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Anticonvulsant Activities of *Ipomea involucrata, Milletia aboensis, and Rauvolfia vomitoria* on 4-aminopyridine Induced seizure in Mice

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Original Research Article

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Abstract: The roots of Ipomea *involucrate* (IP) and Rauvolfia *vomitoria (*RV) and the leaves of *Milletia aboensis* (MA) are reportedly used for the treatment of mental illness. This study investigated the anticonvulsant potentials of these plants. Ethanolic crude extracts of IP and RV (50, 100, and 200 mg/kg bw, ip) and MA (100, 200, and 400 mg/kg bw, ip) were administered to respective groups of mice (n=6). The positive control group received 30mg/kg bw, ip of Carbamazepine, while negative control group received 1% DMSO (vehicle). After 30 min, epilepsy was induced by injection of 4-Aminopyrydine (4-AP) (15mg/kg bw ip). They were observed for the next 30min. The ability of the extracts to delay the onset of trembling, hindlimb extension, seizure, and protect from death as compared to the untreated group was taken as a sign of anticonvulsant activity. Ethanolic crude extracts of MA, RV and IP possess anticonvulsant potencies in the order - MA>RV>IP; with MA protecting two-thirds of animals against death from 4-AP-induced seizures. The plant extracts have the potentials of tackling generalized tonic-clonic and partial seizures. MA and RV may exert their effects by acting on ion channels while IP is most likely to act via other mechanisms.

Keywords: Anticonvulsant; *Ipomea involucrate*; Milletia *aboensis; Rauvolfia vomitoria;* 4-aminopyridine; Mice

INTRODUCTION

Epilepsy is a common and most serious non-infectious brain or neurological disorder with a very high prevalence among developing countries. In Nigeria, its prevalence varies from 15 to 37 per 1000, with 70 – 80% of the people living with epilepsy (PWE) having onset of seizures below 30years of age [1].

Commonly available Antiepileptic Drugs (AEDs) are effective in controlling seizures in about 70% of epileptic patients but their use is limited by serious side effects and drug interactions. The other percentage of patients is refractory to therapeutic intervention, showing that a satisfactory and effective therapy with high tolerability remains a challenge [2-5].

Medicinal plants used for epileptic therapy in traditional medicine possess promising anticonvulsant properties in animal models thus making them invaluable sources of new antiepileptic compound [2]. It is believed that AEDs of herbal sources may possess better safety profiles and effectiveness. Some of the plants evaluated and found to possess anticonvulsant activities among others include *Maerua angolensis* and *Securinega virosa*, *Moringa oleifera*, *Carissa carandus* [6-9]. About a decade ago, as many as 42 plants indicated for the treatment of epilepsy in the Danish folklore was screened for anticonvulsant potential [10]. Later on, an ethnobotanical survey of Akwa Ibom state reported on the use of *Ipomea involucrate*, *Rauvolfia*, and *Milletia aboensis* for the treatment of mental illness among the ethnic nationalities of Southern Nigeria [11]. In this study, we evaluated the anticonvulsant potentials of these three plants.

MATERIALS AND METHODS

Collection of Plant Materials and Extraction

The plant samples were obtained from Abak, Ibesikpo Asutan, and along Calabar-Itu Highway, Itu, all in Akwa Ibom State. They were authenticated at the Botany Department, University of Calabar, Calabar. Fresh leaves of *Milletia aboensis* and the roots of both *Ipomoea involucrata* and *Rauvolfia vomitoria* were washed and allowed to drain completely. They were air-dried under shade for 3weeks and later oven-dried (40˚C) until crispy to aid pulverization by mechanical blender. Pulverized samples were soaked in 80%ethanol (48h for the leaves and 72h for the roots) for extraction. These were then filtered with cheese cloth and further with Whatmann No 1 filter paper; filtrate was then concentrated at 40˚C to yield dry samples. The extracts were kept refrigerated until needed for use at which

point reconstitution was made in 1percent Dimethyl Sulfoxide (DMSO).

Phytochemical screening

The qualitative phytochemical analyses was done for alkaloids, tannins, saponins, flavonoids, phytate, oxalate, glycosides, reducing sugars and phenols using standard methods as reported by Njoku and Obi, (2009) [12], Borokini & Omotayo, [13].

Acute toxicity test (i.p)

The median lethal doses of the plants were estimated using the probit method of Miller and Tainter (1944) as described by Randhawa (2009) [14]. A total of 48 mice of both sexes were used (16mice/plant) and randomly divided into four (4) groups of 4 animals each. The mice were fasted for 8h and various doses of the extracts were administered intraperitoneally to the test groups; 500 mg/kg-2000mg/kg for *Ipomoea involucrata* and *Rauvolfia vomitoria* and 500-5000 mg/kg for *Milletia aboensis*. The animals were closely observed for toxic signs and behavioural changes for the first 2 hours after administration and mortality was recorded within 24 hours. The percentage mortality was calculated from the number of deaths recorded at each dose level. The percentage of animals that died at each dose level was then transferred to probits using the probit table. The probit values were then plotted against log doses. The dose corresponding to probit 5 (50 percent) was estimated from the graph. The $\log LD_{50}$ obtained was converted to LD_{50} by finding the antilog.

Animals

Albino Wister mice were obtained from the animal house of the Department of Physiology, University of Calabar, Calabar. The animals were allowed to acclimatize for two weeks in the animal house of the Department of Biochemistry; kept under controlled environmental conditions of temperature (25±5℃) and 12h light and dark cycle. They were allowed free access to feed (Vital Feed, Grand Cereals and Mills Limited, Jos) and water.

Chemicals and Reagents

Ethanol 95/96 percent (in bond) was obtained from James Burrough Limited, London. All other chemicals and reagents were of analytical grade. 4-Aminopyridine (A78403) 98 percent was purchased from Sigma Aldrich Co. 3060 Spruce Street, St. Louis, USA and Carbamazepine was obtained from Novartis Pharmaceuticals, Switzerland.

Test for Anticonvulsant Potency by 4-Aminopyridine Model

A total of 66 mice of both sexes were divided into 11 groups of 6 animals each. The negative control group received 1% DMSO (vehicle); positive control group – 30 mg/kg bw of Carbamazepine (CBZ), a standard anti epileptic drug (AED); IP treatment groups- 50, 100, and 200 mg/kg bw; RV treatment groups- 50, 100, and 200 mg/kg bw; and MA treatment groups- 100, 200, and 400 mg/kg bw of the respective plant extracts. The choice of dosage range was based on a predetermined LD_{50} of the respective plant extracts [15]. All the drugs and extracts were administered by intra peritoneal (ip) route. All the animal groups were challenged with 4-Aminopyridine (4-AP) (15 mg/kg bw, ip) 30 mins after the administration of the respective vehicle, drug, and extracts [16]. Thereafter, they were observed for another 30 min within which behavioural signs like trembling, hyperactivity, limb extension, tonic-clonic seizures and lethality were regarded as the endpoint. The time taken for the onset of the above signs and the mortality rate was recorded and compared with the respective control groups.

Statistical Analysis

Data were analyzed using one way ANOVA followed by a post hoc LSD test for evaluation of significance between mean values. Significance was accepted at 5 percent probability level ($p<0.05$). Data presented in tables are expressed as Mean and Mean ± SEM.

RESULTS

Acute toxicity Result

The median lethal dose (LD_{50}) for the ethanolic extracts of both *Ipomoea involucrata* and *Rauvolfia vomitoria* roots was estimated as 776.25mg/kg i.p. For the leaves of *Milletia aboensis*, no mortality or toxicity signs were recorded even up to 5000mg/kg. Thus, this puts the LD_{50} at ≥5000mg/kg i.p.

Phytochemical analysis

The qualitative phytochemical screening showed the presence of alkaloids, saponins, tannins, flavonoids, glycosides, phenols, phytate and reducing sugar in the ethanolic extract of *Ipomoea involucrata* root, *Rauvolfia vomitoria* roots and the leaves of *Milletia aboensis*.

Onset of trembling

The result on Fig 3.1 indicates the ability of the plant extracts to delay the onset of trembling as compared to the effect of the standard drug. A dose-dependent delay in the onset of trembling within the IP group was observed with the high dose (200 mg/kg) exhibiting about 61% delay relative to the standard drug CBZ. The percentage delay in the RV group was 37**,** 86, and 82 % (for 50, 100, and 200 mg/kgbw dose groups, respectively). Similar values within the MA dose groups were 62, 58, and 81% respectively, for 100, 200, and 400 mg/kg bw. On the whole, all the extracts significantly ($p<0.05$) delayed the onset of trembling.

The results on hyperactivity, hindlimb extension, onset/duration of seizures, mortality and percentage protection are as recorded in Table 2.

Hyperactivity

The plant extracts were able to prolong (not significantly) the behavioural expression of hyperactivity and wild running. The MA dose groups showed a dose-dependent decrease in latency with the low dose exhibiting a more prolonged effect than the median and high doses. The IP100, RV100, RV 200, MA100 and MA 200 mg/kg doses increased the time of expression of hyperactivity more than the standard drug CBZ.

Hind limb extension (HLE)

All extract-treated groups delayed the onset of HLE. IP 100 displayed higher potency than IP 50 and IP200 in HLE delay. There was a dose-dependent increase in delay within the RV group while the highest effect within the MA group was observed in the low dose (100 mg/kg). Groups treated with RV100, RV200 and MA100 mg/kg were significantly ($p<0.05$) higher than the untreated group.

Onset of seizures

All graded doses of each plant extract delayed the onset of seizures induced by 4-AP. A dose-dependent increase was observed in the RV group with a peak delayed effect evident in the high dose (200 mg/kg). IP 100 mg/kg showed a better delayed effect while the low and high doses of MA (100 mg/kg and 400 mg/kg) delayed the onset of seizures more than the median dose. Groups treated with RV100 and RV200 mg/kg showed a significantly greater ($p<0.05$) latency to seizure than the untreated group

Duration of seizure

No significant difference was observed in all treatment groups compared to the negative control although the duration of seizure tended to increase in all treated groups.

Time to death

All treatment groups showed an increase in the time to death, the peak delayed effect was significant ($p<0.05$) in MA 400 mg/kg (high dose) compared to the negative control. None in IP treated group was protected from death while RV (200 mg/kg) gave a 16.67 percent protection. Within the MA plant group, the median dose (200 mg/kg) and the high dose (400 mg/kg) exhibited 16.67 percent and 33.33 percent, respectively.

Table 1: Qualitative Phytochemical screening

Bioactive principles								
Plants	Alkaloids	Saponins	Tannins	Flavonoids	Glycoside	Phenol	Phytate	Red.Sugar
I.involucrata	+	++	+	+	++	+	++	++
M. aboensis	+	++	+	++	+	++	+	+
R.vomitoria	++	++	++	++	++	++	+	++

+ = Present

++=Moderately Present

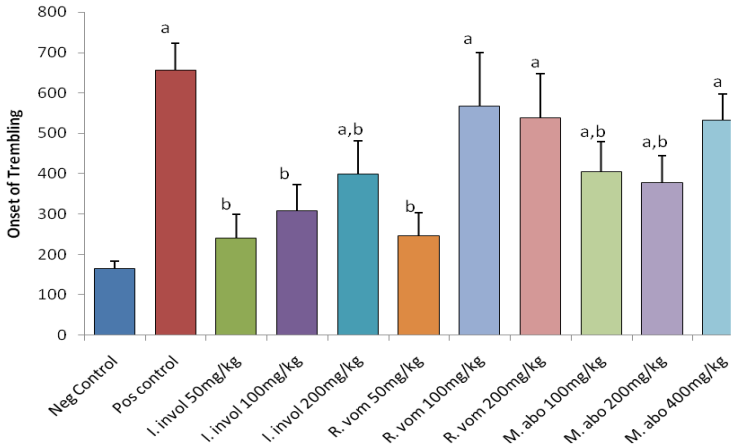

Fig-1: Effect of Treatment and Standard Drug on Onset of Trembling (s)

a – significant at $p< 0.05$ compared to negative control; b – significant at $p< 0.05$ compared to positive control.

Table-2: Anticonvulsant Activity of *Ipomoea involucrata* (IP), *Rauvolfia vomitoria* (RV) and *Milletia aboensis* (MA) on 4-Aminopyridine induced Seizures in Mice

BEHAVIOURAL SCORE/ENDPOINT							
Experimental Groups	Hyperactivity(s)	HindLimb Extension(s)	Onset of Seizure (s)	Duration of Seizure(s)	Time to Death (s)	% Protection	Quantal Protection
1% DMSO	317.33±25.88*	532.17±48.27	538.17±47.59	16.52±5.42	556.67±50.34	0	0/6
IP 50mg/kg	526.33±66.86^{NS}	635.17±135.57^{b}	730.08±157.97^{b}	62.06±24.34^{NS}	836.17±187.17^{b}	0	0/6
IP 100mg/kg	810.17±219.50^{NS}	925.83±207.51^{b}	887.42±246.20^{b}	57.17±18.97^{NS}	1028±221.80^{b}	0	0/6
IP 200mg/kg	597.00±92.85^{NS}	587.00±91.20^{b}	646.83±115.09^{b}	104.83±56.36^{NS}	769.17±149.13^{b}	0	0/6
RV 50mg/kg	514.17±83.20^{NS}	755.83±113.49^{b}	884.33±151.49^{b}	44.69±25.12^{NS}	933.17±161.16^{b}	0	0/6
RV 100mg/kg	914.17±157.88^{NS}	955.33±139.12^{a,b}	1114.00±170.79^{a,b}	79.83±29.66^{NS}	1193.50±155.80^{b}	0	0/6
RV 200mg/kg	771.50±102.33^{NS}	962.50±200.19^{a,b}	1124.67±198.98^{a,b}	48.50±22.22^{NS}	1173.33±207.92^{b}	16.67	1/6
MA100mg/kg	1052.40±209.80^{NS}	1045.00±217.86^{a,b}	918.00±236.21^{b}	28.61±8.89^{NS}	1086.20±204.42^{b}	0	0/6
MA200mg/kg	753.83±166.26^{NS}	713.33±133.45^{b}	795.92±150.79^{b}	48.17±13.23^{NS}	747.40±118.30^{b}	16.67	1/6
MA400mg/kg	609.17±125.75^{NS}	787.00±181.91^{b}	941.08±246.80^{b}	107.84±59.38^{NS}	1712.00±365.84^{a,b}	33.33	2/6
CBZ 30mg/kg	742.50±340.24	-	-	-	-	100	6/6

* Mean ± SEM (n=6). a=significant at p<0.05 compared to negative control, b= significant at p<0.05 compared to positive control, NS= Not significant at p<0.05 to both controls. DMSO: Dimethyl sulphoxide, IP; Ipomoea involucrata, RV; Rauvolfia vomitoria, MA; Milletia aboensis, CBZ; Carbamazepine.

DISCUSSION

In this study crude ethanolic extracts of the plant parts of IP, RV, and MA significantly increased the latency to trembling, HLE, seizures and time to death in treated animals. These results suggest that these plant parts under study contain psychoactive substances with anticonvulsant potentials against 4-Aminopyridine (4-AP)-induced seizures. By offering 16 and 33 percent protection against 4-AP-induced seizures respectively, RV and MA are qualified to be regarded as effective anticonvulsants [16]. In a previous study [17], administration of 200mg/kg bw of RV leaf extract showed a delay in onset of seizures and time to death with without offering protection against STN, PTZ, PTN seizure models. But in our study, a similar dose of RV root extract offered protection against death from 4-AP seizure model, thus indicating that both the leaves and root of RV possess anticonvulsant activities but on different models. The inability of IP to antagonize or attenuate the spread of 4-AP seizures may suggest that it exerts its effects on other cellular targets and not on ion channels [16]. This observation is drawn from reports of studies on other Ipomoea species for example, *I.orizabensis* and *I. aquatica* [18-19] which exhibited anticonvulsant activities in PTZ, STN and picrotoxin seizure models. Thus IP may be acting via other mechanisms such as interaction with glycine or $GABA_A$ receptors, rather than ionic channels.

Phytoconstituents such as alkaloids, lipids, terpenes, saponins, triterpenoids, flavonoids and phenolic compounds like coumarins are reported to have anticonvulsant properties [20]. In our earlier report (David-Oku et al, 2015a) [21], the plant parts under study were found to be rich in reducing sugars, oxalate, phytate, saponins, alkaloids, phenols, and tannins. Furthermore, the rich content of Vit C in MA leaves (295mg/100g), and mineral nutrients of Na, K, Ca, and Mg in both IP and MA [22] may also contribute to demonstrated activities of these phytochemicals.

4-AP is a useful model of generalized tonic-clonic seizures (GTCS) and partial seizures and in evaluating drugs for refractory epilepsy [23-25]. AEDs like Carbamazepine are good antagonists of 4-AP seizures hence effective against GTCS and partial seizures [26]. The finding in this study suggests the ability of these extracts to tackle GTCS and partial seizures.

CONCLUSION

The leaves of *Milletia aboensis* and the roots of both *Rauvolfia vomitoria* and *Ipomoea involucrate* possess anti-convulsant potencies in the order; MA>RV>IP, with MA protecting two-thirds of animals against death from 4-AP-induced seizures. The plant extracts have the potentials of tackling generalized tonic-clonic seizures (GTCS) and partial seizures. MA

and RV may exert their effects by acting on ion channels while IP is most likely to act via other mechanisms

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TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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