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Original Research Article

Preliminary Phytochemical Screening, Acute Oral Toxicity and Anticonvulsant Activity of the Berries of *Solanum nigrum* Linn

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Abstract

Purpose: *To investigate the preliminary phytochemical properties, acute oral toxicity and anticonvulsant activity of the berries of Solanum nigrum Linn (S. nigrum)*
Methods: *Phytochemicals from the ethanol berry extract were screened by standard methods. Acute oral toxicity study was conducted as per Organisation for Economic Co-operation and Development (OECD) 425 guidelines while anticonvulsant activity was evaluated against pentylenetetrazole (PTZ)-induced seizure in mice. The effect of the extract at dose levels of 50, 100, 200 and 300 mg/kg body weight was evaluated in an experimental mice model, using phenobarbital as positive control (100 mg/kg p.o).*
Results: *Phytochemical screening revealed that the berries of S. nigrum contain carbohydrates, flavonoids, saponins, tannins, alkaloids, phenols and steroids. The oral median lethal dose of the extract was 3129 mg/kg body weight. The extract significantly delayed the latency of convulsion ($p < 0.05$) in PTZ-induced seizure mice in at the dose of 300 mg/kg p.o. The extract also reduced the frequency of convulsion and provided up to 100 % protection (300 mg/kg p.o) against death.*
Conclusion: *The results obtained in this study suggest that the ethanol berry extract of Solanum nigrum is safe and possesses anticonvulsant activity in PTZ-induced seizure in mice.*

Keywords: *Solanum nigrum, Phytochemical, Anticonvulsant, Pentylenetetrazole, Lethal dose, Acute toxicity*

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INTRODUCTION

Solanum nigrum (*S. nigrum*) is a widely distributed tropical plant. It is a medicinal plant commonly used in Africa in ethnomedicine to treat a wide range of disorders including epilepsy. The plant has been reported to have antiperiodic, antiphlogistic, diaphoretic, diuretic, emollient, febrifuge, narcotic, purgative and sedative properties [1].

Epilepsy is a brain disorder in which clusters of nerve cells, or neurons, in the brain sometimes signal abnormally. Neurons normally generate electrochemical impulses that act on other neurons, glands, and muscles to produce human thoughts, feelings, and actions. In epilepsy, the normal pattern of neuronal activity becomes disturbed, causing strange sensations, emotions, and behavior, or sometimes convulsions, muscle spasms, and loss of consciousness [2].

The incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing countries is 100 per 100,000 [3]. According to an epidemiological report, epilepsy afflicts 0.44 – 0.55 % of the population of Vietnam. However, only 29/189 patients are treated periodically with anti-epileptic drugs (AEDs). The missed treatment of epilepsy in Viet Nam is very high (84. 7 %) [4].

Modern antiepileptic drugs (AEDs) are unable to control seizure in some patients and also possess adverse effects such as absence of hypersensitivity reactions, weight problems, and drug interactions that cause central nervous system toxicity [5]. It has been observed that the presently available AEDs are unable to control seizures effectively in as many as 25 % of the patients [6]. The conventional antiepileptic agents like phenytoin, carbamazepine and sodium valproate carry with them several serious side effects notably neurotoxicity [7]. Since a majority of AEDs are consumed for life, co-administration of other drugs predisposes to the risk of drug interaction. Thus, it is necessary to investigate new antiepileptic agents that have significant potential for epilepsy treatment as well as are safe in terms of toxicity. Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically proven to possess promising anticonvulsant activities in mouse models [8-10], and thus could be regarded as a source of novel and safe anticonvulsants. Our study was carried out to investigate a non-toxic plant which has a potential in treatment of epilepsy.

EXPERIMENTAL

Plant materials

The berries of *S. nigrum* were collected from a mountain in Krông Nô, Đắk Nông Province of Viet Nam in August 2012. The plant was identified by Associate Prof Dr Tran Van Minh of the Institute of Tropical Biology, Viet Nam. A voucher specimen was deposited in the herbarium of Applied Biochemistry laboratory, Department of Applied Chemistry, School of Biotechnology, International University, Viet Nam National University Ho Chi Minh City, Viet Nam with voucher no. HB-BIO-09-08-12.

Chemicals

Pentylenetetrazole (PTZ) was purchased from Sigma-Aldrich (St. Spruce, Saint Louis, MO 63103, USA), 70% ethanol was procured from Shanghai Demand Chemical Co., Ltd. Phenobarbital (100 mg, Sanofi-Aventis industries, USA). All drugs/chemicals were prepared fresh in distilled water to the desired concentration.

Animals

Healthy Swiss mice *Mus musculus* var. *Albino*, weighing 25 - 30 g, were procured from Pasteur Institute of Ho Chi Minh City. They were housed in clean cages and had free access to standard pallet diet and water *ad libitum*. During the experiment, the mice were kept in a controlled environment of 12 h light/dark cycle. All the animals were acclimatized to laboratory conditions for a week prior to commencement of the experiments. All the animal studies followed the guidelines enunciated in the "Guide for the Care and Use of Laboratory Animals" [11], as well as specific national laws where applicable.

Preparation of *S. nigrum* ethanolic berry extract

Fresh berries of *S. nigrum* were dried in drying oven at 80 °C and ground into fine powder (hereafter referred to as powdered berry). The powdered berry (25 g) was defatted with 350 mL of 70 % ethanol (60 – 80 °C) in Soxhlet apparatus. The extract was then evaporated in vacuum to give a brownish residue. The residue, subsequently referred to as the extract, was stored in a refrigerator until required for further use.

Phytochemical screening

Phytochemical analysis of the extract was carried out for the detection of various constituents [12].

Oral acute toxicity test

Acute toxicity of the plant extract was carried out *in vivo* in healthy female albino mice weighing 25 - 30 g, labeled individually. Prior to dosing, the mice were fasted overnight and the dose for each mouse was determined based on the body weight. Solutions of the dried extracts were prepared using distilled water. The study was conducted as per Organization of Economic Cooperation and Development (OECD/OCDE) Test Guidelines on Acute Oral Toxicity under a computer-guided Statistical Programme-AOT425statPgm, version 1.0. Up and Down protocol and classifying category of toxicity based on the provisions of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) as adopted by the United Nations Economic and Social Council in July 2003 were conducted.

Limit test

Initially, the extract was administered to one animal in a single dose of 2,000 mg/kg by oral gavage using a feeding tube. After administration, food was withheld for 3 - 4 h. The animal was observed once during the first 30 min after dosing, then periodically, during the first 24 h. As the animal was not dead, four additional animals were given the same dose and observed similarly. Two mice were found dead after oral administration of the extract. All the survived animals were then kept for 14 days for further observation.

Next, the extract was administered to one animal in a single dose of 5,000 mg/kg. After administration, food was withheld for a further 3 - 4 h. The animal was observed once during the first 30 min after dosing, then periodically, during the first 24 h. As the animal was not dead, two additional animals were given the same dose and observed similarly. When both animals died, two additional animals were given a dose of 5,000 mg/kg, one at a time. When all the mice died, the main test was conducted.

Main test

Since there was no estimate of the berry extract's lethality available, starting dose was selected as 175 mg/kg. A single animal was dosed in sequence at 48 h intervals. The test was terminated when stopping criteria "5 reversals occur in any 6 consecutive animals tested" was attained. The estimated LD_{50} was calculated from the animal outcomes at termination point using the software program-AOT425statpgm.

Determination of anticonvulsant activity

Mice were randomly divided into six groups of five mice each (n = 5). Group I which served as control received an equivalent amount of distilled water; groups II, III, IV, and V received the extract doses of 50, 100, 200 and 300 mg/kg, p.o., respectively, while group VI received phenobarbital (100 mg/kg p.o) as reference standard. All the extracts and standard drug were administered 60 min before the administration of PTZ (85 mg/kg i.p.) and the mice were observed for convulsions. The mice were immediately placed individually in a cage, observed and monitored to determine the latency and the frequency of convulsions for each mouse. Values were expressed in terms of mean ± S.E.M. Manifestations of seizures were rated on a 6-point scale according to Racine's scale, which is widely used in studies on animal models of epilepsy (Table 1) [13,14].

Table 1: Six-point scale for anticonvulsant activity

Light seizures	Intermediate seizures	Heavy seizures
0.5: Immobility, piloerection, salivation, narrowing of eyes, face and vibrissae twitching, ear rubbing with forepaws	**1.5**: Clonic movements of forelimbs and mild whole body convul-sions, exophthalmia, aggressive behavior	**2.5**: Rearing and falling, eye congestion
1.0: Head nodding and chewing movements	**2.0**: Rearing and running with stronger tonic-clonic motions including hind limbs, tail hypertension, lock jaw	**3.0:** Loss of postural tone with general body rigidity

Statistical analysis

Mean ± standard error of mean (SEM) of the data were computed. Analysis of the data was made using SPSS, version 16.0. The statistical tools used were one-way ANOVA and paired sample t-test. The level of significance applied was $p < 0.05$.

RESULTS

Phytochemical profile

The ethanol berry extract of *S. nigrum* revealed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, phenols and steroids (Table 2).

Table 2: Phytochemistry of the ethanolic berry extract of *S. nigrum*

Phytochemical constituents	Phytochemical test	Inference
Carbohydrates	Barfoed's test	+
Flavonoids	Chloroform	+
	Shinoda test	+
Saponins	Emulsion test	+
	Frothing test	+
Tanins	Ferric chloride test	+
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Hydrochloric acid test	-
Phenols	Ethyl acetate	+
	Diethyl ether	-
Steroids	Chloroform	-
	Hexane	+

**+ indicates positive test result; - indicates negative test result*

Acute oral toxicity study

Data collected from Acute Oral Toxicity test by Up-And-Down procedure was described in Table 3. After 14 days of observation, long-term outcomes were recorded and used to estimate LD_{50} value in Table 4.

Table 3: Dose progression and results

Test Seq.	Animal ID	Dose (mg/kg)	Short-term	Long-term
1	1	175	O	O
2	2	550	O	O
3	3	1750	O	O
4	4	5000	X	X
5	5	1750	O	O
6	6	5000	X	X
7	7	1750	O	O
8	8	5000	X	X

(X = Died, O = Survived); stopping criteria met: 5 reversals in 6 tests. LR criterion

Table 4: Summary of long-term results

Dose	O	X	Total
175	1	0	1
550	1	0	1
1750	3	0	3
5000	0	3	3
All dose	5	3	8

Statistical estimate based on the long-term outcomes; estimated LD_{50} = 3129 (based on an assumed sigma of 0.5); approximate 95% confidence interval is 1750 to 5000

The oral median lethal dose of the extract was estimated as 3129 mg/kg body weight allocated to category 5 based on acute toxicity by the oral route (Table 5). Thus, it was considered to be safe.

Anticonvulsant activity of ethanol berry extract of *S. nigrum*

The ethanol berry extract of *S. nigrum* significantly delayed the latency of convulsions in a dose-dependent manner in PTZ-induced seizure mice.

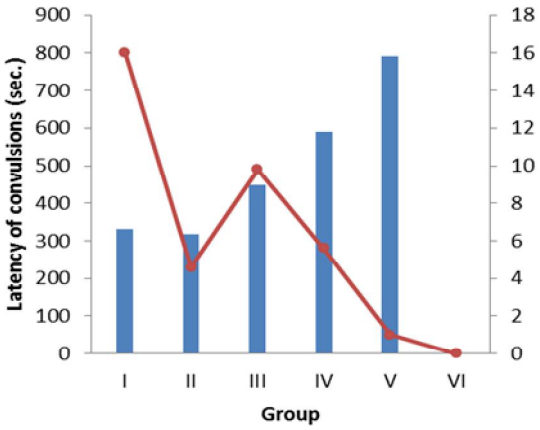

Fig 1: Effect of different doses of ethanolic berry extract of *S. nigrum* (50, 100, 200, 300 mg/kg) on the latency of convulsions and the frequency of convulsions in mice. ***Note:*** Red curve denotes no. of re-occurrence of convulsion

The results are shown in Table 6 and Fig 1; for PTZ-induced seizure, the latency and reoccurrence of convulsions in group I (control) were 330.0 ± 26.0 and 16.0 ± 1.1 sec, respectively.

Table 5: Acute toxicity hazard categories and (approximate) LD_{50} values defining the respective categories*

Acute oral toxicity	Category				
	1	2	3	4	5
LD_{50} value	$LD_{50} \leq 5$ mg/kg	5 mg/kg < LD_{50} ≤ 50 mg/kg	50 mg/kg < LD_{50} ≤ 300 mg/kg	300 mg/kg < LD_{50} ≤ 2000 mg/kg	2000 mg/kg < LD_{50} ≤ 5000 mg/kg

**Chemical hazard classification and labeling: comparison of OPP requirements and the GHS*

Table 6: Effect of ethanol berry extract of *S. nigrum* on PTZ-induced seizure mice

Group (n=5)	Latency of convulsion (second)	Frequency of convulsions (time)	No. of convulsions	No. of deaths	Protection (%)
I	330.00 ± 26.0	16.0 ± 1.1	5	5	0
II	317.60 ± 73.2	4.6 ± 0.3	5	5	0
III	447.20 ± 50.8	9.8 ± 1.2	5	5	0
IV	588.6 ± 109.3	5.6 ± 2.6	5	2	60
V	792.6* ± 157.7	1.0 ± 0.0	5	0	100
VI	0	0	0	0	100

**Each value represents mean ± SEM; *p < 0.05 compared with the control*

In group V, 300 mg/kg of the extract prolonged latency of seizures to 792.6 ± 157.7 and reduced frequency of seizures to 1.0 ± 0.0 s. All the animals in group I died, while in group V the mortality was 100 %. This is comparable to the values obtained for group VI (phenobarbital, reference) where 100 % mortality was recorded. There were slight convulsions in mice of group V but were insignificant ($p < 0.05$). In group IV, protection against convulsion was 60 %, indicating the potential anticonvulsant activity of the extract at low concentration.

DISCUSSION

Data from this study show that *S. nigrum* significantly increases the latency and decreases the frequency of convulsions on PTZ-induced seizures. PTZ exerts its convulsant effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors [15]. GABA is a major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively [16]. Phenobarbital is a known conventional antiepileptic agent that generally inhibits sodium currents and enhances GABA-ergic inhibition. Since the extract delayed latency and reduced the reoccurrence of PTZ convulsions, it is probable that it may be interfering with gabaergic mechanism(s) to exert its anticonvulsant effect.

Phytochemical tests revealed that the extract contains various components. However, it is believed that the saponins and flavonoids present in *S. nigrum* might contribute to the anticonvulsant activity of this plant species as reported in previous studies [17,18]. However, further studies are needed to identify the specific compounds responsible for the anticonvulsant activity.

CONCLUSION

The ethanol extract of *S. nigrum* berry possesses an anticonvulsant activity against PTZ-induced seizure in mice and is safe for oral administration. These findings provide a basis for further pharmacological investigations that could lead to the development of new potential anticonvulsant compounds.

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