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Preliminary Anticonvulsant Screening of *Crinum ornatum* Bulb Extract

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Abstract: The bulb of *Crinum ornatum* (Ait) Bury of the family Amaryllidaceae is a medicinal herb used for the treatment of convulsion. Phytochemical screening revealed the presence of alkaloids and polyphenolic compounds. An LD_{50} of 193.2mg/ml (Best fit value) was obtained when doses 25mg/ml-500mg/ml were applied to groups of seven albino mice of average weight 15g in the acute toxicity study. *C. ornatum* exhibited strong anticonvulsant activity reducing the hind limb tone extension significantly ($P \leq 0.05$) when subjected to anticonvulsant test (Maximal Electro Shock Test {MEST}). The percentage inhibition of shock and response against time observed were 100% at dose 2mg/ml which shows the ability of the crude extract to reduce tonic-clonic seizures.

Key words: Amaryllidaceae · *Crinum ornatum* · Phytochemical screening · Toxicity · Anticonvulsant activity

INTRODUCTION

The genus *Crinum* belongs to the Family-Amaryllidaceae, Phylum- Angiospermae, Subphylum-Liliflorae and comprises approximately 160 species distributed throughout the tropics and warm temperate regions of the world in Africa, America, Asia and Australia. The C*rinum species* are bulbous plant with spirally arranged leaves and conspicuous flowers maturing together. They have commercial, economical and medicinal importance. Hybridization of *Crinum* plants is widespread [1]. The plant attracts considerable attention due to various medicinal properties as antitumor, immunostimulating, analgesic, antiviral, antibacterial and antifungal [2]. The bulb of *Crinum species* is used majorly as an anticonvulsant in folk medicine in Nigeria just like in many other African Countries. One major cause of convulsive seizures has been linked to injury in the brain caused by oxidation reactions. The anticonvulsant effect of the methanolic extract of the bulb of *Crinum ornatum* was therefore determined by electroshock test. This is because it has been established that anticonvulsant drugs that suppress Hind Limb Tone Extension (HLTE) in Maximum Electrode Shock Test (MEST) are effective in the therapy of generalized tonic - clonic and partial seizures [3]. Acute toxicity study was carried out to establish the range of doses producing toxicity and to determine the lethal dose. The plant was also phytochemically screened for secondary metabolites even though a series of work has been conducted on the chemistry of the *Crinum species*. In this research, phytochemical screening is important so as to determine the effect of, if any of seasonal variation on the plant.

MATERIALS AND METHODS

Plant Material/Extraction Procedure: Fresh bulbs of *C. ornatum* were collected in June, at Ife Road in Ibadan North Local Government Area of Oyo State, Nigeria and specimens were identified and authenticated at Forestry Research Institute of Nigeria, Ibadan (No FHI 105367). The bulbs were air-dried under mild sunshine for 10 days and ground into fine powder with a Hammer Mill (Ashai 7500) and kept in non-absorptive nylon for subsequent use. The powdered bulb (1kg) was extracted with methanol by cold extraction method. The filtrates obtained by successive extraction were combined and evaporated to dryness in a rotary evaporator at 37°C and stored in a dessicator prior to further analysis.

Animals: Albino mice (males) weighing 14-16 g bred in the Animal House of the Department of Physiology, University of Ibadan were used for acute toxicity study. These animals which were 12 weeks old were fed on standard mouse cubes (Pfizer Feeds, plc) and allowed free access to water.

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Adult Wistar rats (males) weighing 160 – 220 g (14 weeks old) obtained from the animal House of the Department of Physiology, University of Ibadan were used for anticonvulsant screening. The animals were divided into two sets, one set for the experimental test and the other serving as control test. Five animals were used per group. The rats were fed with balanced livestock feed from Pfizer, plc and water was also given *ad libitum*.

Phytochemical Screening: Alcoholic extract of the bulb of *C. ornatum* was phytochemically screened for the following chemical constituents alkaloids, saponins, flavonoids, tannins, anthraquinones, cardiac glycosides, proteins, carbohydrates, fats and oil using the method of screening as described by Harborne [4].

Acute Toxicity Study: The mice were grouped seven animals per cage. Doses ranging from between 25 to 500mg/ml were employed per group to establish the range of doses producing toxicity and to determine the lethal dose. The methanolic extract of *C. ornatum* was administered intraperitoneally to the animals. 1ml distilled water (vehicle for the extract) was used as control. The animals were observed for 48hrs under room temperature. Percentage mortality was then calculated. LD_{50} was determined by using sigmoidal dose-response (variable slope) model of non-linear curve-fitting.

Screening for Anticonvulsant Activity: Anticonvulsant screening was conducted using the Maximal Electroshock Test (MEST) [5]. Adult Wistar rats (males) weighing 160–220g (14 weeks old) were used. The animals were divided into two sets, one set for the experimental test and the other serving as control test. The rats were grouped five per cage. Each animal served as its own control being placed on stimulator to enable it adapt to it. The crude methanolic extract was dissolved into 2.5% Tween 80 (5ml). Doses ranging from 0.5 to 2mg/ml were injected intraperitoneally into the animals. The Ealing Kymograph with in - built stimulator (Student Kymograph Basic Stimulator) was used to measure the degree of shock or convulsive seizures by considering the hind limb tonic extension (HLTE). The machine was set at two different voltages 25x25v and 15x25v to induce electroshock. Different concentration of the extract was injected intraperitoneally into the animals while 1ml Tween 80 was used for the control animals. The results were noted at 30min, 1hr and 2hrs intervals. Abolition of HLTE was considered as protection from electroshock. Response against time at each applied voltage and the number of animals protected at each concentration were noted [6].

RESULTS AND DISCUSSION

Results of phytochemical screening showed the presence of alkaloids, flavonoids, volatile oil, carbohydrates, cardiac glycoside and tannins while saponin and protein/amino acid are absent. This result is in agreement with other workers. An LD_{50} of 193.2mg/ml (Best fit value) was obtained when doses 25mg/ml-500mg/ml were applied to groups of seven albino mice of average weight 15g (Table 1). In the anticonvulsant screening, the animals served as the control groups and the responses were observed as time, concentration and voltage changes. The degree of response is time and concentration dependent at the two

Table 1: LD_{50} of *C ornatum* extract using sigmoidal dose-response (variable slope) model of non-linear regression curve-fitting

	LD_{50}(mg/ml)		
Extract	Best fit value	95% CI	R^2
Methanol	193.2	192.7-193.7	1.000

Best fit value± S.E. (S.E =Standard error):

Bottom = -5.311e-014±3.506e-009, Top = 100± 4.040e-009, Hillslope = 51.75±1.153

Goodness of fit:

Degrees of freedom = 3, Absolute sum of squares = 1.107e-016, Sy.x = 6.073e - 009

Table 2: Reponse against time for Crude extract at 0.5 - 2mg/ml*

	Control		Crude extract 0.5mg/ml		Crude extract 1.0mg/ml		Crude extract 2.0mg/ml	
Readings	15x25v	25x25v	15x25v	25x25v	15x25v	25x25v	15x25v	25x25v
Initial reading	4	5	4	5	4	5	4	5
30 min	0	0	1.5	3.5	2.5	4.5	3.5	5
1hr	0	0	0.5	2.0	1.5	3.5	2.5	4.5
2 hrs	0	0	0	0	1.0	2.5	1.5	3.5

* The degree of response at the two voltages applied (15x25v and 25x25v) for inducing electroshock at 30min, 1hr and 2hrs interval at 0.5 - 2mg/ml for Crude extract.

Table 3: % Animals Protected from Electroshock Induced Seizures at 25 x 25v and 15 x 25v*

	Dose mg/ml	No of animals protected 30min 1hr 2hrs	% animals protected
Control	0.5-2.0	0 0 0	0
Group 1	2.0	5/5 4/5 3/5	100, 80, 60
Group 2	1.0	4/5 3/5 2/5	80, 60, 40
Group 3	0.5	2/5 1/5 0/5	40, 20, 0

* The percentage of animals protected at the two voltages applied (25x25v and 15x25v) for inducing electroshock at doses ranging from 1.0 - 0.5mg/ml for Crude extract.

voltages applied for inducing the electroshock. The crude extract gave the highest response at 2mg/ml, the response however decreases at 1mg/ml and 0.5mg/ml. Therefore dose dependent anticonvulsant effect was observed, activity being greater at 2mg/ml for the crude extract (Table 2). The% of animals protected in the anticonvulsant effect of *C. ornatum* crude extract on HLTE phase in MEST at 25 x 25V and 15 x 25V at doses 0.5 to 2.0mg/ml is shown in Table 3. Rats treated with the crude methanolic extract were protected against electroshock induced seizure. The protection was dose dependent and was sustained for up to 2hrs. At 30 min after the crude extract was injected, a maximum protection (100%) against electroshock induced seizure was observed at a dose of 2mg/ml. This effect was sustained for up to 2hrs for 2mg/ml and 1mg/ml. However, no animal was protected beyond the first hour at a concentration of 0.5mg/ml.

The anticonvulsant activity of the crude extract at a dose of 2mg/ml in HLTE of MEST suggests that this fraction possess anticonvulsant activity for the treatment of generalised tonic – clonic and partial seizures (Löscher *et al,* 1991 and Raza *et al,* 2001).

CONCLUSION

The anticonvulsant activity of the crude extract by Maximal Electroshock Test showed that *C. ornatum* possesses anticonvulsant activity by reducing the hind limb tonic extension significantly ($p \leq 0.05$) and therefore shows the ability of the crude extract to reduce tonic – clonic seizures. Activity against electrically induced convulsion is a positive index of effectiveness against tonic - clonic seizures (Omogbai *et al,* 2000 and Rang *et al,* 1996). A 100% inhibition was observed and peak responses were observed at 0.5hr at a dose of 2mg/ml, however, no response was observed at the lowest concentration of 0.5mg/ml after 2hrs (Table 3). This study has provided further insight into the bioactivity of *C. ornatum* growing in Nigeria. It has been established that anticonvulsant drugs that suppress HLTE in MEST are effective in the therapy of generalized tonic - clonic and partial seizures (Löscher *et al,* 1991and Trevor and Way, 1995). Since this extract is effective in the MEST, it can be effective in anticonvulsant therapy that involves tonic-clonic and partial seizures. Development of anticonvulsants from *C. ornatum* may produce natural antiepileptic drugs. Its use at larger doses should however be monitored for its potent toxicity. This study has therefore justified the use of this plant in traditional medicine.

RECOMMENDATION

Further studies still needs to be conducted to determine the pure compounds responsible for this activity and compare their mode of action to some tranquilizers like phenothiazines, benzodiazepines and barbiturates. These drugs are used as anticonvulsants and antipsychotics.

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