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Anticonvulsant activity of leaf extract of *Heinsia crinata*

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ABSTRACT

Background: *Heinsia crinata*(Afzel). G. Tayl. (Rubiaceae), is used in Ibibio ethnomedicine for the treatment of various diseases such as CNS disorders. The anticonvulsant activity of the leaf extract was studied.

Method: The ethanol leaf extract of *H. crinata* (450-1350 mg/kg) was investigated for anticonvulsant activity in mice against pentylenetetrazol, aminophylline and isoniazid-induced convulsion models to assess anticonvulsant activity.

Results: The extract was found to significantly ($p<0.005$-0.01) and dose-dependently offered protection against PTZ-, aminophylline and isoniazid-induced convulsions in mice.

Conclusion: The leaf extract of *H. crinata* possess anticonvulsant activity and this supports its use in ethnomedicine for the treatment of central nervous system disorders.

Key words**: —** Anticonvulsant, CNS depressant, *Heinsia crinata,* vegetable, Seizures

1. INTRODUCTION

Heinsia crinata (Afzel). G. Tayl. (Rubiaceae) is shrub with woody stems and branches [1]). It is indigenous to West Africa, especially eastern part of Nigeria, but it is now cultivated in Central Africa, south of Sahara and Francophone Africa [2]). *Heinsia crinata* is casually classified as white and dark by indigenes of Akwa Ibom State in southern Nigeria. Ajibesin *et al.*, [3] reported on the phytochemical constituents of the leaves of the two varieties to be made up of saponins, tannins, flavonoids, cardiac glycosides, terpenes, and alkaloids, with the dark variety having a greater concentration of alkaloids, while saponins were greater in the white variety. Ethnomedically, the decoctions of the leaf are used to treat various diseases and wounds as well as gastrointestinal disorders especially ulcer and spasm [4], while the root is used to treat abscess and hypertension [5]. Two triterpenoid saponins have been isolated from the leaves of the plants [2]. Reports of nutritional values [6, 7] antimicrobial [3, 8, 9], antiplasmodial and antidiabetic activities [4], and antiulcer [10], anti-inflammatory and analgesic [11], antioxidant [12] of the leaf extract have been published. Reports of scientific studies on the leaf of *H. crinata* are few and there is no information regarding the anticonvulsant activity of *H. crinata* leaf extract in rodents. We report in this study the anticonvulsant potential of the dark green variety of *H. crinata* in rodents.

2. MATERIALS AND METHODS

2.1Materials

2.1.1 Equipment

Equipment used in this research were electric water bath, electronic weighing balance (Ohaus, USA), stop watch. Chemicals/reagents were of analytical grades and were obtained from JHD (England).

2.1.2 Plant materials

Fresh leaves of *H. crinata* were procured from Uyo market, Akwa Ibom State, Nigeria, in January, 2019. The plant was identified and authenticated by Prof Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium (voucher no. FPHUU 225). The fresh leaves (2 kg) of the plant were dried on laboratory table for 2 weeks and

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reduced to powder. The powder (100 g) was macerated in 95% ethanol (300 mL) for 72 h. The liquid filtrate obtained was concentrated *in-vacuo* at 40C and all the ethanol was completely removed. The yield was 1.17% w/w. The extract was stored in a refrigerator at 4˚C until used for experiment reported in this study.

2.1.3 Animals
Albino Swiss mice (19 – 28 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. The animals were used in compliance with the National Institute of Health Guide for the Care and use of Laboratory Animals (Publication nos. 85-23, revised 1985) Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

2.2 Methods
2.2.1 Anticonvulsant activity

2.2.1.1 Pentylene tetrazol-induced convulsion
Anticonvulsant effect of the extract was assessed using a modified method of Vellucci and Webster [13] on overnight fasted mice. The mice were divided into five groups of six animals each and treated with 450, 900 and 1350 mg/kg of the leaf extract respectively, phenobarb, 40 mg/kg one hour before induction of convulsion. Seizure was induced in each set of mice with pentylene tetrazol (PTZ) (70 mg/kg i.p). Control group received normal saline. The onset of Clonic/tonic convulsion and the mortality rate were recorded and compared with the respective control group. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity [14].

2.2.1.2 Aminophylline-induced Convulsion
The extract was evaluated for activity against aminophylline–induced convulsion using the method of Juliet et al., [15].The mice were divided into 5 groups of six animals each and treated with 450, 900 and 1350 mg/kg of the extract respectively and phenobarb, 40 mg/kg one hour before induction of convulsion. Seizure was induced using aminophylline (280 mg/kg,i.p). The animals were observed for 120 mins after the administration of aminophylline and the following parameters were noted:

Time to onset of myoclonic jerks in mins.
Time to onset of tonic convulsions in mins.
Time to death during experimental time of 120 mins.
Number of mice dead/alive at 24 hours.

2.2.1.3 Isoniazid (INH)-induced seizures in mice
The method of Nanhakumar and Tyagi [16] was used to evaluate the extract against isoniazid-induced convulsion with slight modifications. Thirty mice were divided into five groups of six mice each. The group I received normal saline10 mL/kg. Groups II,III and IV received 450, 900 and 1350 mg/kg of the leaf extract respectively. The group V received diazepam5 mg/kg body weight. One hour after pretreatment, mice in all groups received INH 300 mg/kg s.c. The animals were observed for about 120 min for the onset of myoclonic/tonic seizures and mortality was recorded.

2.3 Statistical Analysis
Data obtained from this work were analysed statistically using ANOVA (one –way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance i.e. $p \leq 0.05$.

3. RESULTS
3.1 PTZ –induced convulsion
Administration of leaf extract of *Heinsia crinata* (450-1350 mg/kg) provided considerable degree of protection for the mice against seizure induced by pentylene tetrazol. The extract significantly ($p<0.01$-0.001) prolonged the time for onset of myoclonic convulsion in a dose-dependent fashion and these were significant at high doses (900 and 1350 mg/kg; Table 1). The activity of the highest dose (1350 mg/kg) was better than that of the standard drug, phenobarb (Table 1). Similarly, the extract exerted a significant ($p<0.01$-0.001) prolongation of time for onset of tonic convulsion in a dose-dependent manner which was significant ($p<0.05$) at the higher doses (900 and 1350 mg/kg; Table 1). The time of death of treated animal was dose-dependently and significantly ($p<0.01$-0.001) prolonged when compared with the control. The highest dose of the extract (1350 mg/kg) offered 100% protection

to the treated mice. The standard drug, phenobarb was able to offer 33.33% protection to the animals treated with it which was lower than the effect of the highest dose of the extract (1350 mg/kg; Table 1).

Table 1: Effect of ethanol leaf extract of *Heinsia crinata* on Pentylene tetrazol-induced convulsion

TREATMENT	DOSE	Onset of myoclonic	Onset of Tonic	Time of death	No. of death
	mg/kg				
Control normal saline	-	0.32 ±0.08	1.32 ± 0.02	2.89±0.01	6/6
Phenobarb	40	1.23 ±0.09^{c}	18.28±1.82^{c}	30.09±0.01^{c}	2/6
Crude extract	450	0.55 ±0.02	1.87± 0.30	5.92±1.34^{a}	6/6
	900	1.47 ±0.03^{c}	4.48± 0.05^{a}	8.69±0.30^{b}	6/6
	1350	2.68 ± 0.17^{c}	11.99±1.20^{c}	0.00±0.00	0/6

Data are expressed as MEAN ± SEM, Significant at $^{a}p<0.005$;$^{b}p<0.01$;$^{c}p< 0.001$, when compared to control. (n=6).

3.2 Aminophylline-induced convulsion

Administration of the leaf extract of *H. crinata* (450-1350 mg/kg) caused a significant (p<0.01-0.001) delay in the onset of seizure induced by aminophylline in a dose-dependent fashion. The delay was significant (p<0.01-0.001) in both myoclonic and tonic convulsion (Table 2). The time of death of treated animal was prolonged significantly (p<0.05) at the lowest dose (450 mg/kg) when compared with the control, while higher doses (900 and 1350 mg/kg) offered 100% protection to the treated animals. The standard drug, Phenobarb, also offered a 100% protection to the mice treated with it.

Table 2: Effect of ethanol leaf extract of *Heinsia crinata* on Aminophylline-induced convulsion

TREATMENT	DOSE	Onset of myoclonic	Onset of Tonic	Time of death	No. of death
	mg/kg				
Control normal saline	-	5.53±0.02	6.58 ± 0.38	8.03±1.09	6/6
Phenobarbitone	40	12.96±0.76^{c}	0.00 ± 0.00^{c}	0.00±0.00	6/6
Crude extract	450	5.83±0.19	20.98 ± 1.79^{c}	41.30±3.52^{c}	6/6
	900	10.58±0.36^{c}	21.67 ±1.89^{a}	0.00±0.00	0/6
	1350	13.81±0.26^{c}	23.14±0.54^{c}	0.00±0.00	0/6

Data are expressed as MEAN ± SEM, Significant at $^{a}p<0.005$;$^{b}p<0.01$;$^{c}p< 0.001$, when compared to control. (n=6).

3.3 Isoniazid-induced convulsion

Administration of the leaf extract of *H. crinata* (450-1350 mg/kg) caused a significant (p<0.01-0.001) delay in the onset of seizure induced by Isoniazid in a dose-dependent fashion. The delay was significant (p<0.01-0.001) in both myoclonic and tonic convulsion (Table 3). The time of death of treated animal was prolonged significantly (p<0.05) at the highest dose (1350 mg/kg) when compared with the control, while the standard drug, Phenobarb, also offered a more significant (p<0.001) prolongation of time of death to the mice treated with it (Table 3).

Table 3: Effect of ethanol leaf extract of *Heinsia crinata* on isoniazid-induced convulsion

TREATMENT	DOSE	Onset of myoclonic	Onset of Tonic	Time of death	No. of death
	mg/kg				
Control normal saline	-	2.53±0.28	11.50 ± 0.95	36.32±1.70	6/6
Diazepam	5	4.17±0.10^{c}	26.19 ± 0.53^{c}	84.09±4.05^{c}	6/6
Crude extract	450	5.17±0.28	24.70 ± 2.58^{c}	39.73±4.95	6/6
	900	7.45±1.56^{c}	20.67 ±0.31^{a}	35.79±1.61	6/6
	1350	2.64±0.19^{c}	12.84±1.50^{c}	44.19±2.15^{a}	6/6

Data are expressed as MEAN ± SEM, Significant at $^{a}p<0.005$;$^{b}p<0.01$;$^{c}p< 0.001$, when compared to control. (n=6).

4. DISCUSSION

The leaf extract of *Heinsia crinata*, a vegetable, used traditionally for the treatment of convulsion and epilepsy was evaluated for activity against experimentally-induced convulsions. The leaf extract was found to possess significant activity against seizures induced by pentelyene tetrazol, aminophylline and isoniazid, offering 100% protection in some cases. The exact mechanisms of seizures induced by aminophylline appear to be diverse, multiple and complex, and also unclear. Evidence suggests that seizures induced by aminophylline, could be the result of

adenosine receptor antagonism or due to inhibition of cerebral nucleotidase activity [17, 18] which lowers the adenosine content in the brain and eventually lead to a process of disinhibition. However, report has it that di-phenylhydantoin, a potent inhibitor of adenosine uptake was ineffective in preventing these seizures [19]. Apart from non-specific adenosine receptor antagonism [20], aminophylline is thought to have inhibitory influence on adenosine synthesis. At higher doses inhibition of phosphodiesterase activity including mobilization of intracellular calcium ions from labile stores are said to be implicated in aminophylline-induced seizures [21,22]. However, a report by Ray *et al.*, [23], has implicated oxidative stress due to the generation of free radicals and reactive oxygen species to be responsible for the seizures induced by aminophylline. The leaf extract which has been reported to possess significant antioxidant potential [12] may have exerted it activity through the free radical scavenging activities of its phytochemical constituents. Also, the leaf extract may in part have promoted the synthesis of adenosine, thus its anticonvulsant activity. According to De Sarro *et al.*,[24], pentylene tetrazol (PTZ) is suggested to exert its anticonvulsant effect by inhibiting the activity of gamma aminobutyric acid (GABA) at $GABA_A$ receptors. Gamma aminobutyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively [25, 26]. Phenobarbitone and diazepam, standard epileptic drugs,have been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain[27, 28]. These drugs are reported to antagonise PTZ-induced convulsion [29 by enhancing GABA neurotransmission. Phenytoin was unable to prevent PTZ- induced seizure because it is thought to exert its antiepileptic effect by blocking sodium ions into brain cells thus inhibiting generation of repitative action potential [27]. Since the leaf extract of *H. crinata* was able to delay PTZ – induced convulsion and protect the animals, this also confirm its CNS depressant effect and its ability to enhance GABA-mediated inhibition in the brain. Isoniazid, an anti-tuberculosis drug, induces status epilepticus by depleting brain level of Gamma-Aminobutyric Acid (GABA), a major inhibitory transmitter substance in the mammalian brain, through inhibition of pyridoxal-5-phosphate-dependent Glutamic Acid Decarboxylase (GAD) [30, 31]. Pyridoxal-5-phosphate is the active form of pyridoxine, a cofactor for GAD, and an enzyme required for GABA synthesis [30, 31, 32, 33, 34, 35]. The decrease in GABA levels results in recurrent seizures that characterized status epilepticus [30, 31]. Although isoniazid-induced seizure is known to respond poorly to currently available anticonvulsant drugs, intravenous diazepam is still used to control the seizure episodes in the absence of pyridoxine [30, 31, 35, 36]. The leaf extract of *Heinsia crinata* was found to significantly protect the treated mice against INH-induced convulsion. This action may have been due to the extract's ability to enhanced GABA synthesis in the brain through the activity of its phytoconstituents.

5. CONCLUSION

The results of this study have confirmed the anticonvulsant potential of leaf extract of *Heinsia crinata* and justify its use traditionally in the treatment of convulsion.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Contribution of the Authors

The work was carried out in collaboration with all authors. Author JEO designed the work and performed the experimental procedures, KD wrote the first draft of the manuscript, UAE did the statistical analysis, AIB reviewed and edited the manuscript. All the authors read and approved the final manuscript.

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