Research Article

Evaluate the anti-Anxiety, anti-convulsant and CNS depressant activities of ethanolic extracts of leaves of *Pongamia pinnata*

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ABSTRACT

The aim of the present study was to evaluate the anti-anxiety, anti-convulsant and CNS depressant activities of the ethanolic extract of leaves of *Pongamia pinnata* (EELPP). Phytochemicals of the EELPP were screened by standard methods and revealed that the extract contains alkaloid, glycoside, protein and amino acid, gum and mucilage, flavones and flavonoids, saponins, and steroids. The acute toxicity study revealed that the EELPP did not show any signs of toxicity or mortality even at the dose level of 2000 mg/kg body weight. Elevated plus maze, and light and dark model methods in rat were used for study the anti-anxiety activity of the extract; actophotometer method in rat was used for study the CNS depressant activity; and maximal electroshock method in rat was used for study the anti-convulsant activities. In the present study test group (EELPP 400 gm/kg treated) animals has showed significant anti-anxiety, anti-convulsant and CNS depressant activities as that of standard group (diazepam treated) animals. The results of the study indicates the ethanolic extract of leaves of *Pongamiapinnata*is having significant anti-anxiety, anti-convulsant and CNS depressant activities and hence scientifically justifies the use in the folklore remedies.

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INTRODUCTION

A central nervous system depressant activity refers to physiological depression of the CNS, general or local anaesthesia, relaxation of skeletal muscles, or anticonvulsant activities. CNS depressants and anaesthetics acting on the central nervous system by increasing the activity of gamma-aminobutyric acid (GABA), although other targets such as the N-methyl D-aspartate (NMDA) receptor, μ opioid receptor and CB1 cannabinoid receptor also be important.[1] CNS depression most often results from the use of depressant drugs such as alcohol, opioids, barbiturate, benzodiazepines, general anaesthetics and anticonvulsants.[2]

Anxiety is an emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat. As per WHO guideline the definition of anxiety is “anxiety is an emotional characterized by feelings of tension, worried thoughts and physical changes like increased blood pressure”. Though anxiety is a part of normal life, but some psychotic and depressed patients also exhibit pathological anxiety.[1] Anxiety is a generalized mood condition that can often occur without an identifiable triggering stimulus.

There are a group of disorders of the CNS characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodic seizures associated with loss of consciousness with or without characteristic body movement. Episodes are unpredictable and frequency is highly variable. Epilepsy is one of the most common neurological disorders in human. Itis considered to be a chronic brain syndrome characterized by recurrent seizures and generally associated with loss or disturbance of consciousness.[2]

The *Pongamia pinnata*, commonly known as 'Karanj', has been recognized in various systems of traditional medicines for the treatment of different diseases. *P. pinnata* belongs to the family- fabaceae. It is a medium sized evergreen tree with a spreeding crown and a short bole.[3-7]It is indigenous to India and Myanmar and in India it is widely distributed in Rajasthan, Andhrapradesh, Karnataka, Tamilnadu and Odisha. *P. pinnata*plant is used in the traditional systems of medicines as anti-inflammatory, anti-plasmodial, anti-nociceptive, anti-hyperglycaemic, anti-lipid oxidative, anti-diarrhoeal, anti-ulcer, anti-hyper ammonic and antioxidant.[8-14]

Despite the fact that *P. pinnata* is well known to possess medicinal properties, it has not been scientifically studied. Hence this study was aimed to providing scientifical support for

medicinal use of the ethanolic extract of leaves of *P. pinnata*. The study hypothesized that the ethanolic extracts of leaf of *P. pinnata* have anxiolytic and anticonvulsive activity due to CNS depressant properties.

MATERIALS AND METHODS

Plant materials and extraction

The leaves of plant of *Pongamia pinnata* were collected from a local village of Berhampur in the month of September, 2019. The plant materials were authenticated at the department of Pharmacognosy, Royal College of Pharmacy and Health Sciences (R.C.P.H.S.), Berhampur. The leaves werecleaned, dried under shade and stored in an air tight container.

Animals

Young female albino mice weighing 22-28 gm were used for acute toxicity study, and wistar albino rats weighing 180-200 gm were used for pharmacological screening. All the animals were acquired from the animal house of R.C.P.H.S., Berhampur and housed in polypropylene cages. Animals were kept under controlled room temperature (22 ± 2°C) and humidity (55 ± 5°C) with a 12:12 hour light: dark cycle. The animals were fed with laboratory food diet made in-house and pure drinking water *ad libitum*.

The ethical clearance for the study was granted by Institutional Animal Ethics Committee of R.C.P.H.S., Berhampur. After necessary approval the experimental works were carried out during the month of October, 2019 to March, 2020. The animal experiments were conducted according to the ethical norms of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Extraction of plant materials

The dried coarse powders of leaves of *P. pinnata* were extracted by a Soxhlet apparatus. The powder (200gm) was placed in the percolator of Soxhlet apparatus and extracted successively with the each solvent for 72 hr. Petroleum ether (60-80°C) was used in initial step of extraction for defatting the leaves of *P. pinnata*. The marc was air dried and subjected to further extraction by using ethanol. After completion of extraction, the marc was pressed to collect the excess of extract present. The solvent extract was collected separately and dried using rotary vacuum evaporator and stored in desiccator. Yield of each extract of leaves of *P. pinnata* was calculated with respect to the dried plant material. Also the colour and consistency of the extract were tabulated.[15]

Phytochemical screening

Medicinal properties of a plant extracts depends upon the type of phyto-

constituents presents. The ethanolic extract of leaves of *Pongamia pinnata* (EELPP) were analysed for presence of various phytoconstituents by qualitative phytochemical analysis[16, 17].

Acute toxicity studies

As per the OECD guidelines the acute oral toxicity study of the EELPP was carried out. The young female albino mice were divided into six different groups of six animals each. The control group animals administered 5 ml/kg of distilled water orally. The other five test group animals received 100, 500, 1000, 1500, 2000 mg/kg of EELPP through oral route respectively. After oral administration of drugs the animals were observed continuously for the first 4 hr and then occasionally up to 24 hr and recording the mortality at the end of 72 hr, if any. Added observations like behavioural changes, somato motor activity, tremors, convulsions, tonic extension, strub tail, muscle spasm, loss of righting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhoea, salivation, writhing, changes in skin, fur, eyes, mucous membranes etc. were also recorded.[18, 19]

Evaluation of anti-anxiety anti-convulsant and CNS depressant activities[18-28]

Twenty four of wistar albino rats were divided in to four groups (n=6). Then drugs were administered to the different groups of animals through oral route for 5 days prior to study as per the following schedule given in table 1. Thirty minutes after the drug administration on fifth day, the animals were brought for the pharmacological study.

Table 1: Schedule of drug administration to different groupsof animals

GROUP	DRUG	DOSE (mg/kg)	NATURE
I	Distilled water	10 ml/kg	Control
II	Diazepam	2	Standard
III	EELPP-200	200	Test-1
IV	EELPP-400	400	Test-2

Elevated Plus Maze Test

Elevated plus maze originally is designed to evaluate the anti-anxiety agents. Thirty minutes later the drug administration the animals wereplaced on the centre of the maze for 5 minutes by head facing towards open arm. The acute anxiolytic actions of different drugs were noted by measuring number of entries in open and closed arm, and average time spent in each arm.

Light and Dark Model Test:

Light and dark model test is used to study the anti-anxiety activity. Following the elevated plus maze test, the each animal was placed in the light and darkapparatus for 5 minutes and parameters like number of entries into

light and dark chamber and average time spent in each chamber were noted.

Actophotometer Test

A drug increase or decrease CNS activity will also increase or decrease in spontaneous motor activity in the animals. Actophotometer is designed in this principle. The locomotor activity can be easily measured using an actophotometer which is mainly used for study the C.N.S depressant property of the drug.

Following the elevated plus maze test and light and dark method tests, the animal of each group was placed at the centre of the actophotometer for 5 minutes and study the locomotors activity.

Maximal electro shock seizure method

Maximum electric shock induced seizure on rodent is the one of the best method for testing the anti-convulsant properties. One hour later the drug administration on fifth day, following the elevated plus maze test, light and dark model test, and actophotometer test, each animal was applied the current of 150 mA for 0.2 seconds by the electrode of electro-convulsometer,. Then note the time spent by the animals in different phases of the convulsions i.e. a) tonic flexon, b) tonic extensor, c) clonic convulsions, d) stupor, e) recovery or death.

Statistical analysis

The group means were considered to be significantly different at 5% level of significance, $P < 0.05$ by using one-way ANOVA (nonparametric), followed by Bonferroni's multiple comparison test. The values were expressed as mean ± SEM.[29]

RESULTS

Percentage of yield (w/w) and colour of different extracts:

The extract of *Pongamiapinnata*was filtered and then it was concentrated by distilling off the solvent to obtain the crude extract. The extractive values, colours and consistency of the different extracts are tabulated below.

Table 2: Percentage of yield (w/w) and colour of different extracts

Sl. No.	Solvent	% yield (w/w)	Colour	Consistency
1	Pet-ether (60-80)	4.2%	Dark green	Greasy mass
2	Ethanol	9.7%	Brown	Dry powder

Phytochemical studies:

Preliminary qualitative phytochemical screening of EELPP showed the presence of alkaloid, phenolics, glycosides, saponins and steroid (table-3).

Table 3: Phytochemical Study of ethanol extract of leaves of *Pongamiapinnata*

SL. NO.	PHYTOCONSTITUENTS	EELPP
1	Alkaloid	+
2	Carbohydrate	_
3	Protein & Amino acid	+
4	Gum and Mucilage	+
5	Glycoside	+
6	Tannins	_
7	Triterpinoids	_
8	Flavones & Flavonoids	+
9	Saponins	+
10	Steroids & Sterols	+

Pharmacological Study:

Acute toxicity studies:

The results of acute oral toxicity study of EELPP are tabulated below.

Table 4: Acute toxicity studies of EELPP

Treatment	Dose (mg/kg)	No. of Mice	No. of Death	signs of toxicity	LD_{50}
Control (Distilled water)	10 ml/ kg	6	0	-	-
EELPP	100	6	0	-	> 2000 mg/kg
	500	6	0	-	
	1000	6	0	-	
	1500	6	0	-	
	2000	6	0	-	

The acute toxicity study revealed that the EELPP did not show any signs of toxicity or mortality even at the dose level of 2000 mg/kg body weight. As per the ranking system European Economic Community for acute oral toxicity, the LD_{50} dose of 2000 mg/kg and above is categorized as unclassified and low toxicity.[30]

Overall results, suggested the LD_{50} value is greater than 2000 mg/kg. Hence therapeutic dose was calculated as $1/5^{th}$ (400 mg/kg) and $1/10^{th}$ (200 mg/kg) of highest tolerable dose (2000 mg/kg) were used for further pharmacological studies.

Evaluation of CNS depressant, antianxiety and anticonvulsantactivity:

The results of different pharmacological screening process obtained by study the effect of EELPP are tabulated below.

1) Elevated Plus Maze Method:

Table 5: Anxiolytic effect of EELPP on rats by using elevated plus maze apparatus

Group	Treatment	Dose (mg/kg)	Time spend (in sec)		No of entries		Percentile ratio of open/total arm entries
			Open	Close	Open	Total	
Group-1	Distilled water	10ml/kg	25.64±0.702	212.9±0.874	1.62±0.54	6.25±0.056	25.92
Group-2	Diazepam	1	196.38±1.063*	68.92±1.321*	8.65±1.543*	13.66±0.14*	63.32*
Group-3	EELPP-200	200	102.56±2.543	126.66±0.245	3.4±0.874	8.95±1.012	37.98
Group-4	EELPP-400	400	#177.76±0.874*	#74.95±1.984*	#7.25±0.986.*	#12.36±0.54*	#58.65*

2) Light and Dark Model:

Table 6: Anxiolytic effect of EELPP on rats by using light and dark model

Group	Treatment	Dose (mg/kg)	No of entry in to light chamber	Time spent (s) in light chamber	Time spent (s) in dark chamber
Group-1	Distilled water	10ml/kg	2.32±0.356	21±0.947	279±1.056
Group-2	Diazepam	1	5.86±0.623*	204±1.784*	96±0.982*
Group-3	EELPP-200	200	2.27±0-942	53±0.836	247±0.687
Group-4	EELPP-400	400	#5.25±0.652*	#188±0.968*	#112±1.671*

3) Actophotometer method:

Table 7: CNS depressant effect of EELPP on rats by actophotometer apparatus

Group	Treatment	Dose (mg/kg)	Locomotor activity	% Inhibition
Group-1	Distilled water	10ml/kg	219.76±3.659	-
Group-2	Diazepam	0.5	74.48±0.953*	66.21
Group-3	EELPP -200	200	167.64±6.275	23.74
Group-4	EELPP-400	400	#92.33±8.395*	58.12

4) Maximal electroshock method:

Table8: Anticonvulsant effect of EELPP on rats by using MES method

Treatment Groups	Onset Time (in Seconds)				
	Tonic Flexion	Tonic extension	Clonic Convulsion	Stupor	Recovery/ Death
Distilled water	5.12	9.24	13.9	47.15	5/1
Diazepam	1.3*	-	-	-	6/0
EELPP-200	4.53	7.82	12.26	52.92	6/0
EELPP-400	2.18*	2.13*	4.68*	18.08*	6/0

All the above results were expressed as mean ± SEM, n=6, *P< 0.05; compared Standard and Test groups vs control group, '#'- Indicates there is no significant difference between standard and test drug at P< 0.05 significant level.

CONCLUSIONS

In the present study standard group (Diazepam treated) animals and test group(EELPP-400 treated) animals were showed increase in the time spent and rears in the open arms, but reduction in the time spent in closed arms as compared to control group animals in the elevated plus maze.In the bright and dark arena test, standard group (Diazepam treated) animals and test group (EELPP-400 treated) animals were demonstrated a marked increase in the time spent and rearing behaviour in the bright chamber as compared to control group animals. Behavioural changes in both these models suggest that ethanolic extract of leaves of *Pongamia pinnata* plants have significant anxiolytic effects. The ethanolic extracts of *Pongamia pinnata* also shows CNS depressant activity as it was significantly reduced the locomotor activity in actophotometer. This plant extracts was also produced significant anticonvulsant properties when studied in MES induced convulsion.

From the above findings it is suggested that ethanolic extract of leaves of *Pongamia pinnata* have significant anti-anxiety, anti-convulsant and CNS depressant activity effect at 400 mg/kg. Further investigation is expected to isolate and characterize the active principles study their anxiolytic and CNS depressant properties.

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