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Antiepileptic and central nervous system depressant activity of *Sechium edule* fruit extract

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Abstract

The effect of ethanol extract of fruits of *Sechium edule* on antiepileptic and central nervous system (CNS) depressant model was studied in rats. The extract (200 mg/kg body weight, orally) significantly reduced the duration of various phases of convulsions in both MES-induced seizures and in PTZ-induced convulsion. In CNS depressant model, the locomotor activity was also decreased in a dose dependent manner as compared to control group the extract and the rota rod test revealed a significant loss of muscular coordination.

Introduction

Sechium edule, belongs to Cucurbitaceous family vegetable which grows abundantly in the hills of Meghalaya, Mizoram, Manipur, Sikkim and Nagaland. The use of decoctions of leaves and fruits of *Sechium edule* relieve urine retention, burning sensation during urination and also dissolve kidney stones. It is also use for the treatment of arteriosclerosis and hypertension (Gordon et al., 2000). Different pharmacological studies revealed the diuretic properties, anti-oxidant effect (Ordonez et al., 2006), anti-inflammatory and cardiovascular properties of the *S. edule*. It is also used in severe hypokalemia in pregnancy (Jensen et al., 1986), possess trypsin inhibitor activity (Helen et al., 2006). It has been also reported that the extract of *S. edule* having antimicrobial activity (Adriana et al., 2009). The roots, leaves, stems, and fruits of the plant contain five O-glycosyl flavones and three C-glycosyl and were detected by LC-photodiode array-MS (Siciliano and De Tommasi, 2004). The extracts of the seeds of *S. edule* contain twenty known Gibberellins. Gibberellins A₈ and gibberlin A₈ -catabolites are the major gibberellins

found in *S. edule* (Albone et al., 1984).

No major reports on CNS activity of *S. edule* were found. So, in the present study the CNS activities of this fruit were evaluated.

Materials and Methods

Plant

Fresh fruits of *S. edule* were collected from Bangalore and also from Secunderabad. The fruit material was taxonomically identified and authenticated at Regional Research Institute (Ay.), Bangalore, by Dr. Shiddamallayya N. The voucher specimen is conserved under the reference number (RRCBI/MCW/7/2008).

Preparation of ethanol extract

The fruits of *S. edule* were isolated, chopped and dried at room temperature for seven days. The dried materials were powdered by using mixer grinder. The powder was first defatted with petroleum ether (60-80 GR) for 72 hours and then the extraction of dried



powder was done with 99.9% ethyl alcohol. The yield of dried ethanol extract fruits of *S. edule* was approximately 12.1 % w/w.

Experimental animals

Wistar rats of either sex weighing 150-200 g were maintained under controlled temperature ($23 \pm 2^\circ\text{C}$) and humidity ($50 \pm 5\%$) and 12 h day and night cycle. Free access to standard pellet diet and water *ad libitum* were also provided. All the experimental procedures were carried out according to the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) and approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and chemicals

Pentylentetrazole was purchased from Sigma, USA. Phenytoin and diazepam injections were purchased from Ranbaxy, India. PTZ was dissolved in water for injection.

Acute toxicity study

Acute toxicity study of ethanol extract fruits of *S. edule* was performed according to the acute toxic class method of OECD guidelines. In acute oral toxicity study mortality was not observed up to 2,000 mg/kg body weight (OECD 2002).

Maximum electroshock-induced seizures

In this study, animals were divided into four groups (n=6). Group I: Rats were served as a normal control, received vehicle (0.9% w/v NaCl saline, 1 mL/100 g). Group II: Rats were received standard drug phenytoin (25 mg/kg, *i.p.*). Group III: Rats were received ethanol extract of fruits of *S. edule* (200 mg/kg, *p.o.*). Group IV: Rats were received ethanol extract of fruits of *S. edule* (100 mg/kg, *p.o.*).

Inco Electroconvulsimeter model# 100-3 was used to provide maximal electroshock (150 mA) for 0.2 sec through ear electrodes to induce convulsions. The duration of various phases of epilepsy were observed (Sayyah et al., 2000; Balakrishnan et al., 1998).

Pentylentetrazol-induced seizures

The animals were divided into four groups (n = 6). Group I: Rats were served as a normal control, received vehicle (0.9% w/v NaCl saline, 1 mL/100 g). Group II: Rats were received standard drug Diazepam (4 mg/kg, *i.p.*). Group III: Rats were received ethanol extract of fruits of *S. edule* (200 mg/kg, *b.w.*, *p.o.*). Group IV: Rats were received ethanol extract of fruits of *S. edule* (100 mg/kg).

PTZ was administered (80 mg/kg, *i.p.*) 45 min after administration of saline, standard drug and ethanol extracts of fruits of *S. edule*. After injection animals were observed for 30 min and the effect of ethanol extract

fruits of *S. edule* on onset of myoclonic spasm and clonic convulsions were determined (Kulkarni and George, 1999).

Test for locomotor activity

An actophotometer (Inco, Ambala, India) to evaluate the effect of ethanol extracts of fruits of *S. edule* on locomotor activity of rats. An actophotometer consists of a cage which is 40 cm long and 40 x 40 x 40 cm and has a wire mesh at the bottom. Six lights and six photo cells are placed in the outer periphery of the bottom in such a way that a single rat blocks only one beam. Photo cells are activated when the rays of light fall on photocells.

The animals were divided into four groups (n = 6). Group I: Rats were served as a normal control, received vehicle (0.9% w/v NaCl saline, 1 mL/100 g). Group II: Rats were received standard drug diazepam (4 mg/kg, *i.p.*). Group III: Rats were received ethanol extract of fruits of *S. edule* (200 mg/kg, *p.o.*). Group IV: Rats were received ethanol extract of fruits of *S. edule* (100 mg/kg, *b.w.*, *p.o.*).

The beam of light is cut as and when animal crosses the light beam, number of cut offs were recorded for 10 minutes (Goyal, 2006).

Motor co-ordination test (Rota rod test)

In this study, animals were divided into following groups of 6 rats each. Group I: Rats were served as a normal control, received vehicle (0.9% w/v NaCl saline, 1 mL/100 g). Group II: Rats were received standard drug diazepam (4 mg/kg, *i.p.*). Group III: Rats were received ethanol extract of fruits of *S. edule* (200 mg/kg, *p.o.*). Group IV: Rats were received ethanol extract of fruits of *S. edule* (100 mg/kg, *p.o.*).

Motor Co-ordination test was conducted using a Rota rod apparatus (Inco Ambala, India). The animals were placed on the moving rod prior to the treatment and the rats that stayed on the rod without falling for 120 sec were chosen for the study. The fall of time of animals of was noted (Kulkarni, 1987).

Statistical analysis

Statistical analysis of data was done by using the one-way analysis of variance (ANOVA) followed by Dunnet's 'T' test. $p < 0.05$ was considered significant.

Results

The results of anticonvulsant effect of ethanolic extract of fruits of *S. edule* are shown in Table I and II. In MES induced convulsion in rats (Table I), the ethanol extract of fruits of *S. edule* (100 mg/kg, *p.o.* and 200 mg/kg) significantly reduced the duration of various phases of convulsions. In PTZ induced convulsion in rats (Table

Table I							
Effect of ethanol extract of fruits of <i>Sechium edule</i> against MES Induced convulsions rats							
Groups	Treatment	Dose (mg/kg)	Time (sec) in various phases of convulsions (Mean ± SEM)				
			Flexion	Extension	Clonus	Stupor	
I	Control (Saline 1 mL/100 g)	--	6.0 ± 0.6	15.2 ± 0.6	17.5 ± 0.8	94.5 ± 2.1	125.2 ± 0.9
II	Standard (Phenytoin)	25	1.7 ± 0.3 ^b	0.0 ± 0.0 ^b	10.3 ± 0.6 ^b	53.8 ± 1.0 ^b	68.5 ± 2.0 ^b
III	<i>Sechium edule</i> extract	100	4.5 ± 0.4 ^a	13.5 ± 0.4	14.3 ± 0.6 ^a	86.7 ± 1.4 ^b	101.7 ± 2.1 ^b
IV	<i>Sechium edule</i> extract	200	2.0 ± 0.2 ^b	8.8 ± 0.6 ^b	10.8 ± 0.6 ^b	68.7 ± 1.1 ^b	76.5 ± 1.6 ^b

Data were expressed as mean ± SEM. Significant at ^ap<0.05 and ^bp<0.01 when compared to control (n = 6)

Table II						
Effect of ethanol extract of fruits of <i>Sechium edule</i> against PTZ Induced convulsions in rats						
Groups	Treatment	Dose (mg/kg)	Onset time in sec (Mean ± SEM)			
			Jerks	Clonus	Extensor	
I	Control (Saline 1 mL/100 g)	--	51.5 ± 1.3	79.2 ± 1.0	254.0 ± 1.5	Mortality
II	Standard (Diazepam)	4	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	Recovery
III	<i>Sechium edule</i> extract	100	60.8 ± 1.2 ^b	87.2 ± 0.9 ^b	269.8 ± 1.4 ^b	Recovery
IV	<i>Sechium edule</i> extract	200	75.2 ± 0.9 ^b	99.2 ± 1.1 ^b	308.0 ± 1.6 ^b	Recovery

Data were expressed as mean ± SEM. Significant at ^bp<0.01 when compared to control (n = 6)

Table III				
Effect of ethanolic extract of fruits of <i>Sechium edule</i> (SE) on locomotor activity (actophotometer) in rats				
Groups	Treatment	Dose (mg/kg,b.w.)	Score (number of cut off were recorded for 10 min)	
I	Control (Saline 1 mL/100 g)	--	293.7 ± 7.2	--
II	Standard (Diazepam)	4	13.0 ± 1.1 ^b	95.6
III	<i>Sechium edule</i> extract	100	136.3 ± 2.0 ^b	53.6
IV	<i>Sechium edule</i> extract	200	102.0 ± 1.7 ^b	65.3

Data were expressed as mean ± SEM. Significant at ^bp<0.01 when compared to control (n = 6)

Table IV				
Effect of ethanol extract of fruits of <i>Sechium edule</i> on rota rod test in rats				
Groups	Treatment	Dose (mg/kg,b.w.)	Time of fall (Sec)	
I	Control (Saline 1 mL/100 g)	--	312.5 ± 2.0	--
II	Standard (Diazepam)	4	17.5 ± 0.8 ^b	94.4
III	<i>Sechium edule</i> extract	100	141.7 ± 2.4 ^b	54.7
IV	<i>Sechium edule</i> extract	200	104.3 ± 1.6 ^b	64.7

Data were expressed as mean ± SEM. Significant at ^bp<0.01 when compared to control (n = 6)

II) the ethanolic extract of fruits of *S. edule* (100 mg/kg and 200 mg/kg) delayed onset of clonus and extensor and produced significant anticonvulsant activity.

In locomotor activity model, significant (p<0.01) and dose dependent reduction of locomotor activity was observed in rats treated with the ethanol extract of

fruits of *S. edule* (100 mg/kg and 200 mg/kg, p.o) (Table III). The rota rod test revealed a significant (p<0.01) loss of muscular coordination (Table IV). Thus, the ethanol extract of fruits of *S. edule* possessed CNS depressant activity as indicated by the significantly reduced, motor coordination and spontaneous motor activity.

Discussion

The MES induce convulsion model one of the widely used anticonvulsant animal model. This model is helpful for determination of tonic-clonic seizures (Loscher et al., 1988; Oliveira et al., 2001). The repeated electrical pulses by the electroconvulsimeter to neurons produce a standard epileptic characteristic. In this present study, the ethanol extract of fruits of *S. edule* (100 mg/kg and 200 mg/kg) significantly reduced the duration of various phases of convulsions in MES induce convulsion.

Pentylenetetrazole (PTZ) blocks the picrotoxic binding site of GABA type A receptor and interfere with the GABA neurotransmission, produce convulsion (Ramanjaneyulu and Ticku 1984). Benzodiazepines and phenobarbital prevent PTZ induced seizures by increasing the GABA neurotransmission. The ethanol extract of fruits of *S. edule* (100 and 200 mg/kg) delayed the onset of convulsion and produced significant anticonvulsant activity. The ethanolic extract of fruits of *S. edule* (100 and 200 mg/kg) decreased the locomotor activity and also produce loss of muscular coordination. Thus, the ethanol extract of fruits of *S. edule* possessed CNS depressant activity as indicated by the significantly decrease in motor coordination and spontaneous motor activity. This CNS depressant activity may be due to the phytochemicals present in the ethanol extract of fruits of *S. edule*.

Conclusion

The result suggests that the ethanol extract of fruits of *S. edule* possess anticonvulsant and CNS depressant activity.

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