African Journal of Pharmacy and Pharmacology Vol. 3(6). pp.319-322, June, 2009
Available online http://www.academicjournals.org/ajpp
ISSN 1996-0816 © 2009 Academic Journals

Full Length Research Paper

Anticonvulsant activity of *Rauvolfia Vomitoria* (Afzel)

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Accepted 10 June, 2009

The anticonvulsant activity of the aqueous leaf extract of *Rauvolfia vomitoria* (Afzel) was investigated by testing the effects of the extract on strychnine-, picrotoxin and pentylenetetrazole induced seizures in mice. Experiments were carried out on male albino mice and the animals were randomly allotted to the different control and test groups. The extract, at a dose of 200 mg/kg, prolonged the onset of seizures from strychnine, picrotoxin and pentylenetetrazole in the animals. Acute toxicity testing produced a medial lethal dose of 17.5 g/kg. Phytochemical screening of the plant reveals the presence of alkaloids, saponins, tannins and reducing sugars. The results indicate a possible efficacy potential of the aqueous leaf extract of *R. vomitoria* in convulsions.

Key words: *Rauvolfia vomitoria*, convulsion, phytochemical, acute toxicity.

INTRODUCTION

Epilepsy (excluding febrile convulsion) is very common and can affect all ages (Hauser and Annegers, 1993). An epidemiological study suggests a prevalence of 6.8/1000 in the U.S.A (Hauser et al., 1991). It is probable that the prevalence is higher in less developed countries because of higher incidence of antecedent factors such as brain infections, cranial and perinatal traumas and parasitic INFECTIONS (Sander and Shorvon, 1987). Although the prognosis for controlling seizures in most patients in terms of seizure control, remission and withdrawal of medication is good (Goodridge and Shorvon, 1983; Cockerel et al., 1993). Many patients (20 - 30%) however, have seizures that are not adequately managed by the established antiepileptic drugs (AEDs) (Richens and Perucca, 1993), making traditional herbs and herbalists very useful and indispensable especially in underdeveloped countries of Africa (Osuntokun et al., 1987; Shorvon and Farmer, 1988). Thus a need arises for new agents with greater efficacy, negligible or reduced side effects and devoid of unfavourable drug interactions unlike most AEDs in the market today (Triamble, 1990).

Rauvolfia vomitoria (Apocynaceae) is a medicinal plant widely distributed all over the world, especially in Asia and West-African countries. It is a tree that grows to a height of about 15 m and is found in most lowland forest. In Nigeria, especially in Yoruba speaking region, the plant is popularly known as “Asofeyeje” meaning bearing fruits for the birds. It is known as “Akanta” in Ibo and “Penpe” in Ashanti, Twi and Wassaw. The plant has been used extensively for various ailments. It is useful in the lowering of blood pressure (Amole, 2003), as an antimalarial (Amole et al., 1993), as well as an antipyretic (Amole and Onabanjo, 1999). It also possesses analgesic (Amole et al., 2006) and haematinic properties (Amole and Ogunjere, 2001). Information on the anticonvulsant activity of *R. vomitoria* had not been reported; hence this study was designed to investigate the effect of the aqueous extract of the plant on convulsion.

MATERIALS AND METHODS

Collection of *R. vomitoria* and preparation of the extract

Fresh leaves of the plant were collected from a source in Mushin in Lagos State, Nigeria, identified and authenticated by Professor Olowokudejo of the Department of Botany and Microbiology, University of Lagos, Lagos, Nigeria. The collected leaves were dried in the oven at 20°C until a constant weight was attained and then blended. The blended material was extracted in water (50 g / L) using soxhlet apparatus. The extract was weighed on a balance and stored in a refrigerator (4°C) until ready for use. From this, various concentration were reconstituted in a known volume of distilled water before administration.

Experimental animals

Experiments were carried out on male albino mice 18 - 20 g, which were bred in the Laboratory Animal Centre of the Lagos State Uni-

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Table 1. Effect of aqueous leaf extract of *R. vomitoria* on strychnine-induced seizure in mice.

Treatment and route	Seizure onset ± S.E.M. (min.	
	Clonic	Tonic
Control-NS (10 ml/kg, i.p.)	1.2 ± 0.1	1.3 ± 0.1
R. vomitoria (50 mg/kg, i.p.)	2.2 ± 0.3 [a,b]	2.7 ± 0.4 [a,b]
(200 mg/kg, i.p.)	6.2 ± 0.4[a]	7.3 ± 0.7 [a]
Diazepam (10 mg/kg, i.p)	6.5 ± 0.5 [a]	7.7 ± 0.8 [a]

Values are mean ± SEM; [a] Significantly (P < 0.05) different compared to control, [b] Significantly (P < 0.05) lower compared to standard drug-diazepam, Student's *t* test; NS (Normal Saline); n = 5.

Table 2. Effect of aqueous leaf extract of *R. vomitoria* on picrotoxin-induced seizure in mice.

Treatment and route	Seizure onset ± S.E.M. (min.)	
	Clonic	Tonic
Control-NS (10 ml/kg, i.p.)	5.4 ± 0.2	5.8 ± 0.3
R. vomitoria (50 mg/kg, i.p.)	8.6 ± 1.4 [a,b]	9.9 ± 1.7 [a,b]
(200 mg/kg, i.p.)	12.4 ± 1.2 [a,b]	14.6 ± 1.7 [a,b]
Diazepam (10 mg/kg, i.p)	16.3 ± 1.7 [a]	19.2 ± 2.8 [a]

Values are mean ± SEM; [a] Significantly (P < 0.05) different compared to control, [b] Significantly (P < 0.05) lower compared to standard drug-diazepam, Student's *t* test; NS (Normal Saline); n = 5.

versity College of Medicine, Ikeja. The animals were fed with rodents chow and had free access to drinking water *ad libitum*. All experiments were performed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as contained in United States National Institutes for Health Guidelines (1985).

Strychnine induced seizures

Mice were randomly allotted to the different control and test groups. The control mice were administered with strychnine (4 mg/kg, i.m) 30 min after normal saline p.o. The positive control group of mice received 4 mg/kg, strychnine, 15 min after 10 mg/kg diazepam, i.p. Two doses (100 and 200 mg/kg) of the extract were given to each of the test groups i.p. (15 min) before 4 mg/kg strychnine. Any mouse that did not convulse within 30 min after strychnine administration was considered protected (Yemitan and Adeyemi, 2005).

Picrotoxin induced seizures

Mice were randomly allotted to the different control and test groups. Picrotoxin (5 mg/kg, i.p.) was used to induce seizure using the same treatment procedure as described for strychnine (Yemitan and Adeyemi, 2005a).

Pentylenetetrazole (PTZ) induced seizure test

The extract (100 and 200 mg/kg, p.o.), diazepam (2 mg/kg, i.m.) or normal saline (10 ml/kg, p.o.) was administered to groups of mice (n = 5), 30 min before PTZ (75 mg/kg, i.p.) and onset to forelimb cloni, as well as hind limb extension was recorded. The onset and number of deaths after showing tonic hind limb extension were also recorded (Yemitan and Adeyemi, 2005b).

Acute oral toxicity test

A total of 30 albino mice of both sexes were randomly allotted to five groups consisting of the control and four *R. vomitoria*- treated groups. Mice were fasted for 12 h and various doses (50 mg/kg – 20 g/kg) of the extract were administered orally to the test groups. The control group was given distilled water (10 ml/kg) orally. The mice were closely observed for toxic symptoms and behavioural changes for the first 2 h after extract administration and mortality recorded within 24 h. The lethal dose that killed 50% of the mice was estimated after 24 h, applying the method of Miller and Tainter (1944).

Phytochemical test

Phytochemical analysis of *R. vomitoria* for its active biological principles was conducted using the standard methods described by Farnsworth (1989); Sofowora (1993).

Statistical analysis

Values are reported as mean ±.S.E.M. Statistical analysis was carried out using Student's *t*-test to calculate significance of difference. P values < 0.05 were considered significant.

RESULTS

Strychnine induced seizures

The two EXTRACT doses (100 and 200 mg/kg, i.p.), like diazepam (10 mg/kg, i.p.), did not prevent seizure against strychnine (4 mg/kg, i.m.)-induced convulsion test, but a significant (P < 0.05, Student's *t* test) and dose-dependent prolongation of both clonic and tonic seizure latencies were recorded for the extract compared with control mice. *R. vomitoria* dose of 200 mg/kg produced comparable effect to the reference drug, diazepam (Table 1).

Picrotoxin induced seizures

The two extract doses (100 and 200 mg/kg, i.p.), like diazepam (10 mg/kg, i.p.), did not prevent seizure against picrotoxin (4 mg/kg, i.m.)-induced convulsion test, but a significant (P < 0.05, Student's *t* test) and dose-dependent prolongation of both clonic and tonic seizure latencies were recorded for the extract compared with control mice. Both extract doses, however, produced a significantly lower effect compared with the reference drug, diazepam (10mg/kg, i.p.) (Table 2).

Pentylenetetrazole Induced Seizures

Significant (P < 0.05) and dose-related prolongation of onset to seizures and time to death were produced by the two doses of the extract, like phenobarbitone (Table 3) in the pentylenetetrazole (75 mg/kg, i.p.) seizure model.

Table 3. Effect of aqueous leaf extract of *Rauvolfia vomitoria* on pentylenetetrazole induced seizures.

Treatment (mg/kg)	Onset to clonic seizure in minutes ± S.E.M (fraction showing forelimb clonus)	Onset to tonic seizure in minutes ± S.E.M. (fraction showing hind limb tonus)	Time to death in minutes ± S.E.M. (fraction that died within 1 h)
Control	4.34 ± 0.62 (5/5)	6.56 ± 0.68 (5/5)	26.37 ± 3.15 (5/5)
RV (50)	5.57 ± 0.60* (5/5)	6.49 ± 0.77 (4/5)	34.49 ± 2.52* (3/5)
RV (200)	15.44 ± 2.52* (3/5)	27.11 ± 2.49 (1/5)	49.57 ± 3.55* (3/5)
Diazepam (10)	23.32 ± 3.17 (2/5)	-- (0/5)	-- (0/5)

Table showing the anticonvulsant effects and time to death after 30 min pretreatment of mice with *R. vomitoria,* diazepam or distilled water before pentylenetetrazole in mice. * Significant at $P < 0.05$ (Student's *t*-test). RV = aqueous leaf extract of *R. vomitoria*; Control mice received 10 ml/kg distilled water, n = 5 mice per group.

Table 4. Acute i.p (24 h) - toxicity study.

Treatment (g/kg)	Log Dose	24 h Mortality	% Mortality	Probit
Control	–	0/6	0	–
R. vomitoria				
(0.6)	0.2218	0/6	0	–
(1.2)	0.0792	0/6	0	–
(3.0)	0.4771	0/6	0	–
(8.0)	0.9031	1/6	16.7	4.0
(17.5)	1.2430	3/6	50.0	5.0
(37.5)	1.5740	6/7	100.0	(8.7)

Table shows mortality data of 24 h acute toxicity study of orally-administered aqueous leaf extract of *R. vomitoria* in mice (n = 6).

Table 5. Phytochemical constituents of the aqueous root extract of *R. vomitoria.*

Phytochemicals	Remark
Alkaloid	+++
Glycosides	–
Saponins	+++
Tannins	+++
Anthraquinones	–
Carbohydrates	++
Reducing sugars	++
Phenols	–

– = Absent, ++ =abundant, +++ =very abundant.

Acute toxicity tests

Orally administered doses of *R. vomitoria* did not produce any observable toxicity, except for dose-related sedation which graduated to hypnosis as the doses increased. Mortalities were recorded in mice at higher doses, producing a LD_{50} of 17.5 g/kg (Table 4).

Phytochemical screening

Preliminary phytochemical investigations of the aqueous leaf extract of *R. vomitoria* revealed the presence of alkaloids, saponins, tannins and reducing sugars (Table 5).

DISCUSSION

The effectiveness shown by the aqueous leaf extract of *R. vomitoria* against acute seizure induced by chemical convulsants, strychnine and picrotoxin suggests anticonvulsant effects. Strychnine has been demonstrated to have a well defined mechanism of convulsant action reported to be by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine (Biggio et al., 1992) and thus increasing spinal reflexes (Rang et al., 1998). Picrotoxin, on the other hand, is a selective non-competitive antagonist of gamma amino butyric acid (GABA) at $GABA_A$ receptor, which has been widely implicated in epilepsy (Rang et al., 1998). GABA is the major inhibitory neurotransmitter in the brain and its inhibition is thought to be an underlying factor in epilepsy (Gale, 1992). It is therefore, probable that the anticonvulsant effect of the extract might involve both GABAergic and glycinergic inhibitory mechanisms. Preliminary phytochemical investigations of the extract revealed the presence of alkaloids, saponins, tannins and reducing sugars. Finally, the aqueous leaf extract of *R. vomitoria* neither induced lethality nor mortality in mice when administered orally at doses less than 8 g/kg. However, a dose-dependent mortality within a range of doses 8.0 - 20 g/kg was recorded giving an LD_{50} of 17.5 g/kg. Based on the result of toxicity studies in experimental animals, acute exposure of the extract could be said to be relatively safe, because according to Clarke and Clarke (1977), any

plant extract with estimated LD_{50} greater than or equal to 1 g/kg per oral route should be considered safe in experimental animals. Moreover, the dose of the extract giving the minimum mortality, 8 g/kg, is more than the test limit of 5.0 g/kg put forward (OECD, 1996), above which a substance may not be considered to have induced any toxicity that might result thereof. Furthermore, the behavioural components observed during acute exposures to doses above were dose-dependent somnolence and sedation which could easily be likened to side effects of the active constituents of the extract.

In conclusion, the aqueous leaf extract of *R. vomitoria de*monstrated potential anticonvulsant properties and less toxicity in the experimental animals at the doses used. However, further studies still needed to be carried on exposure of the extract to humans, and its use in folk medicine for seizure control should be accompanied by regular assessment of level of consciousness and blood pressure.

ACKNOWLEDGEMENTS

The authors are highly indebted to the authorities of Lagos State University College of Medicine, Ikeja. This investigation was supported by the College of Medicine Research Grant.

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