Anti-seizure activity of the aqueous leaf extract of *Solanum nigrum* linn (solanaceae) in experimental animals

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Abstract

Background: *Solanum nigrum* is claimed in traditional medical practice, to be useful in the treatment of epilepsy in some parts of Nigeria.

Objectives: To study the anti-convulsant property of the aqueous extract of the leaves of *S. nigrum* in chicks, mice and rats.

Method: Aqueous extracts were administered intraperitoneally, at a pre-treatment time of 30 minutes, at graded doses and animals were challenged with different types of proconvulsants.

Results: The aqueous leaf extract produced a significantly ($P<0.05$) dose dependent protection against electrically-induced seizure in chicks and rats, pentylenetetrazole-induced seizure in mice and rats and picrotoxin-induced seizure in mice and rats. The anti-seizure property of the extract was potentiated by amphetamine.

Conclusion: The result obtained in this study suggests that the leaves of this plant may possess anti-convulsant property in chicks, mice and rats.

Keywords: *Solanum nigrum; anti-seizure activity; chicks; mice; rats*

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Introduction

Seizure refers to a transient alteration of behaviour due to disordered, synchronous and rhythmic firing of populations of brain neurons[1]. Epilepsy is a disorder of brain function characterized by periodic and unpredictable occurrence of seizures. Seizures can be "non-epileptic" when evoked in a normal brain by treatment such as electric shock or chemical convulsants or "epileptic" when occurring without evident provocation[1,2]. Modern drug therapy of epilepsy is complicated by the inability of drugs to control seizure in some patients and side effects that range in severity from minimal impairment of the central nervous system (CNS) to death from aplastic anaemia or hepatic failure. Thus its effective and safe therapy remains a challenge[1,3,4,5,6,7]. Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to posses promising anticonvulsant activities in animal models for screening for anticonvulsant activity[8,9,10,11,12] and can be a source of newer anticonvulsants.

Solanum nigrum is a widely distributed tropical plant. The leaves of the plant have been used in some parts of Plateau State, Nigeria for food and medicinal purposes. The plant is used in Nigerian folkloric medicine for the treatment of epilepsy. The plant has been reported to have antipyretic, anticancer activity[13,14]; CNS depressant activity[15,16] and as a promising agent for the control of schistosomiasis[9]. Toxicity studies on the plant showed that the plant is slightly toxic with an LD_{50} of 763 mg/kg[17].

The aim of this study was to investigate the possible anticonvulsant effect of the aqueous leaf extract of *S. nigrum* to justify the traditional use of the leaves of this plant in epilepsy.

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Materials and methods

Plant materials

S. nigrum plant was collected from Vel-Pankshin, Plateau State, Nigeria in June, 2000 and was identified at the Department of Botany, University of Jos, Nigeria and authenticated at the Herbarium Department, Ahmadu Bello University Zaria, Nigeria, where it was deposited as voucher specimen.

Preparation of extract

100 g of the leaves were crushed and macerated in 500 mls of distilled water and exhaustively extracted using the soxhlet apparatus for 72 hours at a controlled temperature of 60°C. The extract was evaporated in a rotary evaporator to dryness to obtain a concentration of 36% w/v. The extract (in form of paste) was stored at 4°C in the refrigerator until use.

Animals

Adult albino rats of either sex (weighing 200-250 g) and mice of either sex (weighing 40-60 g) were obtained from National Veterinary Research Institute

Vom, Nigeria. They were kept in clean cages under a 12/12 hours light/dark cycle at 32±2°C and allowed to acclimatize to the laboratory environment for a period of three weeks before commencement of experiment. Feed (24% protein, Pfizer products Lagos, Nigeria) and water were provided *ad libitum*.

Chicks (weighing 30-40 g) were purchased from Arewa Agricultural Ent. Ltd. Zaria, Nigeria. They were collected a day after hatching and kept in cages for another 24 hours to acclimatize to the laboratory conditions. Feed (Chick mash: Pfizer products Lagos, Nigeria) and water were provided *ad libitum*.

Antiseizure Activity

Electrically-induced seizure

A total of 56 rats and 56 chicks were used in this experiment. An alternating current of frequency 50 Hz, 7.5 V, and pulse width 1.5 ms^{-1} was delivered via steel electrodes clipped on the left and right ear lobes of rats using the Harvard Kymograph, universal model while the chicks received a current of 60 mA, shock duration of 2s, pulse width of 0.4 ms^{-1} and frequency of 100 Hz via steel electrodes clipped on both ear lobes as described in rats.

The extract and distilled water (equi-volume) were administered to test and control groups respectively, 60 minutes before application of electric shock. Duration of tonic convulsions and percentage of seizure protection and mortality were recorded.

Picrotoxin-induced seizures

A total of 56 rats and 56 mice were used in this experiment. Control groups were treated with distilled water (equi-volume) while graded doses of the extract of *S. nigrum* were administered intraperitoneally to the test groups. Chlorpromazine and amphetamine were each co-administered with aqueous extract of *S. nigrum*. Picrotoxin (5 mg/kg) was administered after 30 minutes. The animals were observed for 2 hours for clonic-tonic seizures.

Pentylenetetrazole-induced seizures

A total of 56 rats and 36 mice were used in this experiment. Control groups were treated with distilled water (equi-volume) while graded doses of the extract of *S. nigrum* were administered intraperitoneally to the test groups. Chlorpromazine and amphetamine were each co-administered with aqueous extract of *S. nigrum*. Pentylenetetrazole (85 mg/kg) was administered after 30 minutes. The animals were observed for 2 hours for clonic-tonic seizures.

Chronic administration of extract and seizure activity

A total of 72 rats were used in this experiment. Rats were divided into nine groups of 8 rats each. The following treatments were administered: group 1 (50 mg/kg), group 2 (100 mg/kg), group 3 (50 mg/kg), group 4 (100 mg/kg), group 5 (50 mg/kg), group 6 (100 mg/kg). The corresponding three (3) control groups were administered equi-volume of distilled water. Animals were pre-treated daily with extract as above for 28 days. 24 hours after the termination of administration, they were challenged as outlined below:

Group 1	Electrically-induced seizure
Group2	Electrically-induced seizure
Group3	Picrotoxin 5 mg/kg
Group4	Picrotoxin 5 mg/kg
Group5	Pentylenetetrazole 85 mg/kg
Group6	Pentylenetetrazole 85 mg/kg

Seizure activity, seizure threshold and duration of seizure were observed and recorded.

Statistical Analysis

Values (mean ±S.E.M) were analyzed for statistical significance using one-way ANOVA and all the statistical comparison were by student *t*-test. *P*-value of < 0.05 was considered significant.

Results

The aqueous extract (30-60 mg/kg) produced a significant protection against electrically-induced seizures in rats (Table 1). Concurrent administration of amphetamine (3 mg/kg) with the extract (30 mg/kg) produced a significant protection (87.5%) against electrically-induced seizures while co-administration of the extract (30 mg/kg) with chlorpromazine (5 mg/kg) did not protect the rats against this seizure.

The aqueous extract of *S. nigrum* (10-40 mg/kg) produced a significant ($P<0.05$) dose-dependent protection against electroshock seizure in 3-day old chicks (Table 2). At a dose of 40 mg/kg, seizure activity was completely abolished. Amphetamine potentiated the anti-seizure activity of the extract (10 mg/kg) in 3-day old chicks, as it offered 100% protection in chicks while chlorpromazine (5 mg/kg,ip) neither potentianted nor reduced seizure activity in chicks.

Intraperitoneal injection of the extract (10-60 mg/kg) increased the latency of the convulsions induced by pentylenetetrazole dose dependently, 30-60 mg/kg of the extract produced complete protection against seizure and mortality in rats. Amphetamine potentiated significantly ($P<0.01$) the anti-seizure activity of the extract (10 mg/kg) against pentelenetetrazole-induced seizure in rats co-administration of the extract and

Table 1: The effect of the aqueous leaf extract of *S. nigrum* on electroshock-induced seizure in rats

Treatment (mg/kg)	No. convulsed/ No. used	Onset of seizure (sec)	No. of death s	Protection (%)
Control	8/8	01±1.0	0	0
Extract (5)	8/8	05±1.3	0	0
(10)	6/8	05±1.7	0	25
(30)	**2/8	*10±0.8	0	75
(60)	**2/8	*10±0.2	0	75
Extract (10) + Chlopromazine (5)	8/8	01±0.05	2	0
Extract (10) + Amphetamine (3)	**1/8	*10±1.1	0	87.5

*Values are expressed as mean±SEM *P<0.05, **P<0.01; compared with control*

Table 2: The effect of the aqueous leaf extract of *S. nigrum* on electroshock-induced seizure in 3 day old chicks

Treatment (mg/kg)	No. convulsed./ No. used	No. of deaths	Protection (%)
Control	8/8	3	0
Extract (10)	*5/8	0	37.5
(20)	**2/8	0	75.5
(40)	***0/8	0	100
Extract (10) + Chlopromazine (5)	*6/8	2	35
Extract (10) + Amphetamine (3)	***0/8	0	100

P<0.05,P<0.02, ***P<0.01; compared with control*

chlorpromazine decreased the seizure activity in rats. There was a delay in the onset of seizure but all the animals convulsed (Table 3).

The aqueous extract of *S. nigrum* exhibited a dose dependent protection against leptazole-induced seizure in mice, with a complete protection against seizure achieved with 30 mg/kg of extract. Amphetamine potentiated the anticonvulsant effects of the extract while chlorpromazine, on the other hand reduced the anticonvulsant activity of the extract against PTZ-induced seizures in mice (Table 4). This is indicated in the shortened onset and duration of convulsion.

The extract at doses of 20-60 mg/kg produced a significant increase in seizure threshold. 60 mg/kg gave 75% protection against picrotoxin-induced seizure in rats and also provided complete protection against mortality (Table 5). Chlorpromazine and amphetamine gave a significant protection increase in onset of seizures. Though, the duration was prolonged, there were less episodes of convulsion and the animals recovered (no mortality).

Chronic administration of the aqueous leaf extract of *S. nigrum* did not attenuate seizure activity by any of the methods of seizure induction. There was no protection against seizure and there was no significant increase nor decrease in seizure threshold in the laboratory animals used (Table 7).

Discussion

The present results indicate that the extract exhibited a significant dose dependent protection against electrically-induced seizure in rats and chicks. At higher doses, seizure activity was abolished completely. Amphetamine potentiated the anticonvulsant activity of the extract in rats and chicks, while chlorpromazine enhanced seizure activity. Electroshock causes the inhibition of GABA release and this, in turn, may inhibit GABA synthesis[18]. It is therefore, possible that the

Table 3: The effect of the aqueous leaf extract of *S. nigrum* on pentylenetetrazole (90 mg/kg, ip)-induced seizure in rats

Treatment (mg/kg)	Onset of convulsion (sec)	Duration of convulsion (min)	No of deaths	No. convulsed/ no. used	Protection (%)
Control	35±1.53	7.6±2.08	8	8/8	0
Extract (5)	50±3.07	6.0±2.04	7	7/8	12.5
(10)	*90±4.9	6.0±3.2	2	2/8	75
(30)	-	-	-	0/8	100
(60)	-	-	-	0/8	100
Extract (10) + Chlopromazine (5)	*95±7.8	6.0±2.2	3	8/8	-
Extract (10) + Amphetamine (3)	**105±2.3	6.0±4.1	2	3/8	62.5

*Values are expressed as mean±SEM, *P<0.05, **P<0.01; compared with control*

Table 4: The effect of the aqueous leaf extract of *S. nigrum* on pentylenetetrazole (90 mg/kg, ip)-induced seizure in mice

Treatment (mg/kg)	No. convulsed/ no. used	Onset of convulsion (sec)	Duration of convulsion (min)	No. of deaths	Protection (%)
Control	6/6	4±0.1	7±1.3	6	0
Extract (5)	3/6	7±0.8	10±1.0	1	50
(10)	2/6	*10±1.2	*12±0.1	0	68
(30)	0/6	-	-	6	100
Extract (10) + Chlopromazine (5)	3/6	5±1.0	10±2.3	2	50
Extract (10) + Amphetamine (3)	0/6	-	-	0	100

*Values are expressed as mean±SEM, *P<0.05; compared with control*

Table 5: The effect of the aqueous leaf extract of *S. nigrum* on Picrotoxin (90 mg/kg, ip)-induced seizure in rats

Treatment (mg/kg)	No. convulsed/ no. used	Onset of convulsion (sec)	Duration of convulsion (min)	No. of deaths	Protection (%)
Control	8/8	120±6.1	35±5.7	8	0
Extract (10)	8/8	120±7.4	37±8.5	8	0
(20)	6/8	*180±2.5	40±6.0	3	25
(40)	5/8	*182±7.1	48±8.5	0	37.5
(60)	**2/8	**360±4.3	48±6.0	0	75
Extract (10) + Chlopromazine (5)	**2/8	**240±4.1	*80±6.0	0	75
Extract (10) + Amphetamine (3)	**2/8	**300±5.0	*80±3.0	0	75

*Values are expressed as mean±SEM, *P<0.05, **P<0.01; compared with control*

Table 6: The effect of the aqueous leaf extract of *S. nigrum* on picrotoxin (90 mg/kg, ip)-induced seizure in mice

Treatment (mg/kg)	No. convulsed/ no. used	Onset of convulsion	Duration of convulsion	No. of deaths	Protection (%)
		(sec)	(min)		
Control	8/8	120±5.0	40±3.1	8	0
Extract (10)	8/8	120±4.0	58±4.2	8	0
(20)	*4/8	122±2.0	**240±2.2	4	50
(40)	**2/8	130±6.4	**240±4.8	1	75
(60)	0/8	-	-	0	100
Extract (10) + Chlopromazine (5)	0/8	-	-	0	100
Extract (10) + Amphetamine (3)	0/8	-	-	0	100

*Values are expressed as mean±SEM, *P<0.05, **P<0.01; compared with control.*

Table 7: The effect of chronic administration (28 days) of aqueous leaf extract of *S. nigrum* on seizure activities in rats

Group	Treatment (mg/kg)	No. convulsed/ No. used	Onset of seizure	mortality
Control	Electroshock	8/8	4±0.8	0
1	Extract (50) + Electroshock	8/8	3±0.2	0
2	Extract (100) + Electroshock	8/8	4±1.1	0
Control	Picrotoxin (5)	8/8	120±15.1	8
3	Extract (50) + Picrotoxin (5)	8/8	125±7.8	8
4	Extract (100) + Picrotoxin (5)	8/8	125±10.2	8
Control	PTZ (90)	8/8	30±2.1	8
5	Extract (50) + PTZ (90)	8/8	32±3.3	8
6	Extract (100) + PTZ (90)	8/8	28±4.2	8

*PTZ=pentylenetetrazole, Values are expressed as mean±SEM (n=4) *P<0.051; compared with control.*

aqueous extract of *S. nigrum* increases the release of GABA. It has been observed that an increase in catecholamines, will enhance anticonvulsant activity[19]. It is probable that the aqueous extract of *S. nigrum* mayhave some connection with in the cascade of events in neurohumoral transmission.

Enhancement of central dopaminergic transmission is responsible for anticonvulsant activity[20]. Since concurrent administration of amphetamine with the extract offered protection to chicks and rats against electroshock-induced seizure, and the administration of chlorpromazine (a dopamine antagonist) increase seizure activity in this work. This data shows the possible involvement of the dopaminergic mechanism in seizure modulation in chicks and rats.

In the pentylenetetrazole-induced seizure test, there was a dose-dependent increase in seizure threshold in mice and rats pre-treated with aqueous leaf extract of *S. nigrum*. Higher doses of the extract (30-60 mg/kg) gave complete protection against seizures. The administration of amphetamine enhanced the anticonvulsant activity of the extract in rats and mice while co-administration of the extract with chlorpromazine increases seizure threshold in rats but offered no protection against seizures. However, a 50% protection was recorded in mice. The clinical aspects of certain generalized seizures-especially absence seizures-are highly correlated with experimental seizures produced in animals by administration of

pentylenetetrazol[21]. Thus *S. nigrum* may have some beneficial effect on this type of seizure.

In this work, the aqueous extract of *S. nigrum* offered protection to mice and rats against picrotoxin-induced seizure. There was a significant ($P<0.05$) increase in seizure threshold with higher doses of the extract. Picrotoxin, a pro-convulsant drug blocks chloride channel directly[21]. Thus the pathway of activity of the extract could be through GABA receptor activation. This is further supported by the fact that co-administration of the extract with amphetamine and chlorpromazine produced 75% protection in rats and 100% protection in mice (Tables 5 and 6). This indicates that the mechanism of action of the extract might not necessarily involve dopaminergic receptor.

Glutamate is a major excitatory neurotransmitter in the CNS. Exposure of neurons to high concentration of glutamate can lead to neuronal damage. Glutamate plays an important role in epileptogenesis and expression of epileptic seizure[22]. From observations, though the aqueous leaf extract of *S. nigrum* has some anticonvulsant activity, it does not have antiepileptigenic activity since long-term pretreatment of rats did not attenuate seizure activity by any of the methods of seizure induction used (Table 7).

Conclusion

The aqueous leave extract of *S. nigrum* was found to offer protection against electrically, pentylenetetrazole and picrotoxin-induced seizures. This anticonvulsant property was potentiated by amphetamine, thus, the activity may probably be via the dopaminergic pathway. This result has provided the rationale of the use of this plant in the treatment of seizures.

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