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EVALUATION OF ANTICONVULSANT ACTIVITY OF ALCOHOLIC EXTRACT OF *BENINCASA HISPIDA* (THUNB) COGN. FRUIT EXTRACTS

S K Nimbal[1], N Venkatrao[2], Basavaraj Pujar[3], Shalam[4], Shivakumar Ladde[5]*
[1]Department of Pharmacology, KLES College of Pharmacy, Hubli, India
[2]Department of Pharmacology, VL College of Pharmacy, Raichur, India
[3]Department of Pharmacology, TVM College of Pharmacy, Bellary, India
[4]Dept. of Pharmacology, College of Pharmacy, Al Qassim University, Buraidah, KSA
[5]Department of Pharmacology, Shivlingeshwar College of Pharmacy, Amit Nagar, Hasegaon, Dist-Latur (MH), India

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*Email: shivkumarladde@gmail.com

ABSTRACT
Epilepsy is a common neurological condition, affecting 0.5 to 1% of the population worldwide. According to the Sanskrit texts, *Benincasa Hispida* fruit is useful in insanity, epilepsy, constipation, piles dyspepsia and other nervous diseases; fresh juice is given either with sugar or as an adjunct to other medicines for these diseases. In this work, the anticonvulsant properties of alcoholic extract of *Benincasa Hispida* studied on maximal electroshock test (MEST), pentylenetetrazole and strychnine-induced seizures model in mice. The oral acute toxicity values (LD50) in mice were also evaluated. The alcoholic extract of *Benincasa Hispida* of protected animals against maximal electroshock-induced convulsion and reduced the mean recovery time from convulsion. The alcoholic extract of *Benincasa Hispida* had a also shows anticonvulsant activity against pentylenetetrazole-induced convulsion and protect mice against strychnine-induced convulsions. These results suggest that the ethanolic extract of *Benincasa Hispida* contains pharmacologically active substance(s) like triterpenoids, flavonoids, glycosides and steroids, which may be valuable in the treatment of convulsive disorders, especially Grand mal epilepsy.
Key words: *Benincasa Hispida,* epilepsy, strychnine, pentylenetetrazole, maximal electroshock test (MEST)

INTRODUCTION

Epilepsy is a common neurological condition, affecting 0.5 to 1% of the population worldwide (45-100 million people)[1]. It is characterized by recurrent unprovoked epileptic seizures. These seizures are transient signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. It affects approximately 50 million people worldwide.[2] Conventional antiepileptic drugs (AEDs) phenobarbital, primidone, phenytoin, carbamazepine, ethosuximide and benzodiazepine, are widely used but exhibit an unfavorable site effect profile, including teratogenicity, chronic toxicity and adverse effects on cognition and behaviour.[3] The search for antiepileptic compounds with a more selective activity and lower toxicity continues to be an area of investigation in traditional system. However, only limited efforts have been made to evaluate the potentials of such plants for their use in modern medicine or to scientifically justify their traditional use in the treatment of CNS disorders including epilepsy.

The fruit of *B. hispida* (Thunb) Cogn. Commonly called as ash gourd, belonging to Cucurbitaceae is employed as a main ingredient in kusmana lehyam, in Ayurvedic system of medicine. According to the Sanskrit texts, it is useful in insanity, epilepsy, constipation, piles dyspepsia and other nervous diseases[4,5]. Though some scientific studies have been carried out reveal its Anti-ulcer[6]anti-diarrhoel[7] anti-angiogenic[8], anti-inflammatory[9], anticancer[10], antiasthmatic[11], antioxidant and angiotensin converting enzyme inhibitor[12] anorectic[13], nootropics[14] and hypoglycemia[15] activities. The major constituents of this fruits are triterpenoids, flavonoids, glycosides, sacchrides, proteins, carotenes, vitamins, minerals, ß-sitosterin and uronic acid [16,17].

In the light of above information, the present investigation was undertaken to evaluate the anticonvulsant activity of alcoholic extract of *B.hispida* fruit.

MATERIALS AND METHODS

Collection of plant materials

The fresh fruits were collected during October-November from the local market of Raichur and were identified by botanist of our college.

Extract Preparation[6,7]

After removing the outer skin and the seeds, the fruit of *B.hispida* was mashed using an electric juicer to afford a soft mass. For the preparation of an alcoholic extract, 100ml of fresh juice was mixed with 500ml of ethanol and kept covered for seven days at room temperature with daily occasional stirring. The mixture was then filtered and the filtrate was heated (below 55^0C) and evaporated under reduced pressure. Later the extract was dried completely using a lyophilizer (Lyotap, Germany), brownish sticky mass was obtained, which was protected from direct sunlight. The yield of the extract was 0.733gm/100ml of the fresh juice.

Animals

Swiss albino mice of either sex weighing between 20-30g were procured from Shri Venkateswara Enterprises, Bangalore and were acclimatized for seven days under standard husbandry conditions and water ad libitum.

The approval of the Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy Raichur, Karnataka (Registration number 557/02/c/CPCSEA) was taken prior to the experiments and in order to reduce the day variation, all protocols and the experiments were conducted from 9 to14 h, in a special noise-free room with controlled illumination.

Drugs and Chemicals

The alcoholic extract of *B. hispida* was used as experimental extract, dissolved in 3% tween-80 (s.d.fine Chem.Ltd. Mumbai), Phenytoin [Sun Pharmaceutical India.Ltd, Gujarat, India] and diazepam (Ranbaxy Laboratories Ltd, Mumbai, India) as standard anxiolytic drug was used. Pentylenetetrazole [Sigma-Aldrich, St.Louis, MO 63103 USA], Strychnine [Sigma-Aldrich, St.Louis, MO 63103 USA], Tween-80 [s.d.fine Chem Ltd. Mumbai].

Phytochemical Test

The ethanolic extract of *B.hispida* was screened for the presence of alkaloids, glycosides, tannins, saponins and flavonoids according to standard procedure.[18]

Determination of acute toxicity (LD50)

The acute toxicity of alcoholic extract of fruits of *B. hispida* was determined by using female albino mice (20-30g) those maintained under standard husbandry conditions. The animals were fasted 3 hrs

prior to the experiment, up and down procedure (OECD guideline No. 425) of CPCSEA was adopted for toxicity studies.[19] Animals were administered with single dose of extract and observed for its mortality during 48 hours study period (short term toxicity). Based on short-term profile of drug, the dose of the next animals was determined as per OECD guideline No.425. The LD50 of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA.

Maximum electroshock-induced convulsion in mice

The modified method of Swinyard and Kufferberg (1985); and Browning, (1992) was employed.[20,21] Albino mice (20-30g) were randomly divided into five groups of 6 mice per group. The first group received normal saline (10ml/kg).p.o.; second, third and fourth groups were treated with 100,200 and 400 mg ethanolic extract of *B.hispida* per kg, p.o. and the last group was administered 25 mg phenytoin per kg, p.o.. (positive control). Thirty minutes later, maximal electroshock was administered to induce seizure in the mice using Ugobasile electroconvulsive machine (Model 7801) with corneal electrodes placed on the upper eyelids of the mice. The current, shock duration, frequency and pulse width were set and maintained at 80 mA, 0.80 s, 100 pulse per second and 0.6 ms, respectively. Abolition of Hind limb tonic extension (HLTE) was considered as protection from electroshock.[22,23]

Pentylenetetrazole-induced convulsion in mice

The method of Swinyard et al., (1989) was employed[23]. Thirty mice were divided into five groups of six mice each. The first group received 10 mL normal saline per kg body weight p.o., the second group was given Diazepam 5mg per kg body weight p.o., while the third, fourth and fifth groups received 100,200 and 400 mg ethanolic extract of *B.hispida* per kg body weight p.o. Thirty minutes later, mice in all the groups received 85 mg pentylenetetrazole per kg i.p. Mice were observed over a period of 30 minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold.

Subcutaneous Strychnine-induced convulsion in mice

The method of Porter et al., (1984) was employed[22]. Thirty mice were divided into five groups of six mice each. The first group received 10 mL normal saline per kg body weight p.o., the second group was given Diazepam 5mg per kg body weight p.o., while the third, fourth and fifth groups received 100,200 and 400 mg ethanolic extract of *B.hispida* per kg body weight p.o. Thirty minutes later, mice in all the groups received 1.0 mg strychnine per kg, i.p. Extension onset of tonus was considered an indicator that the testing material could prevent strychnine-induced convulsions.

Statistical Analysis

The results were subjected to statistical analysis by using one-way ANOVA followed by Tukey- Kramer test to assess the significance difference if any among the groups. $P<0.05$ will be considered as significant.

RESULTS

The phytochemical screening of the ethanolic extract of *B.hispida* revealed the presence of triterpenoids, flavonoids, glycosides and steroids.

The alcoholic extract of fruits of *B. hispida* was administered orally to different groups of mice with different dose levels and found that even upto the dose of 2000mg/kg body weight, it has not produced any behavioural symptoms or mortality.

In MES model, different doses of AEBH i.e. 100, 200 and 400mg/kg were administered daily once for seven days, it was found that medium and high doses but not lower dose had produced a significant anti-convulsant effect in above parameters and percentage protection was, 25%, 62.5% and 75%respectively. And Phenytoin and different dose of AEBH decreases hind limb tonic extension (HLTE) as compared to control group (Table 1).

In PTZ induced convulsion model, different doses of AEBH i.e. 100, 200 and 400mg/kg were administered daily once for seven days, it was found that medium and high dose but not lower dose had significantly delayed the onset of clonus i.e.(56.0±4.32, 107.0±5.19 & 111.12±5.47) as compared to control group(48.0±4.35).Standard drug diazepam 5mg/kg) had exhibited a significant anti-convulsant activity and offered 100% protection.(Table 2)

In strychnine induced convulsion model different doses of AEBH i.e. 100, 200 and 400mg/kg were administered daily once for seven days, it was found that medium and high doses but not lower dose had produced a significant anti-convulsant effect by delayed the onset of tonus i.e. 167.75±7.48, 232.0±13.57 & 253.16±16.50 respectively as compared to control group (127.12±6.52). Standard drug diazepam 5mg/kg) does not show any onset of tonus (Table 3).

DISCUSSION

Generally, the data presented in this study suggest that *B.hispida* may contain psychoactive substance(s) with potential anticonvulsant properties. Protection against HLTE in the MEST by the extract predicts anticonvulsant activity of extract. The seizures generated in this model are consistent with the human grand mal epilepsy (Swinyard *et al.,* 1989).[24] AEDs that are effective in the treatment of generalized tonic clonic and partial seizures such as, phenytoin, carbamazepine, oxcarbazepine and lamotrigine suppress HLTE in MEST [25,26] Protection against HLTE also indicates the ability of the extract to inhibit or prevent seizure discharge within the brainstem seizure substrate[25] suggesting that the extract of *B.hispida* may be useful for the treatment of generalized tonic-clonic and partial seizures. The mechanism by which PTZ exert its convulsant action is by acting as an antagonist at the GABAA receptor complex.[27] Drugs offer protections against tonic–clonic seizures induced by PTZ that can raise the seizure threshold in the brain are considered to be useful to control myoclonic and absence seizures in humans[28,29] AEBH delayed the onset of clonus induced by PTZ, AEBH might considered to be useful to control myoclonic and absence seizures Strychnine is another convulsion producing drug used for testing anticonvulsant activity. Glycine is an inhibitory neurotransmitter in the CNS and strychnine is a competitive antagonist of the glycine receptor[30]. Strychnine produces convulsion by antagonizing the inhibitory spinal cord and brainstem reflexes of glycine[31] and AEBH should delay the seizure produced by strychnine. So anticonvulsant effect produced by AEBH might be through suppression of the action of strychnine on glycine inhibitory mechanisms.

It may therefore be concluded based on the data presented, that the use of *B.Hispida* in traditional medicine for the treatment of epilepsy. The plant extract may be valuable in convulsive disorder especially grand mal epilepsy. Further research is going on in our laboratory to isolate the bioactive components responsible for the observed pharmacological activities.

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Table No: 1. Anti-convulsant effect of Alcoholic extract of *B.hispida* in mice with MES induced convulsions model

S.No.	Treatment	Latency (onset of clonus) (Sec/30min) mean±SEM	Duration of tonic extensor (Sec/30min) mean±SEM	Duration of tonic flexion (Sec/30min) mean±SEM	% Protection (24h)
I	Control (3%Tween-80 p.o.)	3.75±0.36	14.0±0.50	NO	12.5
II	Standard. (Phenytoin, 25mg/kg p.o.)	11.12*** ±0.39	NO	5.37±0.46	100
III	AEBH (100mg/kg p.o)	5.0^{ns} ±0.46	12.37^{ns}±0.65	NO	25
IV	AEBH (200mg/kg p.o)	9.62*** ±0.37	7.85***±0.26	NO	62.5
V	AEBH (400mg/kg p.o)	9.0*** ±0.46	8.66***±0.42	NO	75

n=6 Significance at P<0.05*, <0.01**, <0.001*** and ns-not significant

Table No: 2. Anti-convulsant effect of Alcoholic extract of *B.hispida* in mice with PTZ induced convulsions model

S.No	Treatment	Latency (Onset of clonus) (Sec/30min) mean±SEM	Onset of tonic (Sec/30min) mean±SEM	Status of animal (30min) (No. of Animals Alive)	Status of animal (24 hrs) (No. of animals Alive)	% Protection (24h)
I	Control (3%Tween-80 p.o.)	48.0±4.35	223.87±14.65	0	0	0
II	Standard Diazepam5mg/kgp.o.)	NO	NO	ALL	ALL	100
III	AEBH (100mg/kg p.o)	56.0 ^{ns}±4.32	283.87^{ns}±9.98	2	2	25
IV	AEBH (200mg/kg p.o)	107.0***±5.19	613.5***±29.88	6	5	62.5
V	AEBH (400mg/kg p.o)	111.12***±5.47	620.12***±33.64	6	6	75

n=6 Significance at P<0.05*, <0.01**, <0.001*** and ns-not significant

Table No: 3. Anti-convulsant effect of Alcoholic extract of *B.hispida* in mice with Strychnine induced convulsions model

S.No.	Treatment	Onset of tonus (Sec/30min) mean±SEM	Status of animal (after 24 hours)	% Protection (24h)
I	Control (3%Tween-80 p.o.)	127.12±6.52	0	0
II	Standard (Diazepam5mg/kgp.o.)	NO	ALL	100
III	AEBH (100mg/kg p.o)	167.75 ^{ns}±7.48	1	12.5
IV	AEBH (200mg/kg p.o)	232.0 ***±13.57	5	62.5
V	AEBH (400mg/kg p.o)	253.16 ***±16.50	6	75

n=6 Significance at P<0.05*, <0.01**, <0.001*** and ns-not significant

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