Evaluation of the Anticonvulsant Activity of the Hydroethanolic Extract of *Crinum scillifolium* Bulbs (Amaryllidaceae) in Experimental Animals

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Abstract *Crinum* species is frequently used for the treatment of nervous disorders such as epilepsy. This study aimed to investigate the anticonvulsant activity of the hydroethanolic extract of the *Crinum scillifolium* bulbs in chemoconvulsant-induced seizures in mice. The anticonvulsant activity of the extract (25, 50, 100, 200 and 400mg/kg), was investigated in isoniazid (INH)-induced seizures in mice. The hydroethanolic extract protected mice from INH-induced seizures in a dose-dependent manner. 100% of protection was observed when the animals were treated with 200 and 400mg/kg of hydroethanolic extract of *Crinum scillifolium*. At Dose of 50 and 100mg/kg 83% protection was observed; the onset of convulsion significant was delayed, and no mortality was found of the mice against isoniazid-induced convulsion. Mice pretreated with hydroethanolic extract at the dose of 25mg/kg reduced mortality to 27% and significant delayed the onset of death ($p<0.01$). In conclusion, *Crinum scillifolium* was revealed possessing anticonvulsant effects in mice, via the GABAergic neurotransmission.

Keywords Epilepsy, Anticonvulsant, *Crinum scillifolium*, Isoniazid, Mice

1. Introduction

Epilepsy is a chronic brain disorder characterized by recurrent derangement of the nervous system due to sudden excessive disorderly discharge from the cerebral neurons. It is one of the most common serious disorders of the brain, affecting some 50 million persons worldwide [1]. Most

epileptic, they usually suffer from depression, muscular spasms, strange sensations, abnormal behavioural changes, convulsions, loss of consciousness and highly prone to suicide and sudden unexpected death [2].

The prevalence of epilepsy in developed countries is estimated to 5% and Africa 10% [3]. Current epilepsy therapy with modern anticonvulsants is associated with side effect, chronic toxicity as well as teratogenic effects limit their use and 30% of patients continue to have seizures with current anticonvulsant therapies [4].

World Health Organization estimated that approximately 80% people with epilepsy live in developing countries and most of them do not get adequate medical treatment [5].

Therefore, research should continue to develop newer, more effective and safer neuroprotective agents for the treatment of epilepsy.

About 75-80% of the world's population and especially of the developing countries uses medicinal plants for the treatment of epilepsy because of better compatibility with the human body and lesser side effects [6]. Actually, medicinal plants to be an important source of traditional medicines to treat epilepsy. Various plants are used have scientifically demonstrated that they possess promising anticonvulsant activities in animal models for the detection of anticonvulsant activity and various plants are yet to be scientifically investigated [7]. This has accelerated the global effort to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in convulsion [8].

The *Crinum* because of their immense traditional uses, have also been the subject of numerous biological evaluations. Indeed, most of the species evaluated showed, in vitro, important anti-tuberculosis, antioxidant,

anti-inflammatory and anticonvulsant activities [9-11].

This preliminary study was therefore carried out in order to evaluate the anticonvulsant activity of the hydroethanolic extract of the bulbs of *Crinum scillifolium*.

2. Material and Methods

2.1. Plant Material

Bulbs of *Crinum Scillifolium* were collected from Sikensi, at 80 km to Abidjan. The plant was identified and authenticated by the Laboratory of Botanic, University Félix Houphouët Boigny.

2.2. Preparation of Plant Extract

The **Guédé-Guina** method modified [12] was used to obtain 90% *Crinum scillifolium* hydroethanolic extract. After collection, the Bulbs of *Crinum scillifolium* were washed with distilled water. They were dried under the shade at 23°C and the dried bulbs were ground into fine powder. A 90% hydroethanolic solution (ETOH / H₂O, 90:10) was used for extraction. The powdered material was extracted with hydroethanolic solution (50g of powder per 500ml of hydroethanolic solution) by cold maceration for 48 h with stirring. The extract obtained constitutes the hydroethanolic extract. The macerate was filtered through Whatman filter paper and evaporated to dryness in an oven at a temperature of 45°C. The extract was stored in refrigerator (4°C) until ready use. From this various concentration were reconstituted in a known volume of distilled water before administration.

2.3. Material Animal

Swiss albinos mice (22 – 25 g) of either sex were used in this study which were bred in the Laboratory Animal (UFR Pharmaceutical and Biological Sciences; (University Félix Houphouët Boigny). The animals were maintained in standard laboratory conditions (25°C) and light/dark cycles, i.e. 12/12h and fed with standard food and water. In all the experimental studies, each group consisted of six animals. Each animal was used only once. The investigation conforms to the recommendation of OECD in 2008 [13].

2.4. Drugs

Isoniazid 100mg® tablet (laboratory LUTIN, India) was used to trigger convulsions and Clonazepam 100mg® tablet (Laboratory La Roche SA, Switzerland) to protect mice from seizures.

2.5. Isoniazid-Induced Seizures

Animals were divided into seven groups of six mice. Group 1 received respective distilled water (10 ml/kg p.o) ; group 2 was allotted for standard drug (clonazepam 3mg/kg) and group 3, 4, 5, 6 and 7 received graded doses of hydroethanolic extract of *Crinum scillifolium* (25, 50, 100, 200 and 400mg/kg; p.o). One hour later, all animals were administered orally with isoniazid (250 mg/kg) and placed in isolated cages. Animals that did not convulse within the 3 hours of observation were qualified as protected. In unprotected animals, the latency to first convulsion, duration of convulsion, latency of death time, percentage mortality were recorded [14].

2.6. Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Dennett's test using the Graph Pad Prism 4.0 software package. The level of significance was determined in comparison with the control group. Statistical significance was accepted for $p < 0.05$.

3. Results

In the INH test, the standard anti-epileptic drug, clonazepam (3mg/kg) blocked the tonic-clonic seizures and mortality in mice against isoniazid induced seizure (Table I and figure 1).

The hydroethanolic extract protected mice from INH-induced seizures in a dose-dependent manner. Similarly to the reference drug clonazepam, the extract protected the animals with 100% of protection, when was treated with 200 and 400mg/kg of hydroethanolic extract of *Crinum scillifolium*. Mice pretreated with hydroethanolic extract at the dose of 50 and 100 mg/kg, 83% of protection was observed. The dose of 25mg/kg did not blocked the tonic-clonic seizures (Figure 1).

In unprotected animals, the extract showed a significant increase in latency of the first seizure ($P < 0.01$) compared to the control group when the mice were treated with 25, 50 and 100 mg / kg extract (Figure 2).

No dose significantly influenced mean seizure time in unprotected mice compared to mice that received distilled water (Figure 3).

Higher mortality was observed for animals receiving distilled water and hydroethanolic extract prevents the occurrence of death in unprotected animals when treated with 100 and 200mg/kg. Mice pretreated with hydroethanolic extract at the dose of 25mg/kg reduced mortality to 23% and significant delay the onset of death ($p < 0.01$) (Table 1).

Table 1. Effect of hydroethanolic bulbs extract of *Crinum scillifolium* on isoniazid- induced convulsion in mice

Treatment group	Convulsion	Mortality (%)	Latency of death (min)
INH+ Water (10ml/kg. p.o)	6/6	100	72
INH + Clonazepam 3 mg/kg	0/6	0	-
INH + EHE 25 mg/kg	6/6	67	106***
INH + EHE 50 mg/kg	1/6	0	-
INH + EHE 100 mg/kg	1/6	0	-
INH + EHE 200 mg/kg	0/6	0	-
INH + EHE 400 mg/kg	0/6	0	-

The animals (7 per group) were treated with the hydroethanolic extract (EHE), distilled water or clonazepam 1 hour before induction of convulsions with orally administration of 250 mg/kg dose of isoniazid (INH).

Figure 1. Protective effect of hydroethanolic extract (EHE) of *Crinum scillifolium* bulbs against convulsions induced by isoniazid

*** p < 0.01 The data analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test.

INH 250 + ED = Isoniazid 250 mg/kg + Distilled water;

INH 250 + EHE 25 = Isoniazid 250 mg/kg + Hydroethanolic extract 25 mg/kg;

INH 250 + EHE 50 = Isoniazid 250 mg/kg + Hydroethanolic extract 50 mg/kg;

INH 250 + EHE 100 = Isoniazid 250 mg/kg + Hydroethanolic extract 100 mg/kg;

INH 250 + EHE 200 = Isoniazid 250 mg/kg + Hydroethanolic extract 200 mg/kg;

INH 250 + EHE 400 = Isoniazid 250 mg/kg + Hydroethanolic extract 400 mg/kg.

INH 250 + Clonaz 3 = Isoniazid 250 mg/kg + Clonazepam 3 mg/kg.

Figure 2. Effect of the hydroethanolic extract of *Crinum scillifolium* bulbs on latency seizures induced in mice by INH

The data were expressed as mean $\pm$ S.E.M at risk $\alpha = 5\%$. EHE at 25, 50 and 100 mg/kg dose significantly increased seizures latency compared to mice treated with distilled water. $n = 6$ per dose, *** $p < 0.01$. The data analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test.

INH 250 + ED = Isoniazid 250 mg/kg + Distilled water;
 INH 250 + EHE 25 = Isoniazid 250 mg/kg + Hydroethanolic extract 25 mg/kg;
 INH 250 + EHE 50 = Isoniazid 250 mg/kg + Hydroethanolic extract 50 mg/kg;
 INH 250 + EHE 100 = Isoniazid 250 mg/kg + Hydroethanolic extract 100 mg/kg;
 INH 250 + EHE 200 = Isoniazid 250 mg/kg + Hydroethanolic extract 200 mg/kg;
 INH 250 + EHE 400 = Isoniazid 250 mg/kg + Hydroethanolic extract 400 mg/kg.
 INH 250 + Clonaz 3 = Isoniazid 250 mg/kg + Clonazepam 3 mg/kg.

Figure 3. Effect of the hydroethanolic extract of *Crinum scillifolium* bulbs on average duration of seizures induced in mice by INH

The data were expressed as mean $\pm$ S.E.M at risk $\alpha = 5\%$. $n = 6$ per dose, *** $p < 0.01$. The data analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test.

INH 250 + ED = Isoniazid 250 mg/kg + Distilled water;
 INH 250 + EHE 25 = Isoniazid 250 mg/kg + Hydroethanolic extract 25 mg/kg;
 INH 250 + EHE 50 = Isoniazid 250 mg/kg + Hydroethanolic extract 50 mg/kg;
 INH 250 + EHE 100 = Isoniazid 250 mg/kg + Hydroethanolic extract 100 mg/kg;
 INH 250 + EHE 200 = Isoniazid 250 mg/kg + Hydroethanolic extract 200 mg/kg;
 INH 250 + EHE 400 = Isoniazid 250 mg/kg + Hydroethanolic extract 400 mg/kg.
 INH 250 + Clonaz 3 = Isoniazid 250 mg/kg + Clonazepam 3 mg/kg.

4. Discussion

The results of the present study demonstrate that hydroethanolic extract of bulbs of *crinum scillifolium* possessed anticonvulsant activity. Doses of 200 and 400 mg/kg totally antagonized convulsions induced by isoniazid with a 100% protection. The extract had a dose dependent effect which means that at a dose above 200 mg/kg, protection will be maximal. At the doses of 200 and 400 mg/kg, the extract produced the same therapeutic effect as the reference drug Clonazepam. This result suggests that the extract contains an active principle capable of antagonizing the effect of isoniazid.

Dose of 50 and 100 mg/kg significant delayed the onset of convulsion and no mortality was found of the mice against isoniazid-induced convulsion. This suggests that a protective effect has been initiated but this was not sufficient to completely antagonize the isoniazid-induced seizures. The standard anti-epileptic drug clonazepam (3 mg/kg) completely antagonized the seizures produced by isoniazid.

The convulsions produced by isoniazid is primarily due to inhibition of GABA mediated pathway. Indeed, INH induced convulsions are produced due to alteration of GABA level in the brain, it reduces the GABA content in the brain by inhibiting glutamic acid decarboxylase (GAD) [15, 16].

Enhanced GABAergic transmission is reported as antagonizing convulsions, while inhibition of neurotransmission promotes convulsions [17].

The findings of the present study, therefore, tend to suggest that *Crinum scillifolium* hydroethanolic extract have anticonvulsant activity might have inhibited and/or attenuated isoniazid-induced seizures of the mice used by interfering with GABAergic neurotransmission. However, further study is needed to find the accurate mechanism of anticonvulsant effect. We have shown in this study that a species of the genus *Crinum* possesses anticonvulsant activity. Our results are therefore supported by those of de Azikiwe et al. (2012) [10] who have shown in one study that another *Crinum* species of the genus *jagus* would also possess anticonvulsant activity.

5. Conclusions

Our results showed that the hydroethanolic extract of *Crinum scillifolium* bulbs, Amaryllidaceae, demonstrated potential anticonvulsant properties in the experimental animals at the doses used. More studies are necessary to clarify the antiepileptic components and the mechanisms underlying the plant activities.

Ethical Approval

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health

Sciences, University Félix Houphouët-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

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