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Phytochemical screening and oral acute toxicity study of aqueous leaf extract of *Crinum giganteum* (gadalli) in Wistar rats

F. B. Elizabeth[1,2]*, E. N. Obikili[1], A. E. Esom[1], G. E. Anyanwu[1]

[1]Department of Anatomy, University of Nigeria, UNEC, Enugu, Nigeria, [2]Department of Anatomy, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

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***Address for correspondence:**
F. B. Elizabeth,
Department of Anatomy,
University of Nigeria,
UNEC, Enugu, Nigeria.
Tel.: +2348084113179,
E-mail: finbello@yahoo.com

ABSTRACT

Different parts of plants possess variable phytochemical compounds and median lethal dose (LD_{50}). *Crinum giganteum* (gadalli) is one of the plants most commonly consumed in parts of Africa for its various medicinal values. Despite this popularity, the phytochemical screening and the toxicity of the leaf extracts were yet to be evaluated. This study was designed to identify the bioactive agents and estimate the LD_{50} for the aqueous leaf extract of gadalli. Phytochemical screening was done using standard methods. Each bioactive agent and the LD_{50} were estimated by the arithmetic methods of Karber. Phytochemical analysis revealed high presence of alkaloids, saponins, and slight presence of glycosides, whereas the oral LD_{50} was found to be 200 mg/kg. The major active ingredients of aqueous leaf extract of gadalli are alkaloids and saponins. It is observed to be unsafe at 200 mg/kg and above.

KEY WORDS: Alkaloids, *Crinum giganteum*, glycosides, median lethal dose, phytochemicals, saponins

INTRODUCTION

Phytochemicals or bioactive agents describe secondary metabolic compounds found in plants (Engwa *et al*., 2011; Sasidharan *et al*., 2011; Yadav and Munin, 2011; Kennedy and Wightman, 2012) and are synonymously called phytonutrients, phytofood, and nutraceuticals (Prashant *et al*., 2011; Shorbha, 2012). They evolve as chemicals produced by plants against predators and infections, such as bacteria, insects, and fungi (Shorbha, 2012; Engwa *et al*., 2013). These agents can be derived from any plant parts such as the barks, leaves, flowers, roots, fruits, stems, seeds, and bulbs (Yadav and Munin, 2011). Generally, different plants and/or parts have different composition and load of phytochemical agents and the predictions depend on the type of solvents used in the extraction (Shorbha, 2012; Lalnundanga and Lalrinkima, 2012). Several studies have consistently reported the following secondary bioactive substances in medicinal plant extracts: Alkaloids, steroids, phenols, tannins, saponins, flavonoids, and glycosides (Sasidharan *et al*., 2011; Yadav and Munin, 2011).

While the phytochemicals dictate the biological activity of the extract, the median lethal dose (LD_{50}) indicates the degree of safety of a particular plant extract. Studies have shown that extracts of a plant part have different LD_{50} across the different routes of administration and animal species (Rafaat *et al*., 2012; 2013). *Crinum giganteum* (gadalli) is a perennial herb found in parts of Cameroun, Niger, and Nigeria. The plant is also known by other names in different ethnic groups across Africa (Keay 1989). It is called Wadalo by the Bororo's of Cameroun and Daffun of Adamawa in North-East Nigeria. The Bororo's of the Niger Republic calls it Lubo and among the Hausa's in northern Nigeria, it is known as Albacce Buru or Albacce Dawaddi (Keay, 1989). The bulb extract of *C. giganteum* has been found to possess alkaloids and tannins with intraperitoneal LD_{50} of 627 ± 5.8 mg/kg and 520 ± 10.2 mg/kg in mice and rats, respectively and also oral LD_{50} of 1486 ± 18.9 mg/kg and 1023 ± 4.3 mg/kg in mice and rats, respectively (Kapu *et al.,* 2001). There is a paucity of information on the phytochemical agents and LD_{50} of the aqueous leaf extract of *C. giganteum* despite the increasing rate of the consumption of the leaf and other parts of this plant as a

result of its various medicinal values. Hence, this study was designed to evaluate the bioactive agents and estimate the oral LD_{50} for the aqueous leaf extract.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plant material was procured from an open market in Adamawa State, North-East of Nigeria. Identification was done by a taxonomist; Prof. Mrs. M. O Nwosu of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Herbarium sheet was prepared and a voucher specimen (UNH/13/401) was deposited at the herbarium of same department.

Phytochemical Screening

The gadalli leaves were washed with distilled water and air-dried under shade for 7 days. Thereafter, the leaves were pulverized into fine powder by pestle and mortar. Hundred grams of the dried leaf powder were placed in a beaker containing 500 ml of distilled water and heated using a hot plate with continuous stirring at 30-40°C for 20 min. The mixture was then allowed to cool and filtration was done using a mesh cloth. The extract was then evaluated for the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids, and tannins by using the following procedure.

Test for alkaloids

Exactly one milliliter (1 ml) of 1% hydrochloric acid was added to 3 ml of the extract in a test tube. The mixture was heated for 20 min in a water bath, allowed to cool, and then filtered. Two drops of Wagner's reagent were added to 1 ml of the filtrate. A reddish brown precipitate observed indicated the presence of alkaloids.

Test for flavonoids

About 1 ml of 10% sodium hydroxide (NaOH) was added to 3 ml of the extract. Absence of yellow coloration indicated the absence of flavonoids.

Test for glycosides

About 10 ml of 50% tetraoxosulfate (VI) acid (H_2SO_4) was added to 1 ml of the extract in a test tube. The mixture was heated in boiling water for 15 min. About 10 ml of Fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed in the test mixture, which indicated the presences of glycosides.

Test for phenols

A small portion (1 ml) of the extract was added to 1 ml of water and few drops of 5% NAOH were added. Absence of orange coloration is indicative of the absence of phenol.

Test for saponins

The presences of saponins were detected using frothing test. In the test, 2 ml of the extract was vigorously shaken for 2 min. Frothing observed in the extract indicated the presences of saponins.

Test for steroids

Presence of steroids was investigated using Salkowski test in which 5 drops of concentrated (H_2SO_4) were added to 1ml of the extracts. No red coloration was observed in the mixture, indicating the absence of steroids.

Test for tannins

Exactly 2 ml of the extract was boiled gently for 2 min and allowed to cool. Three drops of ferric chloride solution were added. Absence of green coloration is indicative of the absence of tannins.

Procurement and Care of Animals

About 20 adult Wistar rats of both sexes of average weights of 200 g were purchased from the animal house of the College of Medicine, University of Nigeria, Enugu Campus, and housed in the Animal facility of the same institution. The animals were housed in netted iron cages in groups of four, fed with grower's mash and provided water *ad libitum*. The rats were maintained under standard laboratory conditions (temperature 24°C ± 2°C, with relative humidity of 60-70%, and a 12 h light-dark cycle). They were allowed to acclimatize for 2 weeks before the experiment. This study was approved by the University of Nigeria Teaching Hospital, Health Research Ethics Committee, with certificate number NKREC/05/01/2008B-FWA00002458-1RB00002323.

Oral Toxicity Study

The animals were divided into five groups (A-E) of four animals each. Animals in Groups B-E served as the toxicity test groups whereas that in Group A were taken as the control group. Animals in Groups B, C, D, and E were orally administered increasing doses of 200, 400, 800, and 1600 mg/kg of the aqueous extract of *C. giganteum* per body weight, respectively. The control group received 0.1 ml of distilled water orally. The animals were observed for signs of acute toxicity such as behavioral changes and death over 48 h and LD_{50} was estimated using the arithmetic method of Karber as modified by Aliu and Nwude (1982).

RESULTS

Phytochemical Analysis

From the results of the various phytochemical screening, high presences of alkaloids and saponins, and slight presence of glycosides were noted. Tannins, phenols, flavonoids, and steroids were found to be absent in the extracts studied (Table 1).

Oral Acute Toxicity Study

None of the animals in Groups B and C showed any clinical or behavioral changes throughout the observational period. However, depression, weakness, and loss of appetite in the first 3 h were observed in Groups D and E animals treated with the higher doses of the extract. Attempt at recovery from toxicity was observed on the 8th h in two animals in Group D and one in Group E whereas other showed clonic convulsion, anesthesia, tonic extension, salivation, and eventually died within the 48 h after the administration. The LD_{50} of the *C. giganteum* extract was estimated as described by Aliu and Nwude (1982) (Table 2) and calculated to be 200 mg/kg body weight.

The LD_{50} was calculated using the formula:

$$LD_{50} = LD_y - \sum(Ddx\ md)\ N$$

Where,

LD_y = Highest dose

Table 1: Result of phytochemical screening of the aqueous leaf extract

Parameter	Remark
Alkaloids	+++
Saponins	+++
Flavonoids	---
Phenols	---
Steroids	---
Tannins	---
Glycosides	+--

Highly present (+++), Slightly present (+--), Absent (---)

Table 2: Oral acute toxicity (LD_{50}) of *C. giganteum* in rats

Dose (mg/kg)	Number of rats	Death	Dose difference (a)	Mean death (b)	Probit=dose diff/mean death (a and b)
Control (1 ml, 0.9% saline)	4	0	0	0	0
200	4	0	0	0	0
400	4	0	200	0	0
800	4	2	400	1	400
1600	4	3	800	2.5	2000
					2400

LD_{50}: Median lethal dose, *C. giganteum: Crinum giganteum*

N = Number of animals per group

Dd = Dose difference

Md = Mean dead

$$LD_{50} = \frac{2400}{400} = 600$$

800-600 = 200 mg/kg

DISCUSSIONS

The presence of alkaloids, saponins, and glycosides indicate that the plant has some medicinal properties which can be exploited for therapeutic purposes (Jodi *et al*., 2008). Phytochemical analyses of the aqueous bulb extract of *C. giganteum* had revealed the presences of the other bioactive agent such as tannins (Amos *et al*., 2003), which was not found in the aqueous leaf extract in the present study. This confirmed that different parts of a plant could possess different composition or load of phytochemical agents (Shorbha, 2012). The presence of alkaloids agrees with the fact that the *Crinum* species are generally well known rich sources of alkaloids (Rafaat *et al*., 2012; Rafaat *et al*., 2013).

The estimated oral LD_{50} for the aqueous leaf extract of *C. giganteum* (gadalli) in this study was 200 mg/kg. This implies that doses below the LD_{50} can be considered safe for experimental study using this extract. Precisely, one-tenth of the LD_{50} has been advocated for in the choice of safe dose for use in various studies or clinical trials (Jodi *et al*., 2008). The oral LD_{50} of 200 mg/kg of this extract was different from reports of Amos *et al*. (2003) that found intraperitoneal and oral LD_{50} of 627 mg/kg and 1468 mg/kg, respectively, of the aqueous bulb extract of *C. giganteum* in mice.

The part of a plant, route of exposure of drug and the specie of animal used account for the various differences in the LD_{50} values of an extract. The variations observed in the LD_{50} values across different routes of exposure have been attributed to the plasma concentrations and bioavailability of the extracts. Particularly, for an extract, this pharmacological properties might be influenced by the nature or properties of the active principles (phytochemicals) present in the extract.

CONCLUSION

The aqueous leaf extract of *C. giganteum* contain alkaloids, glycosides, and saponins whereas the LD_{50} was found to be 200 mg/kg. However, it is considered safe at doses lower than 200 mg/kg.

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