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Evaluation of the Anticonvulsant Potential of Aqueous Fraction of *Synsepalum dulcificum* Seed Extract in Mice

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Authors' contributions

This work emanated from the M.Sc. thesis of author OJJ and was carried out in collaboration between all authors. Authors OJJ, ORI and MMI designed the study. Author OJJ managed the analyses of the study and literature searches and performed the statistical analysis. Author OJJ wrote the protocol and the first draft of the manuscript. Authors ORI and MMI supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluated the anticonvulsant potential of the aqueous fraction of *Synsepalum ducificum* seed extract in mice. This was with a view to providing information on the possible link between the antioxidant principles earlier reported for the seed and anticonvulsant effect.
Study Design: One-factor, two controls-three test groups experimental design.
Place and Duration of Study: Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, between September and December, 2014.
Methodology: The investigation of the anticonvulsant potential of the aqueous fraction of *S. dulcificum* seed extract (AF) was carried out using pentylenetetrazole (PTZ)-, strychnine- and Maximal Electroshock (MES)-induced seizure tests. For each of the test models, randomly selected albino mice were divided into five groups (n = 5). Group 1 was the control (Normal saline, 10 ml/kg, i.p.), Group 2 was the positive control [Diazepam (1 mg/kg, i.p. against PTZ and 5 mg/kg, i.p. against strychnine); Phenytoin, 25 mg/kg, i.p. against MES] while group 3, 4 and 5 were the test

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groups which received 0.3, 0.6 and 1.2 mg/kg, i.p. of AF respectively. Administration of PTZ (85 mg/kg, i.p.), strychnine (4 mg/kg, i.p.) and MES delivery were done 30 min after the 5 groups of animals were pretreated. The onset of convulsion and the degree of protection against mortality were recorded for the animals in each group. The results were analyzed using one way ANOVA followed by Student-Newman-Keuls test.

Results: The AF gave 33.33% protection against mortality in PTZ- and strychnine-induced convulsion tests and caused a dose dependent reduction in the time of recovery of the animals from MES-induced seizure.

Conclusion: The aqueous fraction of the seed extract of *S. dulcificum* possesses some level of anticonvulsant activity which may be further explored for maximum effect.

Keywords: Synsepalum dulcificum; anticonvulsant activity; maximal electroshock; aqueous fraction.

1. INTRODUCTION

Synsepalum dulcificum (Schumach and Thonn) Daniell (Family- Sapotaceae), also known as miracle fruit is a large shrub with height reaching about 2 - 5 m. It produces red fruits, each being about 2 cm long and containing one seed [1]. It is a native of West and West-Central tropical Africa [2]. The plant is best known for the unique taste-modifying property of its red berries attributed to the presence of a glycoprotein called miraculin in the pulp [3]. On sucking the fruit pulp, this molecule binds to some receptors on the taste buds, causing sour foods (e.g. lime and lemons) subsequently consumed to taste sweet. Hence, miraculin is commercially used as a sugar substitute [2]. Moreover, the fruit has been shown to improve insulin sensitivity [4] while also serving as a low-calorie sweetness enhancer that limits energy intake [5]. Therefore, it is popularly consumed by diabetics and dieters. Proximate and mineral analysis of the seed showed that the seed contains reasonable amount of protein (19.47%), fat (11.93%), total carbohydrate (29.08%) and mineral elements like potassium, sodium, magnesium, among others [6]. Presently, a wide knowledge gap still exists on the possible health benefits of *S. dulcificum* seed which has been shown to be rich in antioxidant principles [7]. Of recent, some central nervous system (CNS) disorders including epilepsy have been linked with oxidative stress [8]. This study is premised on the observation that natural products containing antioxidant properties usually have some pharmacological effects. The aim was to investigate whether or not the aqueous fraction of the seed extract possesses anticonvulsant activity.

An understanding of the meaning of the term-seizure is important in getting a full grasp of the definition of epilepsy. A seizure is the physical findings or behavioural changes that occur after an episode of abnormal electrical activities in the brain [9]. Specific symptoms depend on the part of the brain involved. Symptoms (which rarely last for more than fifteen minutes) occur suddenly and may include: Confusion, sequel to a brief blackout period (a short-term inability to remember things), behavioural changes, eye movements, urinary incontinence, sudden fall as well as uncontrollable muscle spasms with twitching and jerking of limbs (Convulsions) [10].

Recently, a practical definition of epilepsy (with three components) was given by the definitions task force of the International League Against Epilepsy (ILAE). In practical terms, epilepsy can be considered as a disease of the brain defined by any of the following conditions: a) Minimum of two unprovoked/reflex seizures occurring at interval of more than 24 hours; b) a single unprovoked seizure with a recurrence probability similar to the general recurrence risk (≥ 60%) after two unprovoked seizures occurring over the next ten years; c) diagnosis of an epilepsy syndrome: epilepsy can be considered to be resolved in an individual who either had an age-dependent epilepsy syndrome but has now outgrown it (having past the applicable age) or who has remained seizure-free for the last 10 years and without anti-seizure drugs for at least the last 5 years [11].

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Extraction Process

The plant was authenticated in the Department of Botany (Ife herbarium) of Obafemi Awolowo University, Ile-Ife by Mr. B. E. Omomoh and deposited with the voucher number: 17218. Mature fruits were collected from *S. dulcificum* plant domiciled at the parks and gardens of

Obafemi Awolowo University, Ile-Ife around March, 2013. The seeds separated from the skin and the pulp were decoated, oven dried at 40°C and powdered. Cold extraction of the powdered seeds (350 g) was done by soaking it in 1.5 L of absolute methanol with regular stirring for 72 hrs. The resultant mixture was filtered and the filtrate concentrated to dryness in a rotary evaporator under reduced pressure and at 40ºC to prevent decomposition of its constituents. The yield was 70.53 g, being 20.15%$^{w}/_{w}$ of the powdered seeds. For the preparation of the aqueous fraction, a paste of the methanol extract (30 g) was made in a beaker by adding 50 ml of distilled water in portions. This was then transferred into a separating funnel and 75 ml of ethyl acetate added while gently rotating the mixture. The ethyl acetate portion was removed and the aqueous portion was extracted again by adding 75 ml of ethyl acetate while repeating the initial gentle rotation of the mixture to ensure complete separation of the ethyl acetate and aqueous fraction components. The two fractions-aqueous fraction (AF) and ethyl acetate fraction (EAF) were concentrated to dryness separately in a rotary evaporator, the yield being 48.90% and 35.20% $^{w}/_{w}$ respectively.

2.2 Experimental Animals

Albino mice of either sex weighing 18-25 g, bred in the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife were used in this study. They were housed in cages, at room temperature and under natural lighting. Wood shavings were used as beddings and changed at least once in three days. The animals were given free access to guinea feed and clean water. The “principle of laboratory animal care” (National Institute of Health-NIH publication No. 85-23) guidelines and procedures were followed in this study. The University Research Committee through the Faculty Postgraduate Committee of the Obafemi Awolowo University, Ile-ife, Nigeria gave the ethical approval for the research work.

2.3 Drugs and Laboratory Materials

Diazepam (Roche, Basel, Switzerland), Pentylenetetrazol (Sigma Chemical Co., St Louis, USA), Phenytoin (Hospiral UK Ltd., UK), Strychnine HCl (BDH Chemicals Ltd., England), Observation Cage, Mettler balance (USA), Ugo Basile electroconvulsive machine (Model 57800, Ugo Basile Biological Research Apparatus, Italy), Stop-watch, Syringe and needles (1 ml, 2 ml, 5 ml), Distilled water.

2.4 Administration of Extract

The aqueous fraction of the methanol (crude) extract of *S. dulcificum* was administered to mice intraperitoneally throughout the course of this experiment. The volume administered did not exceed 10 ml/kg in all cases. The intraperitoneal route was chosen because it gives faster and more reproducible results as it provides a larger absorptive surface area, hence higher bioavailability compared to oral route [12].

2.5 Acute Toxicity Test

The LD_{50} (an index of acute toxicity) of the aqueous fraction of *S. dulcificum* seed extract was determined by lorke’s method [13]. This is basically divided into two phases (Phase 1 and phase 2). In the first phase, 9 animals were divided into 3 groups (n = 3) representing three dose levels of 10, 100 and 1000 mg/kg, i.p. respectively. The doses were further reduced in phase 2 based on phase 1 results [13].

2.6 Assessment of Anticonvulsive Properties

2.6.1 Pentylenetetrazole (PTZ) induced convulsion

The method previously described in a study [14] was used. Mice were randomly allotted to the control and test groups of six animals each. The control mice were administered with PTZ (85 mg/kg, i.p.) [15], 30 min after normal saline i.p. The positive control group of mice received 85 mg/kg PTZ 30 min after 1 mg/kg diazepam, i.p. Three doses (0.3, 0.6 and 1.2 mg/kg) of the extract (AF) were given to each of the test groups i.p. (30 min) before 85 mg/kg PTZ. The onset of convulsion and the degree of protection (against death) were recorded.

2.6.2 Strychnine induced convulsion

The modified method of a group of researchers [16] was used to assess the possible anticonvulsive properties of the aqueous fraction of *S. dulcificum*. Thirty mice of either sex, weighing 18-25 g, were selected and divided into five groups of six mice each. The three test groups were treated with 0.3, 0.6 and 1.2 mg/kg, i.p. (respectively) of the aqueous extract. The

control animals received 10 ml/kg normal saline while the positive control group was given diazepam (5 mg/kg, i.p.). After 30 min, strychnine (4 mg/kg, i.p.), [17] was administered and the influence on the onset of convulsion and degree of protection (against death) were recorded.

2.6.3 Maximal electroshock-induced seizure model

The animals were divided into five groups of six animals each. The animals in the test groups were given 0.3, 0.6 and 1.2 mg/kg, i.p. of AF. The control animals received 10 ml/kg normal saline while the positive control group was given phenytoin (25 mg/kg, i.p.) [18]. After 30 min of pre-treatment, each mouse was given threshold maximal electroshock of 15 mA for a period of 1sec at a frequency of 100 pulses per second and pulse width of 0.5 ms through a pair of corneal electrodes. UGO BASILE ECT Unit (Italy) was used in delivering this shock. Protection against tonic hind-limb extension or onset of convulsion as well as time of recovery was recorded for each animal. The ear electrodes were dipped in Ringer's solution to soak the felt pads before clipping them to the animal's ears, to decrease contact resistance.

2.7 Statistical Analysis

For Parametric data, results were analysed using Graph pad prism (version 5.01) and expressed as the Mean±SEM (n = 5). These were tested for statistical difference by using one way analysis of variance (ANOVA) followed by Student-Newman Keuls post hoc test. $P \leq 0.05$ was considered as statistical difference between group(s) [19].

3. RESULTS

3.1 Acute Toxicity

The result of acute toxicity test is presented in Table 3.1. All the mice died at doses of 10, 100 and 1000 mg/kg (Phase 1). Doses of 5, 10 and 20 mg/kg also killed the animals in Phase 2 while at 2.5 mg/kg, no death was observed. The inraperitoneal (i.p.) LD_{50} was calculated as 3.54 mg/kg, i.p.

3.2 Effect of AF on the Onset of Seizure and Mortality in Pentylenetetrazole (PTZ) Induced Convulsion in Mice

In PTZ induced convulsion test in mice, 0.3 mg/kg AF gave 16.67% protection against mortality, 0.6 mg/kg did not protect the animal against mortality. At a dose of 1.2 mg/kg, AF gave 33.33% protection against mortality in PTZ-induced seizure test. There was no significant difference in the onset of convulsion between the treatment groups and the control. The reference drug (diazepam, 1mg/ kg) gave 100 % protection against PTZ-induced convulsion and mortality (Table 3.2).

3.3 Effect of AF on the Onset of Seizure and Mortality in Strychnine-Induced Convulsion in Mice

The results of strychnine-induced convulsion test are shown in Table 3.3. Doses of 0.3, 0.6 and 1.2 mg/kg AF all gave 33.33% protection against mortality while the reference drug (diazepam, 5 mg/kg) gave 66.67% protection against mortality. There was no significant difference in the onset of convulsion between all the treatment groups and saline control and also between the reference drug and the saline control.

3.4 Effect of AF on Maximal Electroshock (MES)-Induced Seizure in Mice

The reference drug (phenytoin, 25 mg/kg) fully protected the animals against tonic hind limb extension (HLE) when subjected to MES, compared with saline (control) but 0.3, 0.6 and 1.2 mg/kg of AF did not give this protection (Table 3.4). However, 0.6 and 1.2 mg/kg of AF produced a significant reduction in the time of recovery from the MES-induced convulsion ($P < 0.05$). This is depicted in Fig. 3.1.

Table 3.1. Acute toxicity test for aqueous fraction (AF)

Phase 1	Dose (mg/kg, i.p.)	Mortality ratio
	10	3/3
	100	3/3
	1000	3/3
Phase 2	2.5	0/1
	5	1/1
	10	1/1
	20	1/1

LD_{50} (i.p.) = $\sqrt{(2.5\times5)} \approx 3.54$ mg/kg

4. DISCUSSION

The aqueous fraction (AF) of *S. dulcificum* seed extract was assessed for anticonvulsant activity using pentylenetetrazole (PTZ) - and strychnine-induced convulsion models as well maximal electroshock-induced seizure test (MES). In the PTZ-induced seizure test, AF did not protect the

animals against convulsion and had no effect on the onset of convulsion when compared to normal saline. However, some level of protection against mortality was given by AF. PTZ is a non-competitive antagonist of gamma-aminobutyric acid type A ($GABA_A$) receptor and binding studies have shown that it binds to picrotoxin (PTX) site of this receptor [20]. The result obtained in this study suggests that AF might possess a mild affinity for PTX binding site of the $GABA_A$ receptor complex, hence mediating some level of inhibitory neurotransmission and giving some percentage of protection against mortality. A study has also shown that the seeds are rich in antioxidants [7]. The antioxidant properties of these seeds might be contributing to its ability to give some level of protection against post-convulsion mortality [15].

In the strychnine-induced convulsion test, all the doses of AF gave 33.33% protection against mortality as opposed to 66.67% protection observed with the reference drug (diazepam, 5 mg/kg, i.p.) but AF did not have any effect on the onset of seizure, neither did it protect the mice against convulsion. Strychnine is an antagonist of glycine receptor [21]. It also possesses anti-cholinergic property [22]. It majorly affects the motor nerves present in the spinal cord, glycine being the major inhibitory neurotransmitter in the spinal cord [23]. The result obtained is suggestive of the fact that a component of the extract might have some affinity for glycine receptor or it might be mediating some form of inhibitory neurotransmission (by a mechanism yet to be ascertained), hence counteracting the excitatory effect of strychnine. The mildly protective effect of AF might also stem from the presence of antioxidant activities in the seeds [7]. Epileptogenesis has been linked to oxidative stress as some studies have shown that free radicals might be implicated in epilepsy [24]. Another study has shown that strychnine, pilocarpine, PTZ and PTX tend to increase the lipid peroxidation level, nitrite content and catalase activities which are all parameters of oxidative stress, in the prefrontal cortex, striatum and hippocampus of mice [15]. Hence, AF might have the ability to reduce the oxidative stress induced by the pro-convulsants at the doses used.

Table 3.2. Effect of aqueous fraction (AF) of *S. dulcificum* seed extract on pentylenetetrazole (PTZ)-induced convulsion in mice

Drug/Dose (mg/kg, i.p.) PTZ (85)	Onset of generalized convulsion (min) (Mean±SEM)	Mortality ratio	% protection
Normal saline	1.07±0.06	6/6	0.00
AF (0.3)	1.14±0.08	5/6	16.67
AF (0.6)	1.84±0.37	6/6	0.00
AF (1.2)	1.16±0.07	4/6	33.33
Diazepam (1)	Nil	0/6	100.00

No significant difference in onset of generalized convulsion. n= 5

Table 3.3. Effect of aqueous fraction (AF) of *S. dulcificum* seed extract on strychnine-induced convulsion in mice

Drug/Dose (mg/kg, i.p.) strychnine (4)	Onset of generalized convulsion (min) (Mean±SEM)	Mortality ratio	% protection
Normal saline	2.32±0.21	6/6	0.00
AF (0.3)	3.96±0.47	4/6	33.33
AF (0.6)	4.52±0.59	4/6	33.33
AF (1.2)	3.28±0.27	4/6	33.33
Diazepam (5)	3.60±0.23	2/6	66.67

No significant difference in onset of generalized convulsion. n = 5

Table 3.4. Effect of aqueous fraction (AF) of *S. dulcificum* seed extract on maximal electroshock (MES)-induced seizure in mice

Treatment	% protection against hind limb extension
Normal saline (10 ml/kg)	0
AF (0.3 mg/kg, i.p.)	0
AF (0.6mg/kg, i.p.)	0
AF (1.2 mg/kg, i.p.)	0
Phenytoin (25 mg/kg, i.p.)	100

No protection was given by AF. n = 6

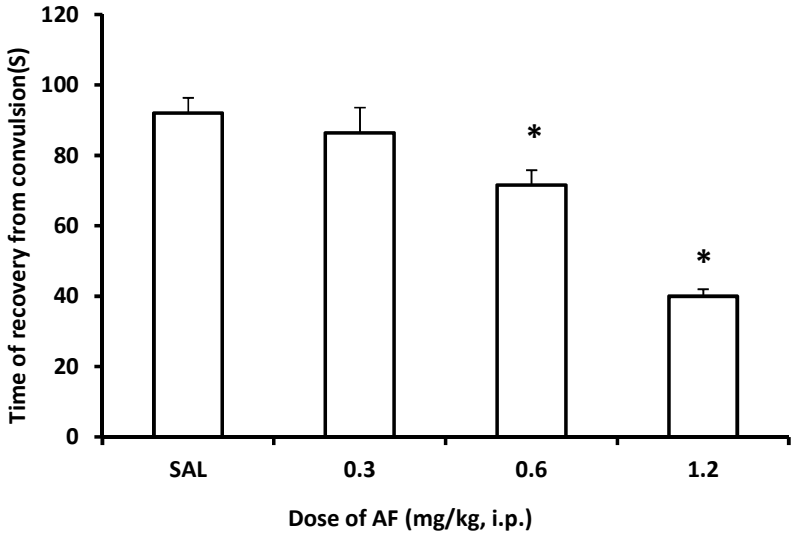

Fig. 3.1. Effect of aqueous fraction (AF) of *S. dulcificum* seed extract on time of recovery from maximal electroshock (MES)-induced seizure in mice

** P < 0.05, compared to saline control. Each bar represents the mean±SEM (n = 5). SAL = Normal saline (10 ml/kg, i.p.)*

In the MES test, none of the doses of AF used (0.3, 0.6 and 1.2 mg/kg, i.p.) protected the mice against tonic hind-limb extension as opposed to the reference drug (phenytoin, 25 mg/kg, i.p.). However, there was a dose-dependent reduction in the time of recovery from the hind limb extension with 0.6 and 1.2 mg/kg, i.p. of AF giving a significant reduction in time of recovery, compared to saline control. This is also indicative of the anticonvulsant potential of one or more components of the extract. The protection observed with phenytoin is in line with a previous study carried out by some researchers [25]. It has been documented that antiepileptic drugs which act by inhibition of voltage-dependent Na^+ channels (e.g. phenytoin, valproate, felbamate and lamotrigine) or blockade of glutamatergic excitation mediated by the *N*-methyl-d-aspartate (NMDA) receptor, such as felbamate have the ability to protect animals against MES-induced tonic hind-limb extension [26].

5. CONCLUSION

This study concluded that the aqueous fraction of *S. dulcificum* seed extract contains some principles which may possess anticonvulsant properties evidenced by the 33.33% protection against mortality in PTZ- and strychnine-induced convulsion and the significant reduction in the time of recovery from MES-induced tonic hind limb extension in mice.

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CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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