

ACUTE TOXICITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *Strophanthus hispidus* STEM BARK

ABSTRACT

The aim of this study was to determine the median lethal dose (LD₅₀) 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 LD₅₀ 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.

Keywords: Medication, exophthalmos, mortality, experimentation.

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 [16]. 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 [6][7][10]. 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 [8].

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. [15]. This work is therefore aimed at providing scientific records as to the amount of *S hispidus* that may constitute potential risk in animal model.

MATERIALS AND METHODS

Animals

Male rats (Wistar strain) obtained from the Department of Anatomy, University of Benin, Benin City, Nigeria were used. The rats weighing between 100-232g 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. (NIH, 1992).

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.

Preparation and Extraction of Plant Material

A modified method of Onoagbe *et al.*, (1999a) 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 72hrs 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 (125mm) filter paper. The filtrates collected were lyophilized using a freeze-dryer and stored in an airtight container for further analysis.

Acute toxicity study

The method of Lorke, (1983) 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}).

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RESULTS

95 The results for the acute toxicity study of both extracts of *S hispidus* stem bark are presented in
 96 tables 1-2. 1000 mg/kg body weight and above in the ethanolic extract (Table 2) and 1600 mg/kg
 97 body weight and above in the aqueous extract (Table 1) resulted in mortality. The LD₅₀ was
 98 beyond 2000 mg/kg for both extracts. (Tables 1-2).

99 **Table 1: LD₅₀ determination on aqueous extract**

PHASE ONE

101	Doses (mg/kg)	Number of rats/group	Ratio of dead rats
102	10	3	0/3*
103	100	3	0/3
104	1000	3	0/3
105	PHASE TWO		
106	1000	1	0/1
107	1600	1	0/1
108	2900	1	1/1
109	5000	1	1/1

110 * Number of dead animals/ number of animals used.

111 LD₅₀ = $\sqrt{a \times b}$. (Where a= highest non-lethal dose, b= least lethal dose) within 24 hours of
 112 extract administration.

113 LD₅₀ = $\sqrt{1600 \times 2900} = \sqrt{4640000} = 2154$ mg/kg (oral)

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120 **Table 2: LD₅₀ determination on ethanolic extract**

121 **PHASE ONE**

122	Doses (mg/kg)	number of rats/group	Ratio of dead rats
123	10	3	0/3*
124	100	3	0/3
125	1000	3	1/3
126	PHASE TWO		
127	800	1	0/1
128	1600	1	0/1
129	2600	1	1/1
130	3600	1	1/1

131 * Number of dead animals/ number of animals used.

132 LD₅₀= $\sqrt{a \times b}$. (Where a= highest non-lethal dose, b= least lethal dose). Within 24 hours of
 133 extract administration.

134 LD₅₀= $\sqrt{1600 \times 2600} = \sqrt{4160000} = 2039$ mg/kg (oral)

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DISCUSSION

149 Diabetes mellitus is a disease that is increasingly affecting millions of people all over the world.
150 And this disease has no known cure in spite of its age long existence. Currently there are over
151 150 millions diabetics worldwide and this is likely to increase to 300 million or more by 2025
152 [19]. Research to provide scientific data for plants with acclaimed hypoglycemic and anti-
153 diabetic properties are on the increase. This became more apparent following WHO (1994)
154 recommendations regarding the need to develop and evaluate better pharmacological agents for
155 improving insulin secretion, enhancing insulin sensitivity, preventing beta-cell destruction,
156 promoting beta-cell regeneration or repair and interrupting pathways leading to the various
157 complications of diabetes. The major contributory factors to this growing interest include these
158 recommendations, the cost and side effects of most orthodox hypoglycaemic agents, low
159 therapeutic index of synthetic compounds and growing incidences of drug resistance [14][18].
160 Ojiako and Igwe, (2009) has reported the use of *S. hispidus* for the treatment of diabetes by the
161 ethnic tribal people of Africa. The major hindrance to the use of traditional herbal preparations is
162 the lack of scientific and clinical data in support of better understanding of the efficacy and
163 safety of the drugs [17]. In screening drugs, determination of LD₅₀ is usually an initial step in the
164 assessment and evaluation of the toxic characteristics of a substance. The oral LD₅₀ of aqueous
165 and ethanolic extracts of *S. hispidus* stem bark were found to be 2154 and 2039 mg/kg body
166 weight (Tables 1 and 2) respectively in the present study. Treatment- related mortality (Tables 1
167 and 2) and signs of sedation, exophthalmos, decreased locomotion and appetite in the aqueous or
168 ethanolic treated animals were observed in the early stages of experimentation at the dose of
169 1000 mg/kg body weight and above in the ethanolic extract and 1600 mg/kg body weight and
170 above in the aqueous extract. According to [3], any substance with oral LD₅₀ above 1000 mg/kg
171 body weight in rats is regarded as being of low toxicity or relatively safe. Based on Hodge and

Sterner scale [2], a test drug administered orally is considered extremely toxic at $\leq 1 \text{ mg kg}^{-1}$, highly toxic at $1-50 \text{ mg kg}^{-1}$, moderately toxic at $50-500 \text{ mg kg}^{-1}$, slightly toxic at $500-5000 \text{ mg kg}^{-1}$, practically non toxic at $5000-15,000 \text{ mg kg}^{-1}$ and relatively harmless at $\geq 15,00 \text{ mg kg}^{-1}$. Agbaje *et al.*, (2009) has reported LD₅₀ 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 leucoxydon* leaf ($>3000 \text{ mg/kg}$), 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 [4]. 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. 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.

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