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Evaluation of the anticonvulsant property of *Russelia equisetiformis* (Schlecht & Chan)

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Abstract

Fits or convulsions are extremely common symptoms in childhood most especially in inflammatory conditions such as malaria and viral infections, tonsillitis and cases of epileptic conditions. Most of the available drugs used in the control of this symptom usually present adverse reactions. Alternative medicine is one of the interesting areas, which is getting more popular and increasingly attractive world wide. In searching for herbal remedy that is safe and efficacious, we therefore, evaluate the anticonvulsant effects of methanol *Russelia equisetiformis* extracts (MERE). Anticonvulsant activity was evaluated in the picrotoxin (PCT) and strychnine (STC) - induced convulsions in mice. MERE (100-400mg/kg), significantly ($p<0.05$) protected mice against picrotoxin - induced seizures. However, the extract did not confer protection against (STC) - induced seizures. n-Hexane fraction (10 mg kg^{-1}), showed the highest percentage level of protection (80%) against picrotoxin-induced seizures, with none of the fractions showing protection against (STC) - induced convulsion. Although, the data obtained in the present study, do not provide convulsive evidence, it would appear that *R.equisetiformis* crude extract (MERE), and its n-hexane fraction produce the observed anticonvulsant activity by enhancing GABAergic neurotransmission, and/or facilitating GABAergic action in the brain. In general, the average onset of convulsion was delayed, while the average duration of convulsion was markedly reduced. These findings, suggest therefore, that the plant could serve as a supplementary therapy for the management and/or control of childhood convulsions and epilepsy.

Keywords: *Russelia equisetiformis* extract Fractions; Anticonvulsants.

1. Introduction

Russelia equisetiformis (Schlect & Chan) belongs to the family Scrophulariaceae [1]. It is commonly known as firecracker, coral and fountain plant [2] *R. equisetiformis* is a native of Tropical America, but can easily be cultivated and freely naturalized in sandy clearings, long streets, roadsides and grow accidentally in quite a number of places by the roadsides [3, 4]. It

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is an evergreen shrub with tiny dark green leaves, scale-like in structure and grow on thin rush-like stems. The branches are arched, bearing 1-2 inch long tubular flowers. The plant blooms abundantly round the year, if given optimum conditions [5]. Medicinally, the plant is used for the treatment of diabetes and leukemia in Southwestern, Nigeria [6]. Phytochemically, the plant has been reported to contain triterpene of lupane type [7]. The extract of the plant has been reported to possess anti-bacterial, analgesic and anti-inflammatory activities [8, 9]. Recently, two flavonoid compounds were isolated and reported to have potential analgesic activity [10]. Some workers have shown the involvement of compounds such as flavonoids, triterpenes saponins in either inhibitory, and/ or stimulatory effects on convulsions produced by various convulsant agents [11, 12, 13, 14, 15, 16, 17]. Also, plants containing phenolic compounds have been shown to possess analgesic, anti-inflammatory and anticonvulsant properties [18 19, 20, 21]. The aim of the present study was, therefore, to evaluate the anticonvulsant activity of *R. equisetiformis* extracts.

2. Materials and Methods

The experimental protocols and procedures used in this study were approved by the Ethical committee, University of Ibadan, Ibadan, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH publications no 85-93, revised 1985).

2.1 Plant material

The plant sample was collected in the month of October, 2005 from Bodija in the South West of Nigeria. The was identified in the herbarium of the Forest Research Institute, Ibadan, Nigeria, where voucher specimen was deposited with voucher number 106998. The plant sample was air-dried at room temperature, and reduced to powdery form using an electric blender.

2.2 Extraction

500 gm of powdered sample of the plant was extracted with 500 ml of 100 % methanol using a soxhlet extractor. The resulting crude methanol extract was then concentrated under reduced pressure at 40°C in a rotary evaporator (Rota vapor) to obtain a solid sample giving 19.7 % yield. This was stored in the refrigerator at 4°C and was used throughout the experiment. The crude extract was dissolved in distilled water and extracted with n-hexane, ethyl acetate, dichloromethane and n-butanol successively.

2.3 Animals

Male mice used for this study weighing between 20-23 g, strain Swiss Albino were housed in a well ventilated pre- clinical animal house, College of Medicine, University of Ibadan. The animals were acclimatized in the laboratory for two weeks before experimentation, and were fed with standard diet (Ladokun Feeds Nigeria Ltd) and water *ad libitum*.

2.4 Evaluation of anticonvulsant activity

The anticonvulsant testing method of, [22] modified by [23] was used to assess anticonvulsant activity of the plant's extracts (MERE) in mice. Standard convulsant agents, picrotoxin (PCT, 10 mg/kg i.p) and strychnine (STC, 3 mg/kg) were used to induce convulsion in mice. Diazepam (DZP, 5 mg/kg) was used as reference anticonvulsant drug for comparison. Following induction of convulsions in the 'test' mice (with intraperitoneal injections of convulsant agents), the animals were observed for signs of neurological deficits, especially hind-limb tonic seizures or convulsions. Hind-limb tonic extensions of the mice were regarded as manifestations of seizures. The ability of the

plant's extract (MERE, 100-400 mg/kg i.p) and its fractions (10 mg/kg i.p) to prevent seizures or delay/prolong or onset of the hind-limb tonic extension was considered as an indication of anticonvulsant activity [24]. The experimental procedures were repeated with other groups of 'test' mice pretreated with graded doses of the plant's extract (MERE, 100-400 mg/kg i.p), and its fractions (10 mg/kg i.p) or with reference anticonvulsant drug used for comparison i.e. diazepam (DZP, 5mg/kg) before administrations of the convulsant agents. In the absence or presence of each of the plant's extract doses, fractions and reference anticonvulsant compound used, the onset and duration of convulsions in the mice were noted and recorded and the percentage of protection by the 'test' compounds used were determined. Because the plant's extract, the fractions and the reference drug used in this study were dissolved in 20% Tween80 each at the beginning of our experiment (10 ml/kg)-treated mice were used as 'control' animals.

2.5 Data analysis

Data are presented as means (± S.E.M). Data from 20% Tween 80 (10 ml/kg) - treat - control - mice were used as baseline values. The differences between the data obtained with the plant extracts and its fractions, and reference anticonvulsant drug - treated - test - mice, were subjected to one way analysis of variance (ANOVA) using version 5.0 Graphpad prism 2007. In all cases, values of ($p<0.05$) were taken to imply statistical significance.

Table 1. Effect of *R .equisetiformis* extract on picrotoxin (PCT)-induced seizures in mice.

Treatment	Dose mg/kg or ml/kg	Latency of convulsion (min)	%Mortality	%Protected
Control 20% Tween 80	10	2.8 ± 0.34	100	0
R. equisetiformis	100	19.0 ± 1.61***	60	40
R. equisetiformis	200	24.0 ± 2.0***	40	60
R. equisetiformis	400	29.0 ± 2.4***	20	80
Diazepam	5	-	0	100

Each value is the mean ± SEM of six mice, ***P<0.001 vs. control.

Table 2. Effect of *R .equisetiformis* extract on strychnine (STC)-induced seizures in mice.

Treatment	Dose mg/kg or ml/kg	Latency of convulsion(min)	%Mortality	%Protected
Control 20% Tween 80	10	2.8 ± 0.34	100	0
R. equisetiformis	100	3.3 ± 0.40*	100	0
R. equisetiformis	200	3.5 ± 0.42	100	0
R. equisetiformis	400	2.8 ± 20.3.4	100	0
Diazepam	5	-	0	100

Each value is the mean ± SEM of six mice, *P<0.05 vs. control.

Table 3. Effect of *R .equisetiformis* fractions on picrotoxin (PCT)-induced seizures in mice.

Treatment	Dose mg/kg or ml/kg	Latency of convulsion(min)	%Mortality	%Protected
Control 20% Tween 80	10	2.40 ± 0.51	100	0
n-Hexane	10	28.40 ± 2.4***	20	80
Dichloromethane	10	15.40 ± 4.52***	40	60
Ethyl acetate	10	6.2 ± 2.4	100	0
n-Butanol	10	4.28 ± 0.67	100	0
Diazepam	5	-	0	100

Each value is the mean ± SEM of six mice, ***P<0.001 vs. control.

Table 4. Effect of *R .equisetiformis* fractions on strychnine (STC)-induced seizures in mice.

Treatment	**Dose mg/kg or ml/kg**	**Latency of convulsion (min)**	**%Mortality**	**%Protected**
Control 20% Tween 80	10	2.40 ± 0.51	100	0
n-Hexane	10	2.42 ± 0.24	100	0
Dichloromethane	10	2.81 ± 1.4	100	0
Ethyl acetate	10	2.0 ± 0.31	100	0
n-Butanol	10	2.70 ± 0.45	100	0
Diazepam	5	-	0	100

Each value is the mean ± SEM of six mice, *P>0.05 vs. control

3. Results

Effect of *R. equisetiformis* crude extract and its fractions on picrotoxin (PCT)-induced seizures

Picrotoxin (PCT, 10mg/kg i.p) produced hind-limb tonic seizures in all the six mice used. Russelia equisetiformis crude extract produced dose-related, significant (p<0.05) protection of the mice against PCT-induced seizures, while its n-hexane fraction showed the highest percentage level of protection of mice against PCT-induced seizures (Tables 1&2). Furthermore, the plant's extract and its n-hexane fraction significantly delayed (p<0.05) in the onset of PCT-induced seizures. The reference anticonvulsant drug used, (DZP, 5 mg/kg i.p), profoundly delayed the onset of, and significantly antagonized (p<0.05) the PCT-induced seizures (Tables 1&2).

Effect of *R. equisetiformis* crude extract and its fractions o strychnine (STC)-induced seizures

Strychnine (STC, 3 mg/kg) produced hind-limb tonic seizures in all the six mice used. *Russelia equisetiformis* crude extract and all its fractions, produced no significant (p>0.05) protection of the mice against (STC) - induced seizures. However, the crude plant's extract (200 mg/kg) showed significant delayed (p<0.05) in the onset of (STC) - induced seizures. The reference anticonvulsant drug used, (DZP, 5 mg/kg i.p), profoundly delayed the onset of, and

significantly antagonized ($p<0.05$) the PCT-induced seizures (Tables 3&4).

4. Discussion

There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and/or treatment of individual with epilepsy. However, most of these synthetic drugs are not only inaccessible and unaffordable, but they also possess many toxic adverse effects. There is, therefore, dire need, for the development of cheap, effective and safe anticonvulsant agents from plants and other natural sources. Although, *Russelia equisetiformis* is used medicinally in the treatment of diabetes and leukemia in the Southwestern part of Nigeria [6], relatively little scientific information exists in biomedical literature on the therapeutic efficacy of the plant product. The results of the present laboratory animal study provide in favour of the anticonvulsant activity of the herb, and show that crude extract of *Russelia equisetiformis* and its n-hexane fractions possess anticonvulsant activity in the experimental animal model used. The effectiveness of the plant's extract in the experimental paradigm used probably suggests that the herb could be used as a supplementary therapy in the management, and/or control of both *petit* and *grand mal* types of convulsions. Picrotoxin and strychnine are two convulsants used to produce convulsions, while ability of an agent to inhibit convulsion in comparison with the untreated mice is taken as a measure of an *in vivo* protection level of the agent. Picrotoxin and strychnine produce their convulsions by blocking GABA and glycine receptors respectively [25, 26, 27, 28]. The reference anticonvulsant drug used in the present study, diazepam (DZP), as well as *equisetiformis* crude extract (MERE), and its n-hexane fraction antagonize picrotoxin (PCT)-induced seizures. GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, acting through two classes of receptors, $GABA_A$ and $GABA_B$ receptors [29]. $GABA_A$ receptors, functionally linked to benzodiazepine receptors and chloride-ion channels to form GABA-chloride ionophore complex, which is intimately involved, in the modulation of neuronal responsiveness (excitability) and activity in the brain by increasing the chloride ion conductance of the synaptic membrane through opening of the chloride ion channel [30, 31].This hypothesis may explain the observed effect and/or antagonistic action of diazepam (a benzodiazepine) against (PCT) - induced seizures. Since *R .equisetiformis* crude extract (MERE) and its n-hexane fraction mimicked the anticonvulsant action of the reference drug used in this study, it is not unlikely that the plant's extract (MERE) and its n-hexane fraction antagonize picrotoxin (PCT)-induced seizures by opening the chloride-ion channel associated with $GABA_A$-receptors. However, both the crude extract and its fractions did not block convulsion produced by strychnine. This probably indicates that the anticonvulsant property possessed by the extracts is through GABAergic and not glycinergic pathway, and that the active component resides in n-hexane fraction. This proposal further buttresses the hypothesis that MERE may be interfering with GABAergic neurotransmission in one way or the other [32]. Since the (PCT)-induced seizures have been shown to be due to inhibition and/or attenuation of GABAergic neurotransmission, it is not unreasonable to speculate that *Russelia equisetiformis* extract probably produces its anticonvulsant activity by enhancing GABAergic neurotransmission. The observed anticonvulsant activity of the plant extract may also be due, at least in part, to its ability to depress the central nervous system (CNS) activity by one or more known mechanism of actions [33] which may include altered Na+-K+ ATPase expression [34],

pyridoxamine-5/phosphate (PMP) metabolism [35] and inhibition of expression of inducible nitric oxide [36]. These mechanisms could explain in part, some of the central nervous system depression and ataxia observed for the extract in the mice. It is impossible for us at this stage, to pin-point and identify with certainty, the anticonvulsant principle's of the extract. Although, two chemicals constituents have been reported in biomedical literature, namely: titerpene (of lupane type) and russelianoside (a flavonoid) 7, 10 and more so, a number of investigators have shown that tannins and other polyphenolic compounds, flavonoids, triterpenoids and a host of other secondary plant metabolites possess analgesic, anti-inflammatory and anticonvulsant properties in various experimental animal models [37, 38, 39]. Therefore, it is not unlikely, that the two chemical constituents (flavonoid and titerpene), may account for the observed anticonvulsant activity of the plant's extract, but there is no sufficient scientific data or evidence to back-up and justify this speculation. In summary, the experimental evidence obtained in the present laboratory animal study suggests that *R. equisetiformis* extract possesses anticonvulsant activity, and may, therefore, be an alternative supplementary therapy for the management and/or control of convulsions and epilepsy.

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