EVALUATION OF THE ANTICONVULSANT ACTIVITY OF THE ETHANOL LEAF EXTRACT OF UVARIA AFZELLI Sc. Elliot (ANNONAECAE) IN MICE

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ABSTRACT

Epilepsy is the fouth most common brain disorder in the world and about 65 million of the global population are victims. Sadly, the currently available conventional drugs are riddled with numerous side effects, expensive and have not demonstrated adequate capacity in managing this disorder. Hence, the need for safer, cheaper and more effective therapy. This study is aimed at investigating the anticonvulsant activity of the ethanol leaf extract of Uvaria afzelii. Twenty-five (25) mice were randomly allotted to five different groups of five mice each. The animals were treated orally with 100, 200 and 400 mg/kg of the extract respectively and 10 ml/kg of distilled water. The standard group received diazepam (5 mg/kg, i.m). Both strychnine (4 mg/kg, i.p) and picrotoxin (5mg/kg, i.p.) were used to induce convulsion 30 minutes post administration of extract and distilled water, and 15 minutes for standard drug. The onset and duration of convulsion for each mouse were recorded. Mice that did not convulse within 30 min of strychnine and picrotoxin injection were considered protected. The extract (400 mg/kg) significantly ($P<0.01$) delayed the onset of convulsion with significant reduction ($P<0.05$) in the duration of seizures induced by picrotoxin with 20% mortality. In the strychnine-induced test, U. afzelii (200 & 400 mg/kg) significantly delayed ($p<0.001$, 0.0001) the onset of convulsion with mortality of about 40%. Preliminary phytochemical screening revealed the presence of tannins, alkaloids, flavonoids, phenols and saponin. The findings in this study show that the ethanol leaf extract of Uvaria afzelii possesses anticonvulsant activity.

Keywords: Anticonvulsant, *Uvaria afzelii,* Diazepam, Strychnine, Picrotoxin .

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Convulsion (seizure) is a symptom of epilepsy, which has been described as the fourth most common serious chronic brain disorder, estimated to affect at least 65 million people in the world, of which 10 million live in Africa alone (WHO, 2019). Epileptic seizures are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking. In epilepsy, seizures tend to recur, and have no immediate underlying cause while seizures that occur due to a specific cause are not deemed to represent epilepsy (Fisher *et al.,* 2014; Magiorkinis *et al.,* 2010; Fisher *et al.,* 2005). Genetic mutations are linked to a small proportion of the disease (Longo and Dan, 2012). About 80% of people with epilepsy are living in low and middle income countries and are being affected by gaps in advocacy, diagnosis and treatment (WHO, 2019).

The burdens of stigmatization, disability, co-morbidities, poverty and death are even more worrisome and alarming in people living with epilepsy (Solomon *et al.,* 2015). Currently, there are several conventional drugs used to treat this disorder but most of these drugs are characterized by life threatening side effects which have ultimately defeated their essence. The newer antiepileptic drugs are more effective with fewer adverse effects but they are expensive and this remains a major headache in the treatment of epilepsy especially in Africa. Against this backdrop, researchers are now working hard in areas of plant medicine and related products which are more effective, less expensive and devoid of life threatening adverse effects.

Uvaria afzelii Scott Elliot (Annonaeceae) is found mainly in tropical region (Graham & Bernard 1978) and has been used traditionally in the

treatment of bronchitis and cough (Burkill, 1985). There are different species of *Uvaria* and they are widely distributed in the tropics, Africa in particular (Graham & Bernard 1978). *U. afzelii* is a small tree or spreading shrub growing up to 5 m tall. The tree is used locally, being harvested from the wild for food and medicines. It is widely distributed and grown in the Southern part of Nigeria, where it is known by various local names such as "gbogbonishe" (Yoruba), "Umimiofia" (Igbo) and "Osu-umimi" (Ukwani) (Odugbemi, 2008). Locally it is used in the treatment of cough, vaginal tumor, breastaches, swollen hands and feet, diabetes and gonorrhea (Kayode *et al*., 2009).The flowers are very heavily scented. The bark or pieces of the stem are sometimes put into palm-wine to add potency, or are added to the distillate to give colour to the spirit (Burkil, 1985). All the parts of the plant are fragrant and as such are used in the preparation of pomade in Ghana. Investigations have revealed its bacteriocidal activity against Gram-positive and acid-fast bacteria (Lawal and Okoli, 2011). Other species of *Uvaria* have been found useful in folklore medicine. This includes *U. doeringii*- the leaf decoction is used for piles, palpitations and pains (Burkill, 1985). *U.scabrida* is used in the treatment of insanity while *U. thomasii* is used in the form of a leaf decoction for catarrh and colic (Kerharo & Adam 1974). *U. tortilis* is used in the treatment of amenorrhoea (Borquet & Debray, 1974). Preliminary ethnobotanical survey conducted on *U. afzelii* revealed its use in the treatment CNS disorders including epilepsy. The aim of this study is to investigate the anticonvulsant activity of the ethanol leaf extract of *U. afzelli* which to the best of our knowledge is yet to be reported.

MATERIALS AND METHODS

Plant collection

Fresh leaves of *U. afzelii* were collected from the environs of Ijebu North-East local Government area of Ogun state, South-west, Nigeria. Identification and authentication were done in the Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria.

Extraction

The fresh leaves of *U. afzelii* were air-dried, grinded, weighed and soaked in 1.5 L of ethanol for 48hours and were filtered. The filtrates were evaporated to dryness at 40^0C under reduced pressure to give a dark brown extract. The dried extract was weighed and dissolved in distilled water to give a desirable working concentration before administration to experimented animals.

Experimented animals

Albino mice (20-30g) of both sexes used in this study were obtained from the animal house, Faculty of Pharmacy, Olabisi Onabanjo University, Ago Iwoye, Nigeria. The animals were kept in well-ventilated hygienic polycyclic cages (38 cm x 23 cm x 10 cm) and maintained under standard environmental conditions (Temperature 25±6°C). They were allowed free access to standard dry pellet diet and water *ad libitum*. The mice were acclimatized for 14 days before the commencement of the experiment. Experimental procedures were carried out in accordance with the United States National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (United States National Institute for Health Publications, 1985).

Preliminary Phytochemical Screening

Preliminary phytochemical screening to detect the presence or absence of saponin, flavonoids, alkaloids, phenols, tannins was carried out according to the procedures of Sofowora and Odebiyi, (1978).

PHARMACOLOGICAL STUDIES

Picrotoxin- Induced Seizures Test

Picrotoxin (5mg/kg,*i.p*.) was administered to the mice 30 min. after treatment with distilled water (10ml/kg, p.o) and extract (100, 200 and 400 mg/kg bodyweight), and 15 min. after diazepam (2 mg/kg *i.m*). The onset and duration of convulsion for each mouse were recorded for 30 min after the administration of picrotoxin (Malami *et al*., 2016, Murtala and Akindele, 2018)

Strychnine- induced seizures test

Strychnine (4 mg/kg,*i.m*.) was administered to the mice 30 min. after treatment with distilled water (10 ml/kg, p.o) and extract (100, 200 and 400 mg/kg bodyweight), and 15 min. after diazepam (2 mg/kg *i.m*). The onset and duration of convulsion for each mouse were recorded for 30 min after the administration of strychnine (Porter *et al*., 1984; Perazzo *et al*., 2003, Gao *et al*., 2018).

Statistical analysis:

Results obtained were expressed as mean ± SEM (n = 5). The data were analyzed using one way ANOVA followed by Dunnett's post-hoc test using Graph Pad

Prism 6 Software. Results were considered significant when $P < 0.05$.

RESULTS

Preliminary Phytochemical Screening

Preliminary phytochemical screening showed the presence of tannins, saponin, phenol, alkaloids, flavonoids and cardiac glycosides in the extract.

Pharmacological studies

Picrotoxin-induced seizure

The extract 100, 200 and 400mg/kg produced a dose dependent increase in seizure latency. The maximum effect of the extract was seen at 400mg/kg with 19.86±1.50 min seizure latency, which was significantly (P<0.01) greater than 3.58±0.41 min seizure latency observed with the control. On the duration of the seizure, at 100 mg/kg, the extract narrowly increased the duration of seizure which was not significant compared with the control. However at 400 mg/kg, the extract, 1.60±0.244 min significantly (P < 0.05) reduced seizure duration compared with the control, 4.97±1.09 min which was longer. Notably, all the extracts-treated mice convulsed, the percentage mortality reduced as the dose increases, 20 % at 400 mg/kg (Table 1).

Strychnine-induced seizure

The extracts200 and 400 mg/kg produced a dose dependent increase in seizure latency in this model with significant (P < 0.001, 0.0001) delay of about12.40±1.806 and 15.40±1.72 min compared to the 0.454±0.029 min seizure latency of the control group. However, the extract did not produce any significant effect on the length of seizure. The percentage mortality was 100% in the control group while it was 60% at both 100 and 200 mg/kg, then 40 % at 400 mg/kg (Table 2).

Table 1. Effects of the Ethanol Leaf Extract of *U. afzelli* in picrotoxin-induced seizure test in mice

Treatment	Dose (mg/kg)	Onset of seizure (min.)	Duration of seizure (min.)	Convulsion ratio	Mortality ratio
Control	10ml/kg	3.58±0.41	4.97±1.09	5/5	5/5
Diazepam	5	22.96±1.35***	1.16±0.63*	3/5	0/5
Extract	100	5.62±0.71	6.31±0.70	5/5	3/5
Extract	200	15.22±6.17*	2.05±0.92*	5/5	2/5
Extract	400	19.86±1.50**	1.60±0.24*	5/5	2/5

Values represent mean ± S.E.M. (n=5) *P<0.05, **P<0.01, ***P<0.001 vs. Control (One way ANOVA followed by Dunnet's multiple comparison test)

Table2: Effects of the Ethanol Leaf Extract of *U. afzelli* in strychnine-induced seizure test in mice

Treatment	Dose (mg/kg)	Onset of seizure (min.)	Duration of seizure (min.)	Convulsion ratio	Mortality ratio
Control	10ml/kg	0.454±0.029	1.144±0.1695	5/5	5/5
Diazepam	5	23.20±2.596****	0.694±0.1809	4/5	1/5
Extract	100	6.800±1.356	2.498±1.657	5/5	3/5
Extract	200	12.40±1.806***	1.210±0.5016	5/5	3/5
Extract	400	15.40±1.720****	0.460±0.1043	5/5	2/5

Values represent mean ± S.E.M. (n=5) *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. Control (One way ANOVA followed by Dunnet's multiple comparison test)

DISCUSSION AND CONCLUSION

DISCUSSION

Essentially, the concept of traditional medicine is gaining more popularity in Africa and even beyond the shores of the continent. The usefulness of these medicinal plant materials is largely due to their phytochemical components or multitude of secondary metabolites present in these plants (Akinmoladun *et al.,* 2007). The preliminary phytochemical screening of the leaf extract of *U. Afzelii* revealed the presence of tannins, alkaloids, saponin, phenol, flavonoids and cardiac glycosides, as previously reported by Sofowara, 1993, Trease and Evans, 1989, Yadav and Agarwala, 2011. These secondary metabolites, singly or in combination, account for some of the pharmacological effects credited to this plant. In this study, it was found that the extracts at 400 mg/kg, significantly increased seizure latency and reduced the duration of seizure in Picrotoxin-induced convulsion test. In strychnine-induced convulsion test, the exract at 200 and 400 mg/kg significantly increased the onset of convulsion but did not affect the length of convulsion. Picrotoxin, a GABA antagonist, was reported to cause convulsion by antagonizing $GABA_A$ receptor-linked chloride ion channels thereby blocking the influx of chloride ions into the brain cells (Leonard, 2000, Nicoll, 2001). Picrotoxin has also been reported to block glycinergic activity. Strychnine, a competitive glycine antagonist, an inhibitory amino acid and neurotransmitter has been reported also to cause convulsion (Ishola *et al*., 2013). The seemingly blocking effects of plant extracts on the actions of picrotoxin and strychnine suggest that the plant may have delayed seizure latency and reduced duration of convulsion by affecting the GABAergic mechanisms and glycinergic pathways respectively (Mora-Perez *et al*., 2016, Murtala and Akindele 2018, Shelar *et al*., 2018). Based on these findings, *U. afzelii* can be said to possess anticonvulsant activity since it increases the seizure latency and decreases its duration. Ayoka *et al*., (2006), reported phenolic compounds, especially flavonoids to exhibit a wide range of neuropharmacological properties such as sedative, anticonvulsant and antipsychotic activities. Murtala and Akindele (2018) reported the involvement of steroids, saponins and alkaloids in the anticonvulsant activity of hydroethanol leaf extract of *Newbouldia laevis*. The anticonvulsant activities of steroids and terpenoids have also been reported in the methanolic stem and root extracts of *Katanchoe pinnata* (Mora-Perez *et al*., 2016). The flavonoids, steroids, saponins and alkaloids present in *U. afzelii* may be responsible for its anticonvulsant activity. However, the involvement of other secondary metabolites in the plant cannot be ruled out (Sowemimo *et al.,* 2012).

CONCLUSION

The findings in this study have shown that the ethanol leaf extract of *Uvaria afzelii* possesses anticonvulsant activity and this justifies the use of the leaf of *Uvaria afzelii* in the treatment of convulsion in traditional medicine. However, there is need for more work to be done to ascertain the actual components involved and the possible mechanism of action.

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