

Anticonvulsive Effect of Seed Extract of *Caesalpinia bonducella* (Roxb.)

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Received May 6, 2009; Revised September 29, 2009; Accepted June 5, 2009

This paper is available online at <http://ijpt.iuums.ac.ir>

ABSTRACT

In traditional system of Indian medicine, *C. bonducella* is widely used for its antipyretic, antiperiodic, anticonvulsive, and antiparalytic activities. For assessing anticonvulsant activity, pentylenetetrazole, maximal electro shock, strychnine- and picrotoxin-induced convulsions models were used. Diazepam was used as a standard reference for all models except maximal electro shock model, wherein phenytoin was used as standard reference. Seed kernels of *C. bonducella* were powdered and subjected to successive extraction with solvents like petroleum ether (PE), ethanol, methanol and water using soxhlet apparatus. All the extracts were administered as suspension in 2% gum acacia in all the experiments. Preliminary phytochemical investigation of petroleum ether extract of caesalpinia bonducella revealed the presence of saponins, glycoside, starch, sucrose, proteins, sterols and reported constituents like homoisoflavone (bonducillin) and a non alkaloid bitter principle (natin). It was found to be non-toxic even up to the dose level of 3000mg/kg (LD50). In pentylenetetrazole, maximal electro shock, strychnine- and picrotoxin-induced convulsion models medium and high doses (600 and 800mg/kg) of the extract showed significant anti-convulsant activity. The present investigation revealed that the PECB possessed anticonvulsant activity which may be contributed to the presence of phytoconstituents such as saponins, proteins, homoisoflavone (bonducillin), carbohydrates and sterols present in the drug, as these are already reported for their anxiolytic and anti-convulsant activities.

Keywords: *C. bonducella*, Seed kernel, Petroleum ether extract, Anti-convulsant activity

Epilepsy is a chronic non-communicable disorder of the brain. It occurs all over the world and in several places over 4% of population is affected. About 40-70 new cases are recorded among every 100,000 persons every year [1]. As reported by WHO, epilepsy is the commonest serious disorder of the brain. An epileptic fit or seizure is caused by brief, excessive and abnormal discharge of nerve cells in the brain, like a small "electrical storm" or "short circuiting" in the brain.

The symptoms of epilepsy are the seizures that occur at unpredictable moments and may vary from frequent brief lapses of consciousness to short periods of automatic subconscious behavior or convulsions of the whole body that make the person fall over and lose consciousness completely. There is sufficient evidence available to show that 70 – 80% of people with epilepsy could lead normal lives if properly treated. Unfortunately, there is also evidence that in many countries more than 50% of people with epilepsy (and in certain areas up to 90%) are not properly treated. Part

of this treatment gap is attributable to insufficient recognition that the symptoms of which these people complain are caused by epilepsy [2].

Epilepsy in the Indian context becomes inevitable as India is the largest populated country next to china. Apart from this, treatment of this disorder under the advanced medication system is not extended as it was expected. The treatment under traditional system was proved to be far more applied. This fact forces the researchers to study the effects of traditional medicines used as the anti-epilepsy to clarify the effects and side effects of these materials.

In traditional system of indian medicine, Ayurveda, *caesalpinia bonducella* (roxb.) is widely used for its antipyretic, antiperiodic, anthelmintic, anti-inflammatory, antimalarial and also for various ailments like skin diseases, leprosy, hydrocele, orchitis, convulsions, paralysis and similar nervous complaints [3, 4]. It is also reported to have Antibacterial and antioxidant, Antidiabetic and Antitumor activities [5-7].

In this work, we have studied the Anticonvulsive effects of *Caesalpinia bonducella* (Roxb.) seeds extract in mice.

MATERIALS AND METHODS

Animals

Swiss albino mice of either sex weighing between 20-30g were procured from Shri Venkateswara Enterprises, Bangalore for experimental purpose. All the animals were acclimatized for seven days under standard husbandry conditions i.e.; room temperature of $24 \pm 10^\circ\text{C}$; relative humidity 45-55% and a 12:12h light/dark cycle. The animals had free access to standard rat pellet diet (Amrut laboratories, Pranava Agro Industries Ltd., Sangli, India), with water provided *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy Raichur (Karnataka) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with registration number 557/02/c/CPCSEA.

Preparation of seed extracts of *C. bonducella*

Seed powder of *C. bonducella* was extracted with petroleum ether by soxhalation and filtered. The filtrate is concentrated by distilling off the solvent and evaporated to dryness on the water bath [4]. We name this extract as petroleum ether extract of *C. bonducella* (PECB).

Drugs

Diazepam [Ranbaxy Laboratories Ltd, Mumbai, India], Phenytoin [Sun Pharmaceutical India.Ltd, Gujarat, India], Pentylenetetrazole, Strychnine, Picrotoxin [Sigma-Aldrich, St.Louis, USA], Tween-80 [S.D.fine Chem Ltd. Mumbai], Petroleum Ether [The Ugar Sugar Works Ltd. Ugar Khurd, Belgaum] and Distilled water [Mysore Petro Chemicals, Raichur, India].

Preliminary phytochemical screening

The preliminary phytochemical investigations will be carried out with the seed extract of *C. bonducella* (PE) for qualitative identification of phytoconstituents.

Experimental design

Acute toxicity test of *C. bonducella*

The acute toxicity of *C. bonducella* was determined by using albino mice of either sex (20-25 g), maintained under standard conditions. The animals were fasted for 3 hr prior to the experiment. Animals were observed for its mortality up to 48 hr study period following the

administration as per OECD guidelines No 425. From the LD₅₀ dose 1/20, 1/10 and 1/5th doses were selected and considered as low, medium and high doses respectively [8].

Grouping of animals and treatment schedule

Male albino mice (22-25g) and wistar rats (150-200g) were divided into following groups each consisting of six animals.

Group A- Normal control (PTZ 80 mg/kg, s.c), Group B- Standard (Diazepam 5mg/kg p.o), Group C- PECB 400mg/kg p.o, Group D- PECB 600 mg/kg p.o), Group E- PECB 800 mg/kg p.o.

All the drugs were administered to the respective groups in all the models for a period of eight days and experiments were performed one hour after the administration of last dose.

PTZ (Pentylenetetrazole)-induced convulsions

One hour after last dose of PECB in respective groups, PTZ 80 mg/kg is administered i.p. Then animals were observed individually in the plastic cage for 30min and later up to 24 hrs. The following parameters were recorded during this period, latency (onset of clonus), onset of tonic-clonic convulsions, status of animal after 30 minutes, status of animal after 24 hrs, and percent protection [9-11].

Maximal electro shock (MES)-induced convulsions

One hr after last dose of PECB in respective groups, MES seizures was induced by electroconvulsimeter (Techno instruments, Lucknow). A 60 mA current was delivered transauricularly for 0.2 sec in mice via small alligator clips attached to each pinna. This current intensity elicited complete tonic extension of the hind limbs in control mice. The following parameters were recorded during 30min test session, Tonic flexion, Tonic extension, Clonus convulsions, Percent protection [12].

Strychnine-Induced Convulsions

One hr after last dose of PECB in respective groups, each animal was placed into individual plastic cage and was observed initially for 30min and later up to 24 hrs. The following parameters were recorded during these sessions, Latency (onset of tonic), Status of animal after 30 minutes, Status of animal after 24 hr, Percent protection [12].

Picrotoxin-induced convulsions

One hr after last dose of PECB in respective groups, all the mice were given 10mg/kg Picrotoxin ip. Each animal was placed into individual plastic cage and following parameters were recorded during test session of initial 30min and up to 24 hrs, Latency (onset of tonic-extensor convulsion), Status of animal after 60 minutes, Status of animal after 24 hr, Percent protection [13, 14].

Statistical analysis

The values were expressed as mean \pm SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dennett's- *t* -

Table 1. Effect of PECB on PTZ-induced convulsions in mice

Treatment	Latency (onset of seizures) (sec/30min)	Duration of tonic clonic (sec/30min)	Status of animal (30min) (no. of animals alive)	Status of animal (24 hrs) (no. of animals alive)	% protection (24h)
Control (2% gum acacia)	47.0±2.113	45.167±9.178	0	0	0
Diazepam (5mg/kg)	NO	NO	ALL	ALL	100
PECB (400mg/kg)	119.17 ns±14.502	22.333 ns±5.004	2	2	33.33
PECB (600mg/kg)	194.83 **±25.579	18.833 **±1.327	6	5	66.67
PECB (800mg/kg)	210 **±60.074	14.167 **±3.027	6	6	100

n = 6, Significant at $p < 0.05^*$ and $< 0.01^{**}$

Table 2. Effect of PECB on MES-induced convulsions in mice

Treatment	Latency onset of clonus (Sec/30min)	Duration of tonic extensor (Sec/30min)	Duration of tonic flexion (Sec/30min)	% Protection (24h)
Control (2% gum acacia)	NO	16.34±1.904	NO	33.33
Standard Phenytoin (25mg/kg)	22.905 **±3.486	NO	NO	100
PECB (400mg/kg)	8.36 ^{ns} ±0.7211	23.54 ⁿ ±1.0228	NO	33.33
PECB (600mg/kg)	19 ^{**} ±0.6831	25.83 ^{**} ±1.548	NO	62.5
PECB (800mg/kg)	20.908 ^{**} ±0.8747	29.22 ^{**} ±1.060	NO	66.67

n = 6, Significant at $p < 0.05^*$ and $< 0.01^{**}$

test test to calculate the significance difference if any among the groups. $p < 0.05$ was considered as significant.

RESULTS

Effect of PECB on PTZ-induced convulsions

As seen in Table 1, it was observed that lower dose (400mg/kg) of PECB had not offered any significant anti-convulsive effect as compared to control. However, medium and higher doses (600 & 800mg/kg) exhibited a significant anti-convulsive effect as evidenced from increased onset time of seizures and decreased duration of tonic-clonic seizures. All three doses offered a protective effect of 33.33%, 100% and 100% up to 30-minute interval and at 24 hour, it was 33.33%, 83.33% and 83.33% respectively. Standard drug diazepam (5mg/kg) had exhibited a significant anti-convulsive activity and offered 100% protection.

Effect of PECB on MES-induced convulsions

PECB at 600 and 800mg/kg had delayed the onset of clonus convulsions and decreased the duration of tonic extensor phase and offered a dose-dependent protection and exhibited significant anti-convulsant activity (Table 2). Standard drug phenytoin (25mg/kg) had exhibited significant anti-convulsive effect and abolished the tonic extensor phase and offered 100% protection.

Effect of PECB on Strychnine-induced convulsions

The data has been shown in Table 3. Medium and high doses of PECB had increased the onset of tonic convulsions and also increased the percentage protection 66.66% and 83.33% respectively. Lower dose had not exhibited any significant effect and percentage protection was only 16.66%. Standard drug diazepam (5mg/kg) had exhibited significant anti-

convulsive effect by abolishing the tonic convulsions and offered 100% protection.

Effect of PECB on picrotoxin-induced convulsions in mice

As evident in Table 4, medium and high doses (600 and 800mg/kg) of PECB but not lower dose (400mg/kg) had delayed the onset of convulsions and onset of tonic extensor convulsion and offered a higher percentage protection survival of the animals i.e., 66.66%, 83.33%, and 33.33% respectively, when compared to control group and exhibited significant anti-convulsant activity. Standard drug diazepam (5mg/kg) had exhibited significant anti-convulsive effect and delayed the onset of convulsions and onset of tonic extensor convulsion and offered 100% protection.

DISCUSSION

There are a number of synthetic anticonvulsant drugs currently available for use in the management and treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. There is, therefore, a dire need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources.

In folk core medicine, *C. bonducella* seed oil is used in the treatment of convulsions and paralysis [15]. Based on the above data, the petroleum ether extract was prepared with seed kernels and studied for its anticonvulsant effect in different experimental animal models. In most common forms of epileptic seizures, effective drugs appear to work either by promoting the inactivated state of voltage-activated Na^+ channels or enhance GABA-mediated synaptic inhibition [16].

Table 3. Effect of PECB on strychnine-induced convulsions in mice

Treatment groups	Onset of tonic convulsions	Status of animal within 1 hour (No. of animals Alive)	% Protection
Control (2% gum acacia)	4.062±0.1738	0	0
Standard (Diazepam 5mg/kg)	NO	ALL	100
PECB (400mg/kg)	3.838 ^{ns} ±0.1923	1	16.66
PECB (600mg/kg)	4.738 ^{**} ±0.1733	4	66.66
PECB (800mg/kg)	5.338 ^{**} ±0.06750	5	83.33

n = 6, Significant at P<0.05* and <0.01**

Table 4. Effect PECB on picrotoxin-induced convulsions in mice

Treatment	Latency onset of convulsion (Sec/50min)	Latency onset of tonic extensor (Sec/50min)	Status of animal after 24 hrs (No. of animals Alive)	% Protection at 24h
Control (2% gum acacia)	283.2±0.2973	301.80±0.2551	2	33.33
Standard Diazepam (5mg/kg)	820.80 ^{**} ±0.7845	1045.80±0.2692	ALL	100
PECB (400mg/kg)	294 ^{ns} ±0.5387	348.60 ^{ns} ±0.5268	2	33.33
PECB (600mg/kg)	304.80 ^{ns} ±0.2587	495 ^{**} ±0.3962	4	66.67
PECB (800mg/kg)	458.40 ^{**} ±0.3864	562.80 ^{**} ±0.5943	5	83.33

n = 6, Significant at p<0.05* and <0.01**

Prevention of PTZ-induced seizures in laboratory animals is the most commonly used preliminary screening test for characterizing potential anti-convulsive drugs. The test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures as PTZ produces clonic and tonic convulsions. It has been demonstrated that, a neural pathway subserving clonic PTZ convulsions is located

in the forebrain while the brain stem is involved in the network of tonic PTZ induced convulsions. The antiepileptic drug should abolish or increase the threshold for clonic and tonic convulsions. The mechanism by which PTZ exert its convulsive action is by acting as an antagonist at the GABAA receptor complex. Drugs offer protections against tonic-clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans [17, 18].

PECB at medium and high doses (600 and 800mg/kg) but not lower dose had significantly increased the threshold for clonic and tonic convulsions and the percentage protection against convulsions were 62.5%, 75% and 0% respectively as compared to control group. Standard drug diazepam (5mg/kg) had abolished the clonic and tonic seizures with intraperitoneal injection of PTZ and offered 100% protection. Diazepam acts through the activation of GABAA receptors and facilitate the GABA mediated opening of chloride channels. A dose depended activity has seen i.e., increase in the latency (onset) of convulsion as well as decrease in duration with tonic-clonic seizure threshold [19, 20]. PTZ induced seizures suggest that, the extract of PECB might have affecting GABA-ergic neurotransmission as PTZ has been shown to interact with the GABA Neurotransmitter.

MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that

produces generalized tonic-clonic seizures. i.e. hind limb tonic extensor, tonic flexion and clonic convulsions. In untreated animals a single MES produced an immediate tonic hind limb extension for 5-10 sec duration followed by clonic seizures. Previous studies have reported that immediate to MES transmitters in vivo increase were over the 20-30 min post ictal period [12]. It has often been stated that antiepileptic drugs that block MES induced tonic extension act by blocking seizure spread, moreover MES induced tonic extension can be prevented either by drugs that inhibit voltage dependant Na⁺ channels (phenytoin, valproate) or by drugs that block glutamergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor (felbamate) [21, 22].

In strychnine-induced convulsion model, PECB at medium and high doses (600 and 800mg/kg) but not lower dose (400mg/kg) had significantly increased the duration of tonic extensor phase and onset of clonus as compared to control and thus exhibited anticonvulsant effect and the percent protection was 62.5%, 62.5% and 25% respectively. Standard drug (phenytoin 25mg/kg) had abolished the tonic extensor phase and showed 100% anticonvulsant effect by preventing seizure spread. The percentage protection (Anticonvulsant effect) was found to be increased dose dependent. Glycine is an inhibitory neurotransmitter in the CNS and strychnine is a competitive antagonist of the glycine receptor which produces convulsion by antagonizing the inhibitory spinal cord and brainstem reflexes of glycine [23, 24].

In picrotoxin-induced convulsion model, PECB at medium and high doses but not lower dose had significantly delayed the onset of tonic convulsions and exhibited anticonvulsant effect and offered 66.66%, 83.33% and 16.66% protection respectively. The anticonvulsant effect (increased percent protection) was

found to be dose dependent. So the anticonvulsant effect produced by PECB might be through suppression of the action of strychnine on glycine inhibitory mechanisms. Picrotoxin, a GABA-receptor antagonist, produces seizures by blocking the chloride ion channel linked to GABA_A-receptors, thus preventing the entry of chloride ions into the brain. This process will in turn inhibit GABA neurotransmission and activity in the brain. So anticonvulsant effect produced by PECB might be through blocking of the chloride ion channel linked to GABA_A-receptors.

CONCLUSION

The results obtained from these experimental models clearly confirmed the anticonvulsant activity of PECB in mice. Phytoconstituents like flavonoids and saponins were FOUND in petroleum ether extract which are also reported for their anti-convulsive effect. So these active components might be responsible for anticonvulsant effect of PECB.

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