

Original Research Article**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 further for 72 hrs. Treatment-related mortality was observed at doses of 1000 mg/kg body weight and above for both extracts. 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 doses of up to 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.

22 INTRODUCTION

23 Plants produce a great diversity of substances that can have therapeutic significance for
 24 maintaining human health and improving the quality of human life, thus justifying their use in
 25 traditional medicine [14]. According to their traditional use, natural compounds are often
 26 assumed to be safe. However, several studies have reported that a great number of plant species
 27 used as food ingredients or in traditional medicine present mutagenic, carcinogenic or toxic
 28 properties [6][7][10]. Drug toxicity can occur on many different time-scales. Acute toxicity
 29 results from a single exposure to a drug, with adverse effects resulting in minutes to hours [5]. A
 30 fundamental goal of toxicology is to determine safe levels of exposure to potentially poisonous
 31 substances for human and the environment [5].

32 The scientific exploitation of herbs used ethno-medically for pain relief, wound healing and
 33 abolishing fevers has resulted in the identification of a wide range of compounds that have been
 34 developed as new therapies for several ailments including diabetes [8].

35 Only few of these herbal products have scientific-research information about their toxic levels in
 36 literatures. Acute toxicity testing can provide a guide to selection of doses of herbal medicines,
 37 in order to avoid potential harmful effects when used. *Strophanthus hispidus* is an African shrub
 38 that belongs to the genus; *Strophanthus*, the family of Apocynaceae. Its popularity in folklore
 39 medicine as well as its bitter taste may not be unconnected with the presence of important
 40 phytochemicals such as saponins, alkaloids, phenols, flavonoids and tannins. [13]. This work is
 41 therefore aimed at providing scientific records as to the amount of *S. hispidus* that may constitute
 42 potential risk in animal model.

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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.

Medicinal plant

The stems of *Strophanthus 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

The stems of *Strophanthus 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 [9] 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 carried on 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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91 Results

92 The results for the acute toxicity study of both extracts of *S. hispidus* stem bark are presented in
93 the tables below:

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

95 PHASE ONE

96	Doses (mg/kg)	Number of rats/group	Ratio of dead rats
97	10	3	0/3 [*]
98	100	3	0/3
99	1000	3	0/3
100	PHASE TWO		
101	1000	1	0/1
102	1600	1	0/1
103	2900	1	1/1
104	5000	1	1/1

105 ^{*} Number of dead animals/ number of animals used.

106 $LD_{50} = \sqrt{a \times b}$. (Where a= highest non-lethal dose, b= least lethal dose) within 24 hours of
107 extract administration.

108 $LD_{50} = \sqrt{1600 \times 2900} = \sqrt{4640000} = 2154 \text{ mg/kg (oral)}$

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Table 2: LD₅₀ 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		
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.
 $LD_{50} = \sqrt{1600 \times 2600} = \sqrt{4160000} = 2039 \text{ mg/kg (oral)}$

141 Discussion

142 Research to provide scientific data for plants with acclaimed hypoglycemic and anti-diabetic
143 properties are on the increase. This became more apparent following WHO (1994)
144 recommendations regarding the need to develop and evaluate better pharmacological
145 agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta-cell
146 destruction, promoting beta-cell regeneration or repair and interrupting pathways leading to
147 the various complications of diabetes. The major contributory factors to this growing
148 interest include these recommendations, the cost and side effects of most orthodox
149 hypoglycaemic agents, low therapeutic index of synthetic compounds and growing
150 incidences of drug resistance [12][16]. [11] Reported the use of *S. hispidus* for the treatment of
151 diabetes by the ethnic tribal people of Africa. The major hindrance to the use of traditional
152 herbal preparations is the lack of scientific and clinical data in support of better
153 understanding of the efficacy and safety of the drugs [15]. In screening drugs,
154 determination of LD₅₀ is usually an initial step in the assessment and evaluation of the toxic
155 characteristics of a substance. The oral LD₅₀ of aqueous and ethanolic extracts of *S. hispidus* stem
156 bark were found to be 2154 and 2039 mg/kg body weight (Tables 1 and 2) respectively in the present
157 study. Treatment- related mortality (Tables 1 and 2) and signs of sedation, exophthalmos, decreased
158 locomotion and appetite in the aqueous or ethanolic treated animals were observed in the early
159 stages of experimentation at doses of up to 1000 mg/kg body weight and above in both extracts.
160 According to [3], any substance with oral LD₅₀ above 1000 mg/kg body weight in rats is regarded as
161 being of low toxicity or relatively safe. Based on Hodge and Sterner scale [2], a test drug
162 administered orally is considered extremely toxic at $\leq 1 \text{ mg kg}^{-1}$, highly toxic at $1\text{-}50 \text{ mg kg}^{-1}$,
163 moderately toxic at $50\text{-}500 \text{ mg kg}^{-1}$, slightly toxic at $500\text{-}5000 \text{ mg kg}^{-1}$, practically non
164 toxic at $5000\text{-}15,000 \text{ mg kg}^{-1}$ and relatively harmless at $\geq 15,00 \text{ mg kg}^{-1}$. [1] 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 leucoxylo*n 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 [4]. The findings from this study indicated that *S. hispidus* stem bark extracts may be slightly toxic at high doses or relatively safe more so that 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 are recommended.

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