-related mortality (Tables 1 and 2) and signs of sedation, exophthalmos, decreased locomotion and appetite in the aqueous or ethanolic treated animals were observed in the early stages of experimentation at the dose of 1000 mg/kg body weight and above in the ethanolic extract and 1600 mg/kg body weight and above in the aqueous extract. According to [17], any substance with oral LD_{50} above 1000 mg/kg body weight in rats is regarded as being of low toxicity or relatively safe. Based on Hodge and Sterner scale [18], a test drug administered orally is considered extremely toxic at≤ 1 mg kg^{-1}, highly toxic at 1-50 mg kg^{-1}, moderately toxic at 50-500 mg kg^{-1}, slightly toxic at 500-5000 mg kg^{-1}, practically non toxic at 5000-15,000 mg kg^{-1} and relatively harmless at≥15,00 mg kg^{-1}. Agbaje et al. [19] has reported LD_{50} for both intraperitoneal and oral routes of *Syzigium aromaticum* (L.) in rodents as 265 and 2500 mg/kg body weight. The oral (rat) LD50 of ethanol extract of *Vitex leucoxylon* leaf (>3000 mg/kg), cold water infusion extract of the same plant (1050 mg/kg), ethanolic extracts of *Ailanthus excelsa* (1000 mg/kg), *Toddalia asiatica* (350 mg/kg) and *Araucaria bidwilli* (250 mg/kg) have been reported [20]. The scarcity of scientific data to validate local claims on the use of *S. hispidus* for the treatment of diabetes as well as lack of data about its tolerable dosage hinders meaningful comparison.

5. CONCLUSION

The findings from this study indicated that *S. hispidus* stem bark extracts may be slightly toxic at high doses. *S. hispidus* may also be considered to be relatively safe since the qualitative and quantitative acute toxicity in animals only has little relevance to human acute toxicity in special cases and after many years of usage. The results also showed that ethanolic extract has lower acute toxicity than aqueous extract of *S. hispidus*. Despite these justifications that *S. hispidus* stem bark extracts may be relatively safe, moderate dosage is advised for purposes of medication. Also, studies to establish safer and therapeutic doses of *S hispidus* stem bark for specific ailments as well as research on its cytotoxic effect on animal model are recommended in order to ascertain its true toxic level.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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