



Anticonvulsant activity of the aqueous extract of *Allium cepa* L. (Amaryllidaceae) in rats and mice

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Abstract

The aqueous extract of *Allium cepa* (ACE) has been reported to be effective in the treatment of convulsion. We therefore investigated its effect on seizures induced by maximal electroshock stimulation (MES) and pentylenetetrazole (PTZ) in rats and mice. The preliminary phytochemical constituents were also elucidated. The extract contained tannins, flavonoids, cardiac glycosides, reducing sugars, saponins and alkaloids. Anthraquinones, steroids and terpenes were absent. In the electrically-induced seizure, ACE significantly ($p < 0.01$) prolonged the onset of tonic convulsion at all doses. It also reduced the duration of the tonic convulsion at all doses but it is only significant at 50mg/kg ($p < 0.02$), 200mg/kg ($p < 0.03$) and 400mg/kg ($p < 0.004$). It was reduced from 3.295 ± 0.448 s (control) to 1.172 ± 0.837 s (50mg/kg); 1.62 ± 0.670 s (200mg/kg) and 0.832 ± 0.376 s (400mg/kg). In the PTZ-induced seizure ACE prolonged the onset of tonic convulsion at 50mg/kg and 200mg/kg dose but it was insignificant. It was increased from 3.469 ± 1.335 min (control) to 3.999 ± 1.658 min (50mg/kg) and 4.261 ± 0.740 min (200mg/kg). It also delayed the time it took for the mice to die at 50mg/kg and 200mg/kg, although not significantly. It is concluded that *Allium cepa* extract has an anticonvulsant effect especially in MES-induced seizure and could serve as a good alternative for the treatment of convulsion. This observation explains, at least in part, the basis for its use by herbalists for the treatment of convulsion.

Keywords: Maximal electroshock stimulation; Pentylenetetrazole; Anticonvulsant; *Allium cepa*

INTRODUCTION

Convulsion may be defined as a medical condition where body muscles contract and relax rapidly and repeatedly, resulting in an uncontrolled shaking of the body. About 7 per 1000 people in the United States have a seizure in a given year. The incidence rates are highest in childhood, plateaus from the age of 15 to 65 years, and

rise again among the elderly (Shovon, 1990; Hauser *et al.*, 1991). From the small number of community based studies available, the point prevalence of epilepsy varies from 5.3 to 37 per 1000 in Nigeria (Osuntokun *et al.*, 1987). In Benin City about, 5.4 percent of children are treated for febrile convulsion yearly and it is more frequent in children less than 2 years of age (Osaghae *et al.*, 2009).

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The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history (Barnes *et al.*, 2007). The reasons for the increased popularity of these herbal medicines may include their relative cheapness compared to orthodox medicines, availability (since they are almost always derived from available plants in the locality), and time-trusted efficacy (Anaka *et al.*, 2009).

The onion (*Allium cepa*), also known as common onion, (Fritsch and Friesen, 2002) is the most widely cultivated and one of the most widely used species of the genus *Allium*, and rank third among produce consumed, after tomatoes and cabbage (Block, 2010). The pungent juice of onions has been used as a moth repellent and can be rubbed on the skin to prevent insect bites. When applied to the scalp it is said to promote growth of hair and on the face to reduce freckling. It has been used to polish glass and copperware and to prevent rust on iron. If boiling water is poured onto chopped onions and left to cool, the resulting liquor can be sprayed onto plants to increase their resistance to pests, and the onion plants when growing are reputed to keep away moles and insects, (Fern and Fern, 2013).

Although *A. cepa* is commonly used as vegetable, we are not aware of any known scientific report whatsoever on its anticonvulsant activity. However, herbalists in Nigeria have through oral communication stated that the aqueous extract of onion bulb is equally effective in the treatment of convulsion. We therefore designed the present study in order to ascertain the claim of anticonvulsant properties.

EXPERIMENTAL

Plant material and extraction. The plant was collected from Satana Market in Oredo Local Government Area of Edo State in the month of June, 2013. Bulbs of *Allium cepa* were peeled and washed to remove debris

from them and the water was allowed to drain off. Then they were weighed into 1 kg (1000g) groups. Then the bulbs were chopped into a blending machine. A brand new blending machine was used for this purpose to eliminate contamination. The plant sample (2kg) was blended using 200ml of distilled water. The blended product was poured into a macerating bottle where 800ml of distilled water was added. It was left to macerate for 24 hours.

After macerating for 24 hours, the product was filtered with a clean cloth into a conical flask; the filtrate was filtered again with cotton wool and a glass funnel. The resultant product was poured into a 2-litre gallon and stored in a refrigerator. This same process was repeated with another 2 kg of onions. The filtrate was concentrated over a water bath by evaporating it almost to dryness. Then it was dried in an oven at 40⁰c for 3 days. After drying, it was weighed and the percentage yield calculated.

Experimental animals. The experiment was performed using adult albino mice and rats of both sexes. They were obtained from a breeding centre in Ibadan. They were transported by road using standard cage from the animal house of the department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. The animals were left for two weeks to acclimatize before the experiment began. They were fed on pellets (Bendel feeds and flour mill Ltd, Ewu, Nig.) and water was freely available to all the animals, although before the experiment, feed and water were removed from their cages for 30 min. Animals were exposed to natural room temperature and lighting conditions and were handled according to standard protocol for the use of laboratory animals (National Institute of Health USA: public health service policy on Human care and use of laboratory Animals, 2009). In this study, animals were assigned to 6 groups with 5 to 15 rats in each

group for the MES and 5 mice each for the chemical groups.

Maximal electroshock seizure model. Rats were treated with intraperitoneal administration of the following to the 6 groups respectively; Phenobarbitone (30mg/kg), crude extract (50, 100, 200 and 400mg/kg), normal saline (2ml/kg). 30 min later, seizure was induced by placing 2 ear electrodes on the ears of the rats and passing voltage through them from an ECT machine (industrial contusion, elettromeccaniche-Milano, Italy. Model no. 840).

The animals were observed and the following parameters were recorded following induction; extensor seizure latency, duration of tonic extension and number of animals convulsing or not convulsing. These parameters in the extract groups were compared to those of the control animals to assess anticonvulsant activity. The ability of the plant extract to prevent the seizures or delay/prolong the latency or onset of the hind limb tonic extension was considered as an indication of anticonvulsant activity (Ojewole, 2008; Okokon and Nwafor, 2009).

Chemical convulsant seizure model. Mice were treated with intra peritoneal (ip) administration of the following to the 5 groups respectively: diazepam (0.5 mg/kg), crude extract (50, 100, and 200 mg/kg), normal saline (2ml/kg). Thirty minutes later, seizure was induced by the IP administration of 70mg/kg of pentylenetetrazol (PTZ). The animals were observed for 30 min and those that did not convulse within this time were regarded as having not convulsed. The following parameters were recorded; onset of tonic convulsion, duration of tonic convulsion and the death or recovery time after convulsion. These parameters in mice in the extract group were compared to those of the control animals to assess the anticonvulsant activity.

Drugs and chemicals. Solutions of pentylenetetrazole 70 mg/kg (SIGMA, USA), diazepam 0.5 mg/kg (Roche, Switzerland) and phenobarbitone sodium 30 mg/kg (BDH Chemicals, England) were prepared fresh in distilled water. All other chemicals were of analytical grade and were manufactured by reputable companies.

Statistics. Data are presented as mean \pm SEM (standard error of mean) and n represents the number of animals used for a particular experiment. Comparisons were made between treated and control groups by the use of student t test. All data were analysed using Graph pad instat software (USA). $P < 0.05$ indicates statistically significant difference.

RESULTS

Extraction. The yield was 5.38% w/w.

Phytochemical contents of aqueous extract of *Allium cepa* (ACE) bulb. Table 1 shows that ACE contained tannins, flavonoids, cardiac glycosides, reducing sugars, saponins and alkaloids. Anthraquinones, steroids and terpenes were absent.

Effect of *Allium cepa* extract (ACE) ACE on MES-induced seizure in rats. In the electrically-induced seizure, ACE significantly ($p < 0.01$) prolonged the onset of tonic convulsion at all doses. It also reduced the duration of the tonic convulsion at all doses but it is only significant at 50mg/kg ($p < 0.02$), 200mg/kg ($p < 0.03$) and 400mg/kg ($p < 0.004$). It was reduced from $3.295 \pm 0.448s$ (control) to $1.172 \pm 0.837s$ (50mg/kg); $1.62 \pm 0.670s$ (200mg/kg) and $0.832 \pm 0.376s$ (400mg/kg). This result is shown on Table 2.

Effect of ACE on PTZ-induced seizure in mice. In the PTZ-induced seizure, ACE prolonged the onset of tonic convulsion at 50mg/kg and 200mg/kg dose but it was insignificant. It was increased from 3.469 ± 1.335 min (control) to 3.999 ± 1.658 min (50mg/kg) and 4.261 ± 0.740 min

(200mg/kg). It also delayed the time it took for the mice to die at 50mg/kg and 200mg/kg, although not significantly. This is show on table 3.

Table 1: Phytochemical constituents of aqueous extract of *Allium cepa* (ACE) bulb

Constituents	Observation
Anthraquinones	Absent
Saponins	Present
Tannins	Present
Alkaloids	Present
Cardiac glycosides	Present
Flavonoids	Present
Terpenes	Absent
Reducing sugars	Present
Steroids	Absent

Table 2: Effect of ACE on MES-Induced Seizure in Rats.

Group (mg/kg)	Extensor Seizure Latency (s)	Duration of Tonic Convulsion (s)	Number of animals Protected/used	Percentage Protection (%)
Control (ml/kg)	3. 288± 0. 365	3. 295± 0. 448	0/13	0
PHB	10. 465±0.913*****	0.00±0.00	15/15	100
(ACE) 50	8. 89 ± 2. 872***	1. 172± 0. 837**	3/5	60
(ACE) 100	5. 478± 1.193*	2. 591± 0. 701	4/9	44. 44
(ACE) 200	7. 011± 1. 122*****	1. 62± 0. 670*	5/10	50
(ACE) 400	7. 632±1. 529 *****	0. 832±0. 376*****	2/5	40

Values are expressed as mean ± SEM, *p< 0.03, ** p< 0.02, ***p< 0.007, **** p< 0.004, ***** p< 0.002, *****p< 0.001, *****p< 0.0001, significantly different when compared with control group, n=5-15, PHB = Phenobarbitone, ACE = *Allium cepa* extract

Table 3: Effect of ACE on PTZ-Induced Seizure in Mice

Group mg/kg	Onset of Tonic Convulsion (min)	Duration of Tonic Convulsion (min)	Death time (min)
Control (ml/kg)	3.469±1.335	0.161±0.024	4. 561±2.001
PHB	0.00	0.00	0. 00
DZP	24. 217± 4.690***	0.213± 6.002	17. 300±7.581
(ACE) 50	3. 999±1.658	0. 588±0. 388	6. 031±2.828
(ACE) 100	3. 105±1. 178	0. 548±0. 190*	4. 536±1.356
(ACE) 200	4. 261±0.740	0. 253±0.021**	5. 730±0. 715

* p< 0.04, ** p< 0.02, *** p< 0.003 significantly different when compared with control group, n = 5, PHB = Phenobarbitone, ACE = *Allium cepa* extract, DZP= Diazepam

DISCUSSION

Currently available anticonvulsant drugs are able to efficiently control epileptic seizure in about 75% of the patients. Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult. So there are demands for new types of anticonvulsant drugs. One of the approaches is to search for new antiepileptic drugs in the naturally occurring compounds which may belong to new structural classes (Azadeh et al., 2011).

In the present study, the effect of *Allium cepa* extract on MES-Induced convulsion in rats and PTZ-induced convulsion in mice were evaluated and the result evidently demonstrated for the first time that *Allium cepa* extract is able to produce potent anti-convulsant activity in MES seizure.

In MES-induced seizure model, ACE increased the latency of tonic seizure in a dose dependent manner at all the doses and also reduced the duration of tonic seizure at all doses, although only significant at

50mg/kg, 200mg/kg and 400mg/kg. This finding suggests that the extract has good anticonvulsant protection in the MES-animal model. It resulted in significant protection against MES-induced seizure following prior administration of the extract hence ACE can be considered a good alternative anticonvulsant of plant origin. The direct mechanism of action of MES induced convulsion is not known but it can be assumed that ACE provided protection against MES-induced seizure by inhibiting GABA receptor nerve transmission.

In PTZ-Induced seizure model, ACE prolonged the onset of tonic convulsion at 50mg/kg and 200mg/kg, although it was not significant, and also delayed the time it took for the mice to die at 50mg/kg and 200mg/kg although insignificantly. It resulted in protection of mice in the PTZ-induced seizure model following prior administration although not comparable to standard drugs used (Phenobarbitone and Diazepam). PTZ is generally assumed to exert its action by acting as an antagonist at the picrotoxin sensitive site of the GABA receptor complex (Ramanjaneyulu and Ticku, 1984). Since PTZ has been shown to interact with the GABA neurotransmission (Loscher and Schmidt, 1988; De Deyn *et al.*, 1992), PTZ-induced seizures can be prevented by drugs that enhance GABA_A receptor-mediated inhibitory neurotransmission such as benzodiazepines and Phenobarbital (Coulter *et al.*, 1989; McDonald and Kelly 1995; Senthil Kumar and Raj Kapoor, 2010). Antagonism of PTZ-induced seizure with the administration of ACE suggest that ACE interacts with GABA neuro-transmission.

The efficacy of most herbal remedies is attributed to the combination of various active principles. The observed pharmacological actions of the *Allium cepa* extract may be due to the presence of saponins, tannins, flavonoids, alkaloids, reducing sugar and cardiac glycosides, as

indicated by the results of preliminary phytochemical screening; as triterpenoids (Datta *et al.*, 2004), saponins (Dubois *et al.*, 1986), flavonoids (Datta *et al.*, 2004; Dubois *et al.*, 1986) from other plants have been reported to have central nervous system depressant activity. Flavonoids, an important class of naturally occurring compounds, have demonstrated CNS activities such as affinity for GABA_A receptors and anticonvulsant effects (Miliauskas *et al.*, 2004, Huen *et al.*, 2003). Some researchers have reported anticonvulsant activity of apigenin as glucoside flavonoid of *T. polium* (Abdollahi *et al.*, 2003, Kawashty *et al.*, 1999). Triterpenes are reported to possess anticonvulsant activity in some experimental seizure models like PTZ and MES. Monoterpenes also have protective effects against PTZ-induced convulsions (Librowski *et al.*, 2000; Brum *et al.*, 2001). Presence of Saponins, like tropeoside and ascalonides in *Allium cepa* have been reported (Leung's encyclopedia of natural ingredient, 2010). Existence of saponins in *Allium cepa* may explain some of its anticonvulsant activity. Also the presence of phenolic substance in *Allium cepa* has been reported. Phenolics present in phenyl propanoid moieties of caffeoyl-quinic acid had sedative effects in pentobarbital-induced sleeping time in mice and anticonvulsant effect in PTZ-induced mice (Nugroho *et al.*, 2012). The presence of phenol in phenobarbitone, a potent anticonvulsant drug, which was also used as a standard drug in this experiment, explains the anticonvulsant activity demonstrated by the extract of *Allium cepa*. Phenobarbitone is a long acting barbiturate used as an anticonvulsant, sedative and hypnotic [Miller, 2003]. Phenobarbitone acts by interfering with GABA receptors, blocking nerve impulse transmission in CNS, which reduces motor activity and raises seizure threshold (Nursing Spectrum Drug Handbook, 2009). Currently used anticonvulsant drugs (e.g.

phenobarbitone, phenytoin) effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test (White, 1997; Macdonald and Kelly 1993). Also phenols are reported to have anticonvulsant activities in PTZ-induced convulsion in Mice (Nugroho *et al.*, 2012).

Conclusion. It is concluded that *Allium cepa* extract has an anticonvulsant effect especially in MES-induced seizure and could serve as a good alternative for the treatment of convulsion. However, further research is necessary to determine the exact mechanism by which it works.

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