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Original Article

Anticonvulsant Activity of Aqueous Root Extract of *Annona Senegalensis* Pers

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ABSTRACT

Objective: *Annona senegalenesis* Pers is a sprawling shrub belonging to the family Annonaceae, and is mainly used in ethnomedicine. The use of decoction of *A. senegalenesis* is very prominent in the management of various conditions ranging from bacterial infection, worm infestation, snake bites, pyrexia and febrile convulsion in folk medicine. **Methods:** The investigation on anticonvulsant activities of *A. senegalenesis* was undertaken using animal models with a view to elucidating its mechanism of action **Results:** Results revealed that the aqueous extract of the root of *A. senegalenesis* was safe at high doses (LD_{50} 954.99±2.86 mg/kg body weight), protected drug–induced convulsion in mice, prevented electroshock in mice, being more effective against generalized than partial seizures and prolonging drug-induced sleep in mice. We conclude that the roots of *A. senegalenesis* have definite anticonvulsant activity and there is valid pharmacological basis for employing same for this purpose by the local people.

1.INTRODUCTION

In ethnomedicine, febrile convulsions are treated by the use of different methods and techniques. The treatment modality varies from one locality to the other but one common feature is the use of heat, strong lubrication with palm kernel oil along with plant decoctions and or cow urine (Ojewole, 2005; Ajibesin et al, 2008). Among the Igbos who inhabit south eastern Nigeria, *Annona senegalenesis* Pers (Annonaceae) stands out as the most popular plant for the treatment of various disorders. It is a sprawling shrub whose different parts are utilized in ethnomedicine for the treatment of various ailments. *Annona senegalenesis*, commonly known as wild custard apple is a shrub or small tree widely distributed in Africa (Adzu et al, 2003; Adzu et al, 2005; Ogbadoyi et al, 2007) and particularly in the savannah areas and near streams, and enjoys great reputation for its immense medicinal value (Abubakar et al, 2007). Earlier workers had described it to be a potent drug against a variety of ailments (Dalziel, 1948; Irvine, 1961). The roots and leaves have been claimed to be efficacious in the treatment of venereal diseases, intestinal disorders, worm infestation, sleeping sickness, and in combination with other drugs or herbs is used for dysentery, diarrhea, snake bites, toothache and guinea worm sores. The use of *Annona senegalenesis* in the treatment of sores and cold has been reported by other workers (Irvine, 1961; Nadkarni, 1985). Febrile convulsions have also been successfully managed with the decoction of the roots of

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Annona senegalenesis by the local people thereby giving this shrub prominence in ethnomedicine or folk medicine. The present study is aimed at evaluating the anticonvulsant activities of the aqueous extract of the roots of *Annona senegalenesis* using animal models.

2. MATERIALS AND METHODS

Plant material was collected from Awka, Anambra State, Nigeria during the last quarter of the year 2012. Botanical identity was authenticated by Prof. C.U. Okeke, Department of Botany and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria with voucher specimen number: (DBB/NAU/146) deposited in the University herbarium.

2.1. Extraction procedures

The roots of *Annona senegalenesis* were air-dried and reduced to coarse powder with a hand mill. 250g of the powdered drug was macerated with 1 litre of double distilled water for 24h, then filtered with a Whatman number 1 filter paper. The filtrate was concentrated to about 25% volume using a rotary evaporator at 4^0C and standardized. The concentrate was stored in a refrigerator until required for use and the yield was 30%.

2.2. Experimental animals

Convulsive experiments were performed on adult albino mice of either sex weighing between 20 and 35g. All experimental procedures followed the recommendations of the Committee for Research and Ethical Issues of the International Association on Ethical Standard for Investigations of Experimental Animals (Zimmerman, 1983). The number of experimental animals was kept to a minimum. Three sets of mice each set containing 60 mice, divided into 6 groups of 10 animals each, were maintained in separate mice cages at ambient room temperature. Twelve hours before the experiment, food was withheld but the mice in each set had access to water. All experiments were performed during the light phase with each animal being used only once. The following studies were undertaken:

2.2.1. Phytochemical studies

The aqueous extract of the root of *Annona senegalenesis* was chemically tested with specific reagents for the presence of saponins, tannins, carbohydrate, resins, glycosides, alkaloids, flavonoids and sterol using standard methods, techniques and thin layer chromatography (Marini-Bettolo, 1981; Wager and Bladt, 2001).

2.2.2. Acute toxicity studies

Adult albino mice of either sex weighing between 20 and 35g (mean 25.6±1.3 g) were used in the study. The mice were provided with pelleted mice feed manufactured by Livestocks Nigeria Plc. The animals were divided into 6 groups of 10 mice per group. The extract or drug was administered intraperitoneally (i.p) in doses 50, 100, 200, 400, 800 and 1000mg/kg body weight and mice in each group received the same quantity of the extract or drug per body weight. The animals were observed for 24h for toxic symptoms, such as wet fur, restlessness, morbidity, oedema etc and the percentage mortality calculated. The percentage mortality was plotted against the log dose and the LD_{50} determined.

2.2.3. Pentylenetetrazole-induced convulsions

This study was carried out as described by (Parmar et al, 1972). Seventy albino mice of either sex were randomly (weight 20-35g, mean 22.5±1.5g) divided into eight groups of 5 mice per group. Groups 3 to 8 received 50, 100,200, 400, 800, 1000mg/kg body of the aqueous root extract respectively while the first group (negative control) received 25ml/kg body weight of normal saline intraperitonealy while mice in the 2^{nd} group (positive control) received phenobarbitone sodium 35mg/kg intraperitoneally. Thirty minutes later, 70mg/kg of pentyleneterazole was administered to each mouse in the groups intraperitoneally. From preliminary experiments, it was discovered that the minimum dose of pentylentetrazole which caused 100% mortality in 24h in mice was 70mg/kg body weight. The effect of various doses of *A. senegalenesis* on mortality due to this dose of pentylenterazole was determined. The animals were observed for 60 minutes for seizures. An episode of clonic spasm that persisted for a minimum of 30 seconds was interepreted as threshold convulsion. Animals devoid of a threshold during the 60 minutes of observation were considered protected. The results are presented in Table 1.

2.2.4. Electroshock-induced convulsion

The method of (Dikshit, 1972) was used. Another set of seventy mice of either sex weighing between 20 and 30g (mean 23.4±0.8g) were randomly divided into 8 groups of 5 mice per group. Groups 3 to 8 received 50, 100, 200, 400, 800, 1000mg/kg of the root extract of *A. senegalenesis* respectively while the first group (negative control) received 25ml/kg (i.p.) of normal saline per body weight, while mice in the 2^{nd} group (positive control) received phenobarbitone sodium 35mg/kg (i.p.). Each mouse in the various groups was given an electroshock (Ugo Basile, Electroconvulsive Treatment Unit) via ear clip electrodes previously soaked in normal saline to make for better conduction thirty minutes later. The stimulation parameters used were 45mA, 0.2 second

and 100Hz which produced maximum shock without being lethal.

2.2.5. Phenobarbitone-induced sleeping time in mice

The method of (Wambebe, 1985) was used with modifications. Another set of forty mice of either sex, weighing between 26 and 30g (mean 25.5± 0.9g) were randomly divided into eight groups of 5 mice per group. Mice in groups 2-7 received 50, 100, 200, 400, 800, 1000mg/kg of the root extracts of *A senegalenesis* intraperitoneally, while mice in group 1 served as positive control. Group 1 received normal saline (ip) 25ml/kg body weight. Each animal was observed for onset and duration of sleep. The time from induction of sleep to loss of righting reflex was considered as onset of sleep while that between loss and recovery of righting reflex was recorded as duration of sleep.

2.3. Drugs Used

Pentylentetrazole (PTZ) and sodium chloride were purchased from Sigma USA, while Phenobarbitone sodium was obtained from BDH Chemicals and were prepared fresh prior to use.

2.4. Statistical Analysis

Statistical analysis was done using two-tailed Student "t" test assuming equal variance at 95% confidence interval. Results are expressed as mean±SEM for 6 experiments in each group. Differences between groups were considered significant at $p<0.05$.

3. RESULTS

Preliminary phytochemical analysis with thin layer chromatography (TLC), using specific reagents showed that the extract contained some phytoconstituents including: alkaloids, glycosides, tannins, flavonoids, carbohydrates and saponins. Acute toxicity study in mice: The LD_{50} value following intra-peritoneal administration of the aqueous extract of the root of *A. senegalenesis* in mice was 954.8±2.9mg/kg body weight. It was also observed during the study that the extract did not produce any extreme symptoms such as wet fur and the animals moved freely.

Pentylenetetrazole–induced convulsion: Table 1 shows the results obtained during the study. Protection against drug–induced convulsion was dose dependent and *A. senegalenesis* protected 50% of the mice from death due to leptazole (pentylenetetrazole) at a dose of 562.3± 1.3mg/kg body weight or a therapeutic index of 1.7 (LD_{50} 954.86±mg/kg body weight). Electoshock–induced convulsion: Table 2 shows the results obtained during the study. There was no protection in the control as well as the groups that received 50 and 100 mg/kg respectively, while protection againstelectroconvulsion increased as the dose of the extract increased thusoffering 40% protection at the maximum dose of 800 mg/kg.

Phenobarbitone-induced sleeping time: Table 3 shows that the aqueous extract of root of *A. senegalenesis* shortened the latency of sleep (onset) and prolonged the sleeping time (duration) in a dose dependent manner.

4. DISCUSSION

Plants have always been used as medicine from ancient times. Among several traditional claims, the usefulness of *A. senegalenesis* in the management of febrile convulsion has been manifest. Hence, the present study has given scientific authentication to the traditional claims. The present investigation revealed that the aqueous extract of the root of *A. senegalenesis* was fairly nontoxic and did not induce any change in animal behavior as food and water intake were normal during the acute toxicity study. The LD_{50} calculated graphically was 954.8±2.9 mg/kg body weight.

The treatment of febrile convulsion is still far from satisfactory and a number of new anticonvulsant remedies and measures have been introduced during the last century some of which are related to compounds already known and the search for drugs with reduced toxic effect is continuing. Assessment of anticonvulsant activity revealed increased seizure latency, shortened duration of seizure and protection of treated mice from seizure-induced deaths. PTZ-induced convulsion occurs through the antagonism of gama-aminobutyric acid (GABA) receptor-chloride ion channel complex (Corda et al, 1990), thereby attenuating GABA-dependent inhibition. Thus, the anticonvulsant activity of aqueous root extract of this plant and its use are mediated by effect on GABAergic mechanisms. Agents that protect against tonic-clonic seizures induced by pentylenetetrazole are considered useful in humans (Nisar et al, 2008). The present study provides evidence for the anticonvulsant activity of aqueous extract of the root of *A. senegalenesis* in mice, being more effective against drug-induced (generalized) convulsion than electroshock-induced (partial) convulsions. Although in both versions of experimental convulsion, the time of onset of seizure increased as the dose of the extract is increased, but on weight for weight basis, the delay in onset was longer in drug-induced convulsion than electroshock model. However, with a therapeutic index (TI) of 1.7, caution must be exercised whenever the extract is used, because drugs with high TI are considered safer and carries less risk of toxicity than those with lower TI. Although, in practice, these types of seizures are distinct in their clinical manifestations and responses to therapy; only very few drugs can be effective against both seizures. The clinical aspect of generalized seizures are highly correlated with experimental seizures produced by subcutaneous administration of pentylenetetrazole while partial

seizures in humans correlate positively with experimental seizures elicited by the maximal electroshock test (Porter and Meldrum, 2004). These seizures respond differently to drugs through different mechanisms of action. Ethosuximide, for instance, which is effective against generalized seizures act through a reduction of Ca^{2+} entry into excitable membranes, while phenytoin which is effective against partial seizure produces anticonvulsant effects through alteration of ionic transport across excitable membrane (Jusko, 1977). It is, therefore, interesting that the aqueous extract of the root of *A. senegalenesis* was effective against both seizure types and may be acting through synergistic effects in ionic movements across the seizure foci. Such preparations are, therefore, of great benefits in folk medicine because at that level convulsions are not usually classified. Further, studies on the sedative activity of the extract revealed reduced latency and increased duration of phenobarbitone-induced sleep.

CONCLUSION

In conclusion, the present study has given credence to the traditional claims suggested for *A. senegalenesis,* although the active compounds responsible for the pharmacological actions are yet to be identified, work is currently going on in this direction.

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Table 1:

Effects of Pentylenetetrazole on mice pretreated 30 minutes earlier with the aqueous extract of *Annona senegalenesis* (n=6)

Dose of extract (mg/kg)	Onset of Convulsion (min)	Deaths (%)	Protection (%)
Control	9.3±1.3	5	0
50	15.5±1.0	5	0
100	18.7±1.2	1	20
200	26.8±1.0	28	40
400	29.9±1.5	36	60
800	35.2±1.6	44	80
1000	42.5±1.8	28	40

Table 2:

Effects of Electroshock on mice pretreated 30 minutes earlier with the aqueous extract of *Annona senegalenesis* (n=6)

Dose of extract (mg/kg)	Onset of Convulsion (min)	Deaths (%)	Protection (%)
Control	0.9±0.1	5	0
50	1.2±0.6	5	0
100	1.6±0.3	5	0
200	1.8±0.2	1	20
400	2.6±0.9	2	40
800	3.4±1.0	3	60
1000	10.2±0.5	2	40

Table 3:

Effects of aqueous root extract of *A. senegalenesis* on Phenobarbitone-induced sleeping time in mice

Group	Dose of extract (mg/kg)	Sleeping time (Min)		
		Onset	Duration	Prolongation (%)
1	Normal Saline (25ml/kg)	26.1±0.6	95.5±6.5	0
2	50 (extract)	26.2±0.7	86.6±4.5	NP
3	100	24.4±0.5	112.6±5.2	16.7
4	200	20.3±0.4	120.4±6.2	24.8
5	400	18.2±0.4	124.6±6.4	29.1
6	800	15.2±0.3	130.7±7.5	34.5
7	1000	10.2±0.2	135.8±8.5	40.7

*NP: Not Prolonged