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ACUTE TOXICITY OF AQUEOUS AND ETHANOL EXTRACTS OF *DIALIUM GUINEENSE* STEM BARK

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ABSTRACT

Background and objective: The safety of plant-derived bioactive compounds has become a global concern. The present study investigated the acute toxicity of aqueous and ethanol extracts of *Dialium guineense* stem bark using Wistar rats. **Methods:** Adult male Wistar rats (n = 13) weighing 150 – 180 g (mean weight = 165 ± 15 g) were used for this study. The aqueous and ethanol extracts of the plant stem bark were obtained using cold maceration method. Lorke method was used to determine oral LD_{50} of the extracts. Signs of toxicity and possible death of rats were also monitored for twenty-four (24) h. **Results:** The major signs of toxicity observed within 24 h were: difficulty in breathing, loss of appetite and general weakness. No deaths were recorded in both phases and all the animals in each phytochemical group survived. The oral LD_{50} of aqueous and ethanol extracts of *D. guineense* were greater than 5000 mg/kg body weight (bwt). **Conclusion:** The results of this study suggest that aqueous and ethanol extracts of *D. guineense* stem bark are not toxic at doses not exceeding 5000 mg/kg bwt.

Keywords: *Dialium guineense*, Acute toxicity, Rats, Extract, Lethal dose.

INTRODUCTION

In recent times, plant-derived substances have become of huge importance to man due to their many applications. Extraction methods involve the separation of medicinally active portions of plant tissues from the inactive/inert components using selective solvents [1 – 3]. These plant components exist as complex mixtures of many medicinal metabolites, such as alkaloids, glycosides, terpenoids, phenols, flavonoids and lignans [4 - 6]. Medicinal plants have long been recognized as important sources of therapeutically active compounds [7]. Evidence-based research supports the medical and pharmacological benefits of plant-derived compounds with interest in the identification and characterization of bioactive compounds from natural sources [8 - 10].

Dialium guineense (Velvet Tamarind), is a tall, tropical, fruit-bearing tree which belongs to the *Leguminosae* family. It has small, typically grape-sized edible fruits with brown hard inedible shells. *Dialium guineense* grows in dense forests in Africa along the southern edge of the Sahel and it can be found in West African countries such as Ghana where it is known as "Yoyi", Sierra Leone, Senegal, Guinea-Bissau and Nigeria where it is known as "*Awin*"or "*Igbaru*" in Yoruba, "*Icheku*" in Igbo, "*Tsamiyarkurm*"in Hausa and "*Amughen*" in Edo. The bark and leaves have medicinal properties and are used against several diseases [11]. Despite the widespread use of *D. guineense* in medical research, the safety of its bioactive components is rarely reported. Extracts of the plant are reported to be rich in important phytochemicals [12, 13]. The present study investigated the acute toxicity of aqueous and ethanol extracts of *Dialium guineense* stem bark using Wistar rats.

METHODS

Experimental rats

Adult male Wistar rats (n = 13) weighing 150 – 180 g (mean weight = 165 ± 15 g) were obtained from the Department of Anatomy, University of Benin, Benin City. The rats were housed in metal cages under standard laboratory conditions: temperature of 25 °C, 55 – 65 % humidity and 12-h light/12-h dark cycle. They were allowed free access to rat feed (pelletized growers mash) and clean drinking water. Prior to commencement of the study, the rats were acclimatized to the laboratory environment for one week. The study protocol was approved by the Faculty of Life Sciences Ethical Committee on Animal Use.

Collection of Plant Material

The stem barks of *D. guineense* were obtained from Auchi Area of Edo State, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

Plant preparation and extraction

The stem bark was brushed and shade-dried at 30 °C for a period of two weeks and crushed into small pieces using clean mortar and pestle. Aqueous and ethanol

extracts of the stem bark were obtained using cold maceration method as described previously [14 - 16].

Acute toxicity test

This was carried out using the Lorke's method [17], which comprised two phases. In the first phase, nine (9) rats were divided into three groups of three (3) rats each. Each group of rats was administered aqueous or ethanol extract of *D. guineense* stem bark orally at doses of 10, 100 and 1000 mg/kg bwt. The rats were placed under observation for 24 h to monitor their behavior and mortality. In the second phase, 4 rats were randomly assigned to four groups of 1 rat each. The rats were administered higher doses of aqueous or ethanol extract of *D. guineense* stem bark orally at doses of 1600, 2900 and 5000 mg/kg bwt, and then, observed for 24 h for signs of behavior and mortality.

The lethal dose (LD_{50}) of aqueous and ethanol extract was calculated thus:

$$LD_{50} = \frac{\sqrt{D0 + D100}}{2}$$

where D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality

Table 1: Acute toxicity study on aqueous and ethanol extracts of *D. guineense* stem bark on Wistar rats

Phase 1		
Groups	**No. of rats**	**Dose (mg/kg bwt)**
1	3	10
2	3	100
3	3	1000
Phase 2		
1	1	1500
2	1	2500
3	1	2900
4	1	5000

STATISTICAL ANALYSIS

Data are expressed as mean ± SEM, and statistical analysis was performed using GraphPad Prism Demo (6.07).

RESULTS

Results of acute toxicity study on aqueous and ethanol extracts of *D. guineense* stem bark on Wistar rats.

No mortality was recorded even at 5000 mg/kg bwt in the saponins and tannins groups (Tables 2 & 3).

Table 2: Outcome of acute toxicity study of aqueous extract of *D. guineense* stem bark

Dose (mg/kg bwt)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
1600	1	0	1	0/1
2900	1	0	1	0/1
5000	1	0	1	0/1

Data are number of death and survival of rats.

No. of deaths recorded = Nil

No. of rats that survived = All

Mortality ratio = no. of death/no. of survival

Oral LD_{50} > 5000 mg/kg bwt.

Table 3: Outcome of acute toxicity study of ethanol extract of *D. guineense* stem bark

Dose (mg/kg bwt)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
1600	1	0	1	0/1
2900	1	0	1	0/1
5000	1	0	1	0/1

Data are number of death and survival of rats.

No. of deaths recorded = Nil

No. of rats that survived = All

Mortality ratio = no. of death/no. of survival

Oral LD_{50} > 5000 mg/kg bwt.

DISCUSSION AND CONCLUSION

There have been growing interests in the toxicity of substances purified from plants basically to determine their safety. This study investigated the acute toxicity of aqueous and ethanol extracts of *Dialium guineense* stem bark using Wistar rats.

The results showed that no death was recorded in both phases after 24 h and all the rats in each group survived. The major signs of toxicity observed within 24 h were: difficulty in breathing, loss of appetite and general weakness. Thus, the median lethal dose LD_{50} (oral) of aqueous and ethanol

extracts of *D. guineense* stem bark were greater than 5000 mg/kg bwt. These results suggest that the aqueous and ethanol extracts may be relatively safe [16]. One major and overriding criterion in the selection of herbal medicine for use in health services is safety. Phytochemicals present in plant extracts should not only be clinically effective, but safe for consumption. Therefore, screening of bioactive components present in plant extracts to identify their toxic potentials is necessary for selection of plants for drug formulations [18 - 20].

SIGNIFICANT STATEMENT

The results obtained in this study show that aqueous and ethanol extracts of *D. guineense* stem bark are not toxic at doses not exceeding 5000 mg/kg bwt.

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