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ANTICONVULSANT ACTIVITY OF THE CRUDE FLAVONOID FRACTION OF *PITHECELLOBIUM DULCE* LEAF

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ABSTRACT

Pithecellobium dulce is a species of flowering plant in the pea family, Fabaceae that is native to Mexico, Central America and South America. The leaves have been used to treat ear ache, leprosy, peptic ulcer, tooth ache and convulsions. The Preliminary Phytochemical Screening revealed the presence of Flavonoids, Glycosides, Tannins, Saponins. The present investigation was intended to evaluate the Anticonvulsant activity of the crude flavonoid fraction of the leaf of *Pithecellobium dulce* (CFFPD) using the subcutaneous Pentylenetetrazole (PTZ) and Maximal Electroshock Test (MES) models in rats, respectively at the doses of 250 and 500 mg/ kg body weight. The crude flavonoid fraction exhibited significant reduction in the duration of hindleg extension and onset of convulsion at a dose of 500 mg/ kg body weight i.p. (comparable to Phenytoin at 20 mg kg^{-1}) in both Maximal Electroshock Test (MES) and Pentylenetetrazole (PTZ) model.

KEYWORDS

Pithecellobium dulce, Flavonoid fraction, Anticonvulsant

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INTRODUCTION

Epilepsy is characterized by recurrence of seizures associated with loss or disturbance of consciousness, usually but not always with characteristic body movements (convulsion) and always correlated with abnormal and excessive EEG discharge. An imbalance between the excitatory and inhibitory neurotransmitters is responsible for seizures. At neuronal level, seizure activity often occurs when glutaminergic excitatory neurotransmitters over rides gamma-aminobutyric acid (GABA) mediated inhibition[1].

Epilepsy has now become the most serious brain disorder and affects about 40 million people, which accounts for about 1% of the world's burden of diseases. The incidence rate for primary generalized tonic-clonic and absence seizures are highest in infants and children[2].

Pithecellobium dulce Benth. (Leguminosae) is a small to medium sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans[3]. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and is useful in dermatitis and eye inflammation. Roots have been reported to possess estrogenic activity[4]. The leaves have been reported to be a folk remedy for ear ache, leprosy, peptic ulcer, tooth ache, and venereal disease. It also acts as astringent, emollient, abortifacient, antidiabetic, anodyne and larvicidic in folk medicines. Leaves can be used as a plaster to allay pain even from veneral sores and can relieve convulsions[5]. So, the present study has been undertaken to evaluate the anticonvulsant activity of crude flavonoid fraction of *Pithecellobium dulce* leaf.

MATERIALS AND METHODS

Plant Material

The leaves of *Pithecellobium dulce* were collected in the month of January, from local area of Nellore, Andhrapradesh, India and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tamilnadu, India. A voucher specimen was deposited in the herbarium for future reference.

Extraction

The leaf of *Pithecellobium dulce* Benth were dried and coarsely powdered. About 300 gms of powder were extracted with Ethanol by cold maceration method. After the maceration process, the supernatant was decanted. The residual marc was strained with a clean and dry net sieve with fine mesh to free more supernatant. This was added to the already decanted supernatant and filtered. The filtrate thus obtained was concentrated to dryness on water bath and stored in a vaccum desiccator.

Fractionation of the Plant Extract

The dried Ethanol Extract of *Pithecellobium dulce* (EEPD) (12 gm) was dissolved in hot water and filtered. Then the following method[6] was adopted to partition the flavonoid portion from the filtrate. The crude flavonoid fraction was then evaporated to dryness on water bath to obtain 1.40g mass with percentage yield of 11.2%.

Preliminary Phytochemical Screening

The ethanol extract of *Pithecellobium dulce* was evaluated for the presence of Flavonoids, Glycosides, Tannins, Saponins in Table No.1.

Test for Flavonoids on the Crude Fraction

A pinch of fraction was dissolved in ethanol, mixed thoroughly and filtered. To the filtrate, magnesium metal pieces and concentrated hydrochloric acid were added and heated gently. Appearance of magenta colour indicates the presence of flavonoids[7].

Animals

Wistar rats (180 – 200 gm) and male Albino mice of either sex obtained from the laboratory animal's centre were used for various studies. They were kept in a well ventilated environment, had free access to food and water ad libitum and kept in the laboratory environment (12 h dark/ 12 h light cycles) for seven days for acclimatisation. Animals were fasted overnight and weighed before the experiment.

Acute Toxicity Study

Acute toxicity up and down procedure was carried out as per guidelines by Organisation for Economic co-operation and Development (OECD) 423[8].

Six groups (n = 6) of male albino mice were used to study the acute toxicity of extract administration. Animals were fasted overnight and administered with ethanol extract and crude flavonoid fraction (50, 500, 2000 mg/ kg). A group of animals which received equal volume of saline (10 mL/ kg) served as control. Mortality was assessed 24 hr after administration. Treated animals were further observed for up to 14 days for signs of toxicity.

ANTICONVULSANT ACTIVITY

Maximal Electro Shock induced Convulsions

Albino rats of 150-200 g body weight were divided into six groups of six animals each. Group 1 received

1% w/v SCMC, (1ml/100 g) as negative control. Group 2 received Phenytoin (25mg/kg) as standard. Group 3 & 4 were respectively received 250 & 500 mg/kg, p.o of *Pithecellobium dulce* ethanolic extract (EEPD). Group 5 & 6 were respectively received 250 & 500 mg/ kg , p.o of crude flavonoid fraction of *Pithecellobium dulce* (CFFPD). MES seizures were induced by an electroconvulsometer. A 60 mA current was delivered transauricularly for 0.2 sec in mice using corneal electrodes. This current intensity elicited tonic hindlimb extension (THE) in control mice. For recording various parameters, rats was placed in a clear rectangular plastic cage with an open top, permitting full view of the animal's motor responses to seizure. The various phases of convulsions, viz. tonic flexion, extension, clonus, stupor and mortality due to convulsions were timed. To evaluate the drug effect on the seizures severity, the duration of THE and mortality due to convulsions were selected as the parameters. Each animal was individually observed for 2 hr after MES seizures and at 24 hr for mortality. A compound is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES[9].

Pentylenetetrazole Induced Convulsions

Albino rats of 150-200 g body weight were divided into six groups of six animals each. Group 1 received 1% w/v SCMC, (1ml/100 g) as negative control. Group 2 received Diazepam (4mg/kg) as standard. Group 3 & 4 were respectively received 250 & 500 mg/ kg, p.o of *Pithecellobium dulce* ethanolic extract. Group 5 & 6 were respectively received 250 & 500 mg/ kg , p.o of crude flavonoid fraction of *Pithecellobium dulce*. Pentylenetetrazole is a central nervous system stimulant. The PTZ-induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in the treatment of absence seizures suppress PTZ- induced seizures, which is the most commonly used initial screening test for recognizing anticonvulsant drugs or traditional herbs. The animals in each group were pretreated with a definite concentration of the extract and fraction orally. After 30 minutes, PTZ was administered at the dose of 80 mg/kg subcutaneously. After the injection of PTZ, the convulsive behavior was observed for 30 min. The parameters measured were: a) onset of myoclonic jerk i.e. latency b) clonus c) tonic flexion and d) mortality. The efficacy of the test drug to protect the animals against lethal seizures was measured in terms of extended latency period and decreased mortality rate of each group and compared with the respective control group[10].

Statistical Analysis

The duration of THE phase of MES convulsions and seizure latency of PTZ induced seizures expressed as the arithmetic mean ± SE and was analysed by one-way analysis of variance (ANOVA) followed by Dunnet's 't' test. *P* value less than 0.05 ($P<0.05$) was the critical criterion for statistical significance.

RESULTS

Acute Toxicity study

Acute oral toxicity was carried out by up-down regulation method. It is found that EEPD and CFFPD were safe at limit dose 2000 mg/kg with no mortality in studied subjects. Hence, $1/4^{th}$ (500mg/kg) $1/8^{th}$ (250mg/kg) of these oral doses were used in the subsequent study respectively in Table No.2.

Anticonvulsant Activity

Among the many methods used for screening and evaluation of anti convulsant drugs, the most commonly employed models are MES and PTZ-induced seizure method.

Effect on MES Induced Epilepsy

In MES induced epilepsy, negative control group (vehicle treated) showed the duration of tonic hindleg extension which was 16±0.3651 seconds. The CFFPD at dose of 250 mg/kg and 500mg/kg significantly ($p<0.01$) reduced the duration of tonic hindleg extension to 4.67±0.2108 and 2.17±0.1667 seconds, respectively as compared to control. The rate of mortality protection was significant for standard, CFFPD (500 mg/kg) as compared to control in Table No.3.

Effect on Pentylenetetrazol Induced Convulsions

In drug induced convulsion model, the seizure latency was prolonged by all the test groups as compared to control. The CFFPD at dose of 500

mg/kg significantly delayed the onset of clonic convulsions for 623.83±3.458 (p<0.01) seconds, respectively. 100% protection was achieved with CFFPD at a dose level of 500 mg/kg. Mortality of EEPD and CFFPD at dose of 250 mg/kg and 500 mg/kg treated rats is reduced as compared to negative control in Table No.4.

The protection was more significant against PTZ induced convulsions compared to MES.

DISCUSSION

Phytochemical study

Preliminary phytochemical screening was carried out to identify the chemical constituents present in Ethanol extract. It showed the presence of constituents such as Flavonoids, Tannins and Saponins.

MES induced Convulsions

GABA plays a critical role in the etiopathology of epilepsy. GABA ergic mechanism has been implicated in protection from variety chemo- and electroshock-induced seizures. Benzodiazepine (BZD) agonists, like diazepam are positive allosteric modulators of GABA-mediated neurotransmission in central nervous system[11].

The Crude flavonoid fraction when compared with the Ethanol extract had given potent effect and when compared with the control it decreased by nearly half the extension time. This indicates its ability to prevent the spread of seizure in the central nervous system.

The MES model has served to identify antiepileptic drugs that are functionally similar to phenytoin and most of these compounds display, the same ability to inactivate voltage dependent Na^+ channels in a use dependent fashion. Such compounds have been shown to suppress sustained repetitive firing in cultured neurons[12].

Thus, *Pithecellobium dulce* is expected to have a similar type of mechanism, and may be effective against partial and secondary generalized seizures.

PTZ induced Convulsions

The CFFPD has been found to be effective against PTZ-induced convulsions. PTZ has been classified as a central benzodiazepine receptor antagonist. The probable protection in this model is mediated either by: 1) Suppression of Ca^{2+} conductance across low threshold T- type Channels or 2) Enhancement of $GABA_A$ receptor mediated Cl^- conductance[12]. The protection offered by the EEPD and CFFPD could be described by the synergistic effect of its principle constituents such as tannins, steroids, terpenoids and flavonoids. Further the fractionated compound also showed significant anticonvulsant activity at higher dose comparable with the total extract.

This model reveals that *Pithecellobium dulce* may be clinically effective against generalized-spike-wave absence epilepsies.

So the effect of fraction against MES and PTZ-induced convulsions may be due to the presence of flavonoid.

Table No.1: Preliminary Phytochemical Screening

Chemical Test	Ethanol Extract
Alkaloids	-
Carbohydrates	-
Glycosides	+
Tannins	+
Proteins & Amino acids Phenolic	-

Terpenoids	-
Flavonoids	+
Anthocyanin	-
Gums & Mucilage	+
Steroids	-
Saponins	+

(+) = Present (-) = Absent

Table No.2: Acute Oral Toxicity Studies of *Pithecellobium dulce* extract and fraction

S. No	Groups	Dose/kg b.w	Weight of animals		Signs of Toxicity	Onset of Toxicity	Duration of Study
			Before Test	After Test			
1.	EEPD	50 mg	175 g	180 g	No signs of Toxicity	Nil	14 days
2.	EEPD	500 mg	190 g	200 g	No signs of Toxicity	Nil	14 days
3.	EEPD	2000 mg	185 g	205 g	No signs of Toxicity	Nil	14 days
4.	CFFPD	50 mg	180 g	200 g	No signs of Toxicity	Nil	14 days
5.	CFFPD	500 mg	165 g	185 g	No signs of Toxicity	Nil	14 days
6.	CFFPD	2000 mg	190 g	210 g	No signs of Toxicity	Nil	14 days

Table No.3: Effect of EEPD and CFFPD on MES induced epilepsy

Group	Design of treatment	Stages of convulsion (sec)					% protection
		Flexion	Extensor	Clonus	Stupor	Recovery	
I	Vehicle control	8.33±0.2108	16±0.3651	19.17±0.3073	39.17±0.7032	187.67±2.333	-
II	Phenytoin 25mg/kg,i.p.	2.67±0.2108**	0.00±0.0000**	10±0.3651**	18.33±0.5578**	89.83±1.276**	100
III	EEPD 250mg/kg,p.o	6.33±0.3333**	8.17±0.3073**	15.5±0.4282**	35.67±0.6667	143.83±1.222**	48.93
IV	EEPD 500mg/kg,p.o	4.83±0.3073**	5.33±0.2108**	13.83±0.5426**	29.17±1.0140**	132.50±1.258**	66.68
V	CFFPD 250mg/kg,p.o	5.33±0.2108**	4.67±0.2108**	14.83±0.3073**	28±0.7303*	124.50±1.176**	70.81
VI	CFFPD 500mg/kg,p.o	4±0.2582**	2.17±0.1667**	12.17±0.3073**	23±0.3651**	110.83±2.286**	86.44

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

Table No.4: Effect of EEPD and CFFPD on PTZ induced convulsion

Group	Design of Treatment	Onset of convulsion(sec.)	Duration of convulsion (sec.)	Protection convulsion%	Protection mortality%
I	Vehicle control	173.83±3.114	80.17±1.276	0	33.33
II	Diazepam(4mg/kg,i.p)	725.33±4.551**	12.17±0.4773**	84.82	100
III	EEPD 250mg/kg,p.o	358.17±1.973**	41.83±0.9458**	47.82	50
IV	EEPD 500mg/kg,p.o	453.17±3.410**	32.17±0.7481**	59.87	83.33
V	CFFPD 250mg/kg,p.o	467±3.307**	29.67±0.7601**	62.99	94
VI	CFFPD 500mg/kg,p.o	623.83±3.458**	20.83±0.4773**	74.02	100

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test *p<0.05;** p<0.01

CONCLUSION

The anti convulsant activity of EEPD & CFFPD was evaluated by MES & PTZ method. Thus our results fortify that the Crude flavonoid fraction showed more potent activity than the extract and thus it reveals the ethano-pharmacological importance of CFFPD as an anti convulsant agent. It is concluded from this study that the drug possesses anticonvulsant activity in different neuro pharmacocological models.

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