



Neuropharmacological effects of *Crinum Zeylanicum* alkaloid fraction in laboratory animals

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Abstract

Preparations of *Crinum zeylanicum* (CZ) bulb extract are used in traditional medicine in Nigeria for management of malaria, general debility, childhood convulsions and epilepsy. The objective of this study was to investigate some neuropharmacological effects of alkaloid extracted from *Crinum zeylanicum* bulb. The oral acute toxicity of *Crinum zeylanicum* alkaloid was carried out in mice using modified L. Orke's method. Pentobarbitone-induced sleep was used to study the sedative effect at 3 dose levels of 10, 20, and 40 mg extract/kg body weight. The anti-emetic effect was evaluated using the Copper sulphate-induced emesis in one day old chicks; the hypothermic effect was evaluated in normopyretic mice while the anticonvulsant effect was evaluated using Pentylenetetrazole (PTZ) - and picrotoxin (PCT) -induced seizure models in mice. The oral acute toxicity of the extract was found to be 770 mg extract/kg body weight. The alkaloid significantly ($p < 0.01$) shortened the onset and prolonged pentobarbitone-induced sleeping time. The extract at 10, 20 and 40 mg/kg body weight produced inhibition of retching in one day old chicks by 27.14, 41.53 and 50.81% respectively. The alkaloid significantly ($p < 0.05$) decreased the body temperature of normopyretic mice and protected against PTZ- and PCT-induced seizures in a dose dependent manner. These results indicate that the alkaloid fraction of Cz possesses antiemetic, hypothermic, anti-convulsant and sedative effects.

Author Keywords: *Crinum zeylanicum*, Alkaloid, emesis, pentobarbitone, hypothermia, epilepsy

Introduction

Epilepsy is still a major devastating disorder which affects an estimated 40-50 million people worldwide (Chindo et al., 2009). The incidence and prevalence of epilepsy varies with age (Banerjee and Hauser, 2008) and is highest among children below 7 years of age and in individuals above the age of 55 years (Jalalpure et al., 2009). In Nigeria, the prevalence of epilepsy is about 5.5 to 10 per 1000 people, which is about 1/10th of the world population (Osuntokun et al., 1978). The available synthetic antiepileptic drugs (AEDs) such as diazepam (DZP), phenytoin (PHE), carbamazepine, phenobarbital, felbamate, valproate, and lamotrigine used for the treatment of epilepsy are unable to control epileptic seizures in as many as 25% of patients (Jalalpure et al., 2009). In developing countries the treatment of epilepsy is inaccessible and unaffordable (Ojewole, 2008). Moreover, the current available therapeutic agents may possess serious adverse effects such as infertility, cognitive impairment and reproductive organ toxicities (Jalalpure et al., 2009). These undesirable effects and cost of the available conventional antiepileptic drugs makes the development of newer effective and safe agents from plants and other natural sources (Gupta et al., 1999; Gupta and Malhotra, 2000; Ojewole, 2008.) highly imperative.

Crinum zeylanicum (L.) (Family; Amaryllidaceae) is used in Sri Lanka traditional medicine for management of rheumatic pain and ear ache (Yakandawala and Samarakoon, 2006; Tsuda et al., 1984) and in the Dominican Republic for management of Malaria (Fennell and Staden, 2001). In western Nigeria, the bulb is used externally for skin trouble, injuries and on refractory ulcers while the Ibinis in southern Nigeria use juice obtained from the bulb for management of childhood convulsions, epilepsy, general debility and malaria (Jayeoba, personal communication). Preliminary studies in our laboratory showed that the methanolic bulb extract of *Crinum zeylanicum* possesses sedative, anti-convulsant and anxiolytic effects (Tijani et al., 2010). The isolation of crinidine (synonymous with crinine), flexinine, 6-

hydroxypowelline, zeylamine, lycorine, hamayne, 3-acetylhamayne, crinamine, 6-hydroxycrinamine and 6-methoxycrinamine from *Crinum zeylanicum* have been reported by Fales et al., (1959), Tsuda et al., (1984), Doepke et al., (1986) and Trimiño et al., (1988). In spite of the progress made in the isolation of these alkaloids from *Crinum zeylanicum* bulb there is little information on the basic central nervous system effects of these compounds in animal models. Therefore in continuation of our evaluation of biological effects of this plant on the central nervous system, the need to extract and evaluate the central nervous system (CNS) effect of its alkaloids arose, since the CNS effects of other members of the family amaryllidaceae have been reported to be mainly due to their alkaloids (Elgorashi et al., 2004). The scientific studies on antiepileptic and CNS depressant activities of *Crinum zeylanicum* alkaloids (Cz-alkaloid) have not been reported till now. In view of the above, we hypothesized that Cz alkaloid, in part, could be involved in the CNS related properties of *Crinum zeylanicum*. To test this hypothesis, we evaluated some neuropharmacological effects of *Crinum zeylanicum* bulb alkaloid in one-day old chicks and mice respectively.

Methods

Plant material and extraction of alkaloid

The whole plant (bulb, leaves and flower) of *Crinum zeylanicum* was collected by Mr. Goodluck Jaiyeoba, a traditional herbal medicine practitioner from Rafin sayan, a village in Suleja, Niger state of Nigeria. The plant was identified and authenticated by Mrs. Jemilat Ibrahim, a taxonomist with the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja where a voucher sample (NIPRD/H/6258) was prepared and deposited. Homogeneous powder of *Crinum zeylanicum* bulb (200 g) was moistened with water. The mixture of the plant material and water was treated with lime and ammonia solution, filtered and the filtrate

extracted with chloroform to obtain aqueous phase and organic phase. The aqueous phase was collected and treated with ammonia, followed by chloroform. The chloroform portion was evaporated at room temperature of 25 °C to leave behind the crude alkaloid.

Drugs and Reagents

Pentobarbitone sodium, pentylene tetrazole, picrotoxin, diazepam anhydrous copper sulphate and metoclopramide were obtained from Sigma Chemical Company (USA), chloroform and Ammonia solution were purchased from Sigma-Aldrich Chemie GmbH (Switzerland).

Animals

One-day-old chicks of both sex (35 - 40 g), and male albino mice (20 - 22 g) were used in the study. The chicks were obtained from Echo Poultry in Kubwa, Abuja, while the mice were obtained from the Animal Facility Centre of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. They were fed with starter's mash and given water *ad libitum*. All experiments performed on laboratory animals in this study followed the NIH Guidelines on the care and use of Laboratory Animal.

Acute toxicity (LD₅₀) study

The acute toxicity profile of the alkaloid was evaluated in mice orally using Lorke (1983) method modified by Salawu *et al*, (2009). The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice each given 10, 100 and 1000 mg extract/kg body weight orally. The mice were observed for signs of toxicity such as paw-licking, stooling, micturition, lying flat on belly, erect fur, reduced activity, sedation, convulsion and death. In the second phase of the study, doses of 225, 370 and 600 mg extract/kg body weight were administered orally to another twelve groups of three mice each.

These mice were also observed for signs of toxicity and mortality for 24 h.

Pentobarbitone-induced hypnosis in mice

This test was performed in 6 groups of 5 mice each. Three groups received doses of 10, 20, and 40 mg C_z-alkaloid /kg orally, while distilled water (5 ml/kg) was administered (orally) to the fourth group, which served as control. The last group which served as the reference standard group received diazepam (1 mg/kg). Thirty min after extract and diazepam treatment, pentobarbitone sodium (25 mg/kg i. p.) was administered to each mouse. Each mouse was observed for the on-set and duration of sleep in minutes. The criterion used for determination of sleep was loss of righting reflex, indicated by the animals' inability to resume or return to its upright position on all four limbs after being gently rolled sideways (Miya *et al.*, 1973; Roland *et al.*, 1991) three times. The interval between loss and recovery of righting reflex was recorded as the index of hypnotic effect.

Bioassay for anti-emetic activity

The one day old chicks were divided into five groups of five chicks each and each chick was kept in a large beaker at 25 °C for 20 min. Group 1 served as the negative control and was treated with 5 ml normal saline/kg. Groups 2, 3, and 4 were treated with 50, 100, and 200 mg extract/kg body weight orally, while group 5 was treated with 50 mg metoclopramide/kg body weight intraperitoneally. One hour later 50 mg anhydrous copper sulphate/kg body weight was administered orally to each chick, and then the number of retches (an emetic action without vomiting gastric material) was counted for 10 min. The antiemetic effect was assessed as the decrease in number of retches in the treated group in contrast to the control. The inhibition (%) was calculated as follows: Inhibition (%) = [(A-B)/A] x 100. Where A is the control frequency of retching and B is the frequency of retching of the treated group.

Effect of Alkaloid on normothermic animals

The procedure described by Nikolov and Yakimova (2010) was adopted for this study. Twenty mice of either sex were randomized into four groups of five mice each and then treated as follows: group I received normal saline, while groups II, III and IV received 10, 20 and 40 mg extract /kg orally respectively. The base line rectal temperature of all the mice was recorded before drug administration by inserting digital thermometer (Omron Digital Fever Thermometer, Omron® Healthcare, China) into the rectum of each mouse and thereafter at 30 and 60 minutes.

Pentylene tetrazole (PTZ) - induced seizures

The anti-convulsant method described by Mahomed and Ojewole (2006) was used for this study. Adult mice of both sexes were randomized into five groups of seven mice each. Group I received 5 ml distilled water/kg. Groups II to IV received 10, 20 and 40 mg/kg body weight of the alkaloid orally respectively while group V mice received 4mg diazepam/kg intraperitoneally. After 1 hour of alkaloid and thirty minutes of diazepam administration, all the mice were given 85mg PTZ/kg body weight intraperitoneally. The mice were then observed for onset and duration of tonic hind limb extension for 30 minutes. The ability of the extract to prevent tonic hind limb extension or prolong its onset was considered as an indication of anticonvulsant activity (Amabeoku et al., 1998).

Picrotoxin - induced seizures

The anticonvulsant method described by Salih and Mustafa (2008) was used. Adult mice of both sexes were randomized into five groups of seven mice each. Group I mice received 5 ml normal saline/kg body weight. Mice in groups II to IV received 10, 20 and 40 mg/kg body weight of the alkaloid respectively, while mice in group V received 4mg diazepam/kg body weight intraperitoneally. One hour after extract treatment and thirty minutes

after diazepam administration, 10 mg Picrotoxin /kg body weight was given to all the mice intraperitoneally. They were then observed for hind limb tonic seizures for thirty minutes. Delay/prolongation of the latency or onset of the hind limb tonic extension was considered as an indication of anticonvulsant activity (Navarro-Ruiz et al, 1995).

Statistical analysis

All numerical data are expressed as the mean \pm standard error of mean (SEM). Statistical analysis was carried out using student's t-test and differences between means were considered to be significant when $p < 0.05$.

Results

Acute toxicity study

The alkaloid when administered at 100 mg /kg body weight orally produced decreased respiratory rate, decreased activity, sedation and no mortality. There was 100% mortality at 1000 mg extract/kg body weight within 24 h post-treatment. The oral median lethal dose (LD_{50}) of the alkaloid in mice was calculated to be 774 mg/kg body.

Pentobarbitone induced sleep

The alkaloid at 40 mg/kg and 1 mg diazepam/kg significantly reduced the onset of sleep. The extract significantly ($p < 0.05$) and dose- dependently prolonged duration of sleep in mice (fig. I and II) while diazepam significantly ($p < 0.05$) prolonged duration of sleep.

Effect of *Crinum zeylanicum* alkaloid on emesis

The alkaloid at doses of 50, 100 and 200 mg /kg body weight and significantly ($p < 0.001$) and dose –dependently decreased the frequency of retching. At 5 mg/kg body weight the standard reference drug metoclopramide also significantly ($p < 0.001$) decreased the frequency of retching in one –day old chicks (Fig III).

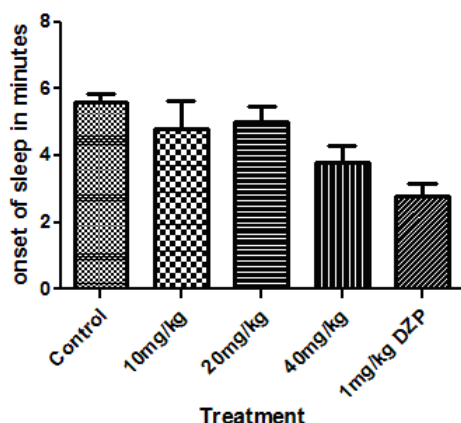


Fig I: Effect of *Crinum zeylanicum* alkaloid on onset of sleep

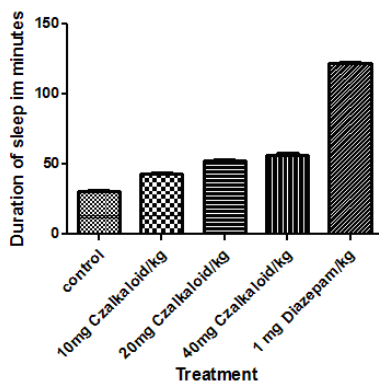


Fig II: Effect of *Crinum zeylanicum* alkaloid on duration of sleep in mice

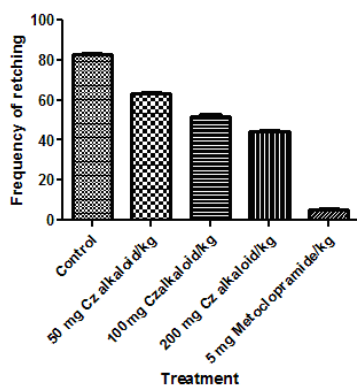


Fig III: Effect of *Crinum zeylanicum* alkaloid on copper induced retching in chicks

Effect of *Crinum zeylanicum* alkaloid on body temperature

The alkaloid produced significant ($p < 0.05, 0.005$)

dose dependent reduction in body temperature of normothermic mice (Table 1).

Treatment	Temperature in °C at 0 minute post-treatment	Temperature in °C at 30 minute post-treatment	Temperature in °C at 60 minute post-treatment
5ml distilled water/kg	37.00±0.09	37.00±0.08	37.00±0.06
10 mg Cz alkaloid/kg	37.00±0.04	37.00±0.09	36.00±0.06 *
20 mg Cz alkaloid/kg	37.00±0.07	36.00±0.12**	36.00±0.09**
40 mg Cz alkaloid/kg	37.00±0.02	36.00±0.09**	35.26±0.12 **

Table I: Effect of *Crinum zeylanicum* alkaloid on body temperature of normothermic mice

*Significantly different from the control at $p < 0.05$ and ** at $p < 0.001$

Effect of *Crinum zeylanicum* alkaloid on pentylenetetrazole (PTZ)-induced seizure in mice

The alkaloid significantly ($p < 0.005$) and dose –dependently increased latency of P TZ- induced seizures in mice (fig IV).The reference standard drug diazepam at 4 mg/kg also significantly ($p < 0.001$) increased latency of PTZ-induced seizure in mice. At 10, 20 and 40 mg/kg the alkaloid protected 20, 40 and 60 % of mice against PTZ-induced seizures, while the reference standard drug diazepam protected 100% of the mice (Table II)

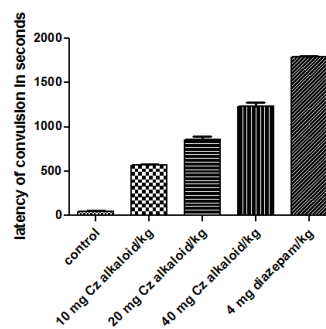


Fig IV: Effect of *Crinum zeylanicum* alkaloid on pentylenetetrazole-induced seizure in mice

Treatment	% protected	% mortality
5ml distilled water/kg	0.00	100.00
10 mg Cz alkaloid/kg	20.00	80.00
20 mg Cz alkaloid/kg	40.00	60.00
40 mg Cz alkaloid/kg	60.00	40.00
4 mg diazepam/kg	100.00	0.00

Table II: Percentage of mice protected by *Crinum zeylanicum* alkaloid against PTZ-induced seizure

C z: *Crinum zeylanicum*

Effect of *Crinum zeylanicum* alkaloid on picrotoxin (PCT)-induced seizure in mice

The alkaloid significantly ($p < 0.005$) and dose-dependently increased latency of PCT-induced seizures in mice (fig V). The reference standard drug diazepam at 4 mg/kg, significantly ($p < 0.001$) increased latency of PCT-induced seizure in mice. At 10, 20 and 40 mg/kg the alkaloid protected 20, 40 and 60% of mice against PCT-induced seizures, while the reference standard drug diazepam protected 100% of the mice (Table III)

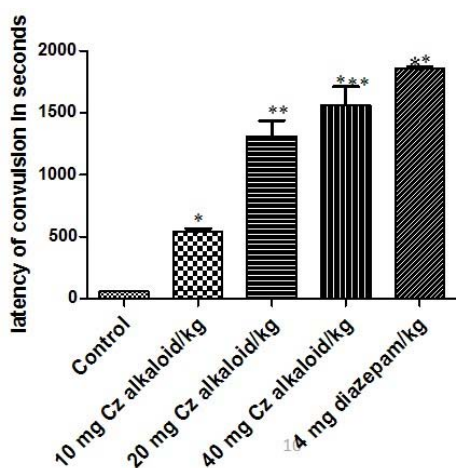


Fig V: Effect of Cz alkaloid on picrotoxin-induced seizure in mice

Cz: *Crinum zeylanicum*

Treatment	% protected	% mortality
5ml distilled water/kg	0.00	100.00
10 mg Cz alkaloid/kg	20.00	80.00
20 mg Cz alkaloid/kg	40.00	60.00
40 mg Cz alkaloid/kg	60.00	40.00
4 mg diazepam/kg	100.00	0.00

Table III: Percentage of mice protected by *Crinum zeylanicum* alkaloid against PCT-induced seizure

Cz: *Crinum zeylanicum*

Discussion

In the present study, the crude alkaloid extracted from *C. zeylanicum*, showed sedative, anti-emetic, body temperature lowering and anticonvulsant effects in the experimental animals. In the pentobarbitone-induced sleep model, the alkaloid

dose-dependently potentiated the effect of pentobarbitone. The prolongation of pentobarbitone-induced sleeping time may be attributed to an inhibition of pentobarbitone metabolism or to an action on the central mechanism involved in the regulation of sleep (N'Gouemo et al., 1994), thus suggesting *Crinum zeylanicum* alkaloid as a neurosedative drug (Capasso et al., 1996). The copper sulphate-induced retching was used for evaluating anti-emetic effect of Cz alkaloid in one day old chicks. The alkaloid at doses of 10, 20 and 40 mg/kg significantly reduced the frequency of retching in chicks. Copper sulphate induces vomiting through excitation of visceral afferent nerve fibres of the gastro intestinal tract. It has also been established by Jordan et al., (2007) that the peripheral 5-HT₄ located in the stomach plays an important role in copper sulphate induced emesis. The potent anti-emetic effect produced by the alkaloid may be mediated via antagonism of serotonin receptors in the gut and or possibly through facilitation of central inhibitory GABAergic effect. The alkaloid produced significant reduction in rectal temperature of normothermic mice. Thermoregulation is a complex physiological process involving both central and peripheral autonomic mechanisms. The primary thermoregulatory center resides in the preoptic area of the hypothalamus and controls the balance between heat gain and heat loss. GABAergic terminals and GABA_A receptors on the neurons of the preoptic area of the hypothalamus have been reported by Gritti et al., (1993) to be involved in the process of thermoregulation. In addition studies carried out by Frosini et al., (2004) have also showed that systemic administration of either GABA or GABA_A agonists usually produce hypothermia. The alkaloid-induced hypothermia in normothermic mice may therefore be mediated via facilitation of GABAergic inhibitory tone at the preoptic area of the anterior hypothalamus. The involvement of other mechanisms such as opioidergic and serotonergic pathway will need to be further explored.

The anticonvulsant study revealed that PZ and PCT (epileptogenic chemicals) produced marked

seizures in the control animals. In these tests, the animals behaved in a characteristic manner and showed progressive signs of epilepsy such as ear and facial twitching, convulsive waves axially through the body, myoclonic body jerks, straub tail, generalized clonic convulsions, twitching and turning of animals to one side, hind limb tonic extension (HLTE phase) - the severe generalized tonic convulsion phase, stupor, and recovery or death. In the PTZ -model, Cz alkaloid (10, 20, and 40 mg/kg, i.p.), prevented P TZ-induced seizures in mice. The P TZ test represents a valid model for human generalized myoclonic seizures and also generalized seizures of the petit-mal (absence)-type (Malawska & Scatturin, 2003). Drugs protecting against seizures induced by P TZ, reduce T-type calcium currents (M urali et al., 2008) and are considered to be useful for control of myoclonic and absence seizures (Nisar et al., 2009). Since P TZ is a potent GABA_A receptors antagonist (Jalalpure et al., 2009) and Cz alkaloid is effective in this model, it is likely that its anticonvulsant effect is mediated through Gabaergic mechanism stimulation. In the picrotoxin (PCT) - model, prior treatment with Cz alkaloid (10-40 mg/ kg, p.o) significantly ($p < 0.01$) attenuated the latency of HLTE phase. Recently it has been reported that PCT blocks the GABA_A receptor-linked chloride ion channels (M eldrum & Rogawski, 2007) which normally open to allow increased chloride ion conductance into brain cells (Amabeoku et al., 2007) and thus elicits seizures by inhibition and/or attenuation of gamma aminobutyric acid (GABAergic) neurotransmission (Leonard, 2000; Rang et al., 2003; Ojewole, 2008). The seizures induced by PCT are analogous to the petit mal type of epilepsy in humans (M urali et al., 2008). Inhibition of PCT-provoked convulsions by Cz alkaloid may be attributed to the augmentation of GABAergic transmissions. It is likely that antiepileptic effects of Cz alkaloid may be attributed to the opening of chloride ion channels and enhancing GABAergic neurotransmission which plays an important role in epilepsy (Ito et al., 2005; Perucca, 2005).

Conclusion

The present study for the first time reports sedative, hypothermia, anti-emetic and anticonvulsant effects of Cz alkaloid. The possible mechanisms for its sedative, hypothermic and anticonvulsant effects could be the facilitation of chloride ions through GABA_A receptors while its anti-emetic effect may be mediated via antagonism of 5HT₄ receptor at the gastrointestinal tract. Our results support the traditional claims of *Crinum zeylanicum* for the treatment of epilepsy and related disorders. Future study shall focus on the identification of exact mechanisms of action of the alkaloid for the potential CNS modulating properties using different animal models.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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