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Screening of anti-convulsant potential of *Plumeria pudica* leaf extract using animal models

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Abstract

The present investigation was undertaken with an objective to evaluate the antiepileptic activity of *Plumeria pudica* flower extract. The chloroform extract (CEPP) was found to contain most variety of phytochemicals and was evaluated for their anticonvulsant efficiency by MES and pilocarpine induced convulsions method using 200 mg/kg and 400 mg/kg doses. The CEPP (150 mg/Kg) exhibited a significant reduction in the duration of hind limb extension (10.8 ± 1.788) and was able to avert the occurrence of clonus in the animals in the MES method. At dose of 75 mg/Kg, CEPP was unable to significantly reduce the duration of convulsions. It could be concluded from the present investigation that the anticonvulsant effects of the chloroform extract of leaves of *Plumeria pudica* may be attributed to non-specific mechanisms.

Keywords: *Plumeria pudica*, antiepileptic, maximal electroshock, pilocarpine, phenolics, seizure

Introduction

Epilepsy is a chronic disorder of the brain that affects people worldwide and is characterized by brief episodes of seizures and excessive EEG discharge [1].Epilepsy is a major neurological disorder and upto 5% of the world population develops epilepsy in their lifetime. Approximately 50 million people currently live with epilepsy worldwide. An estimate shows that people suffering from epilepsy would be 50 million worldwide of which 80% belong to countries with low or middle income [2].

An estimated of 1% Indian population (more than 10 million people) might be suffering epileptic seizures in India. The occurrence is higher in the rural population (1.9%) compared to urban (0.6%) [3].

The current therapy of epilepsy with modern antiepileptic drugs (AEDs) is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy [4]. The discovery of novel antiepileptic drugs relies upon the preclinical employment of animal models to establish efficacy and safety prior to the introduction of the AEDs in human volunteers. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles [5].

Traditional systems of medicines are popular in developing countries and upto 80% of the population relies on traditional medicines/ folk remedies for their primary health care need.

From the literature it was evident that the *Plumeria* species especially *Plumeria acuminata* has being studied widely for its pharmacological potential [6-9]. It was also found that the antioxidant potential, the related anti inflammatory and antidiabetic property of the plant has also being scientifically explored by researchers. It was therefore envisioned to extracting the leaves of the *Plumeria pudica* in various solvent and studying the anticonvulsant potential of the extracts.

Materials and Methods

Pilocarpine nitrate solution and diazepam injection were purchased from local pharmacy store. All other reagents and chemicals used in the study were purchased from Oxford Fine Chemicals Pvt. Ltd., Mumbai and were used as received. The leaves of *Plumeria pudica* were collected from the local surrounding of Bhopal, Madhya Pradesh.

Preparation of the plant material

The collected plant leaves after authentication was washed with distilled water and was dried under shade. The completely dried leaves were converted to fine powder form using a blender at low speed.

Extraction of leaves [10]

The leaves powder prepared using the above procedure was used for extraction process. Hot continuous extraction was performed for extracting out the phytochemicals from the leaf powder. 500 g of the leaf powder was evenly packed in the extractor of the soxhlet apparatus and extracted successively with various solvents of increasing polarity. The solvents used for extraction included hexane, chloroform, methanol and water. The extraction process was carried out for about 12-14 h for each solvent. The extracts were concentrated on water bath and dried in desiccator.

Preliminary phytochemical testing [11]

The test was performed for alkaloids, triterpenes /steroids, glycosides, tannins, flavonoids, saponins, and phenolic acids. The color, intensity of color or the precipitate formation was used as observational responses to the reactions occurring in these tests.

Pharmacological Evaluation

Animals

Healthy male Wistar rats weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Acute Toxicity Study

A total of three animals were used which received a single oral dose (2000mg/kg) of chloroform extract of *Plumeria pudica*. Animals were observed individually at least once during the first 30min after dosing, periodically during the first 24 h and daily thereafter for a period of 14days. Once daily observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the period of 2 weeks. [12]

Evaluation of anticonvulsant action

Maximal Electroshock Seizure (MES) method

The animals were divided into four groups of 5 animals each.
Group I - Normal saline i.p (Vehicle Control)
Group II – (Diazepam 4 mg/kg i.p (Standard)
Group III – *Plumeria pudica* chloroform extract (75 mg/Kg)
Group IV – *Plumeria pudica* chloroform extract (150 mg/Kg)

Animals in the control group [Group 1] were administered normal saline by i.p route. Animals in Group 2 were administered standard drug Diazepam. In Groups 3 and 4 *Plumeria pudica* extracts were administered by oral route in 1% Sodium lauryl sulphate solution at dose of 75 and 150 mg/kg respectively. After 30 minutes of administration of above drugs, all the rats were given electroshock with electro convulsiometer through ear electrodes [after moistening the ear of animals with drop of normal saline] at intensity of 150 mA, 60Hz for 0.2 seconds. The hind limb extensions and clonic seizures were observed. The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group. [13]

Pilocarpine induced seizures method

The animals were divided into four groups of 5 animals each.

Group I - Normal saline i.p (Vehicle Control)
Group II – (Diazepam 4 mg/kg i.p (Standard)
Group III – *Plumeria pudica* chloroform extract (75 mg/Kg)
Group IV – *Plumeria pudica* chloroform extract (150 mg/Kg)

Animals in the control group were administered normal saline by i.p route. Animals in Group 2 were administered standard drug Diazepam. In Groups 3 and 4 *Plumeria pudica* extracts were administered by oral route in 1% Sodium lauryl sulphate solution at dose of 75 and 150 mg/kg respectively.). All animals were treated with scopolamine (muscarinic antagonist) (1 mg/kg i.p.) to reduce peripheral cholinergic agonist-induced side effects. After 30 minutes of administration of above drugs, all the rats were administered with pilocarpine (300 mg/Kg i.p). The animals were observed for 30 min for tonic convulsion episodes. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity. [14]

Statistical Analysis

The results of the duration of seizures were analyzed by one way ANOVA followed by Dunnetts test. A p value of<0.05 was considered as statistically significant.

Results and Discussion

The extraction abilities of different solvents for recovering extractable components from leaves followed the order: methanol>water>chloroform> hexane. For detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids a small fraction of all the dried extracts were subjected to the phytochemical evaluation tests. The findings suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, proteins and flavonoids in the leaf of the plant (Table 1). The presence of ursolic acid, stigmast-7-enol and lupeol in the leaves of the plant has also been reported by Shinde et al [15] in their review on *Plumeria*.

Table 1.Phytochemical screening of *Plumeria pudica* leaf extracts

Chemical Tests	Pet. Ether extract	Chloroform extract	Methanolic extract	Aqueous extract
Alkaloids	-	-	-	+
Glycosides	-	-	-	-
Saponins	-	+	-	-
Phenols/Tannins	+	+	+	+
Flavonoids	-	+	+	+
Proteins	-	-	-	+
Sterols/triterpenoids	-	-	-	-

The chloroform extract was found to contain glycosides along with phenolics and flavonoids and hence it was considered for anticonvulsant evaluation.

Anticonvulsant Evaluation

The chloroform extract of *Plumeria pudica* leaves (CEPP) were evaluated for their anticonvulsant efficiency by MES and pilocarpine induced convulsions method. The results of total duration of HLTE, onset of clonus and percentage protection against mortality are presented in table 2 and 3.

Table 2. Effect of CEPP on MES induced convulsions

Treatment	Total duration of of hind limb extension (Seconds)	Duration of onset of Clonus (rhythmic involuntary movements of limbs) (Seconds)	% Protection against mortality
Saline	20.00 ± 3.807	35.6 ± 2.408	0
Diazepam	0.00 ± 0.000***	0.00 ± 0.000***	100
CEPP (75 mg/Kg)	17.2 ± 1.923^{ns}	0.00 ± 0.000***	60
CEPP (150 mg/Kg)	10.8 ± 1.788***	0.00 ± 0.000***	80

n =5, ns- not significant, ***- significant with $p<0.05$

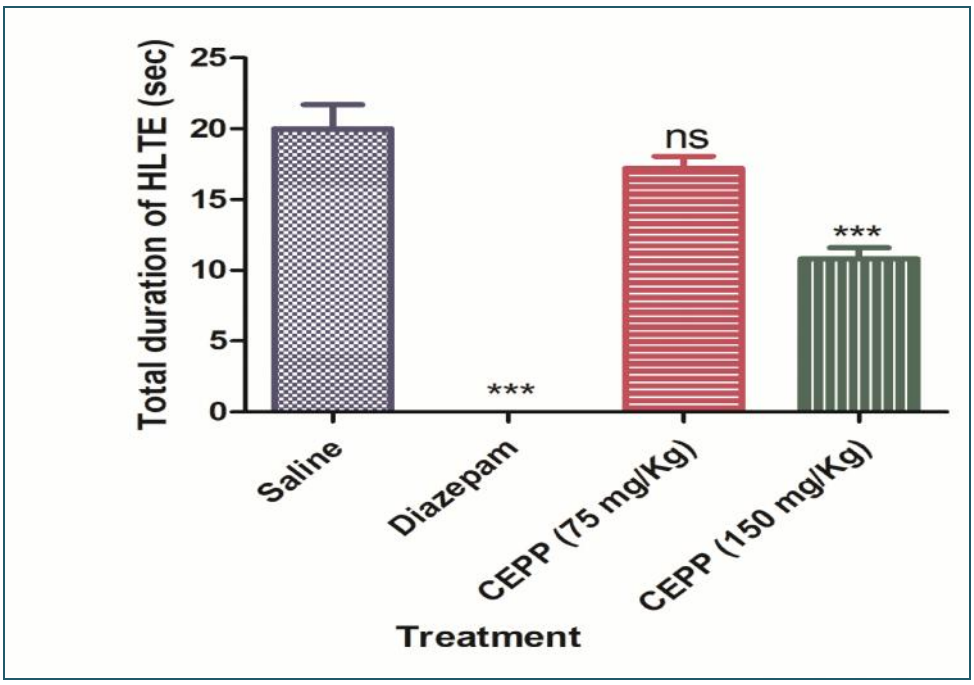

Figure 1. Statistical comparison: One way ANOVA, followed by Dunnett's multiple comparison test compared to control (MES method)

Table 3. Effect of CEPP on Pilocarpine induced convulsions

Treatment	Total duration of hind limb extension (seconds)	Death Latency (seconds)	% Protection against mortality
Saline	100.6 ± 5.549	182.8 ± 4.381	0
Diazepam	0.00 ± 0.000***	0.00 ± 0.000***	100
CEPP (75 mg/Kg)	127.0 ± 3.391***	214.4 ± 4.560***	0
CEPP (150 mg/Kg)	260.8 ± 5.674***	330.8 ± 6.870***	0

n =5, ***- significant with p<0.05

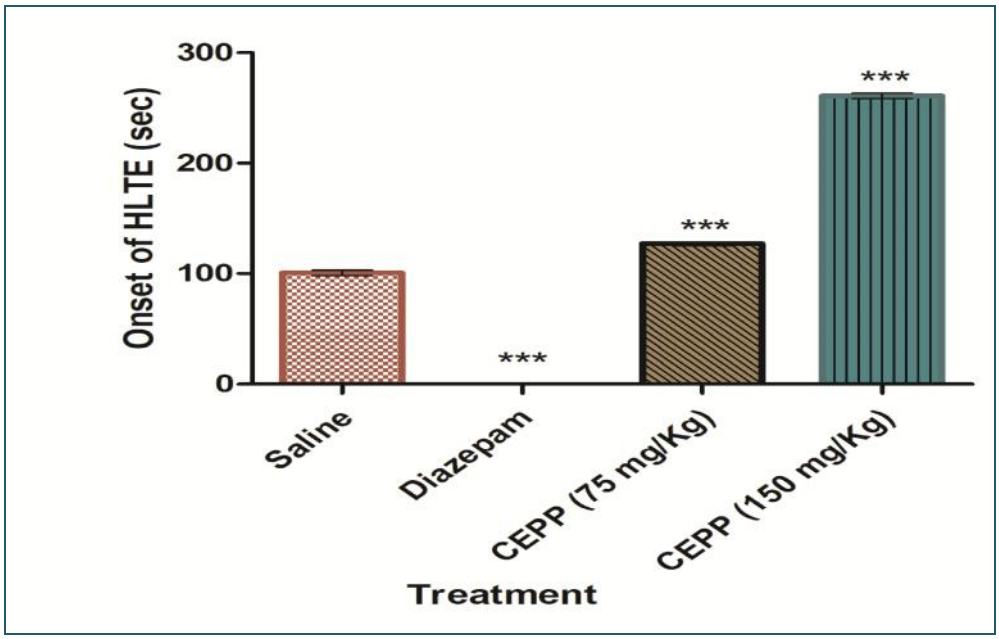

Figure 2. Statistical comparison: One way ANOVA, followed by Dunnett's multiple comparison test compared to control (Pilocarine method; Onset of HLTE)

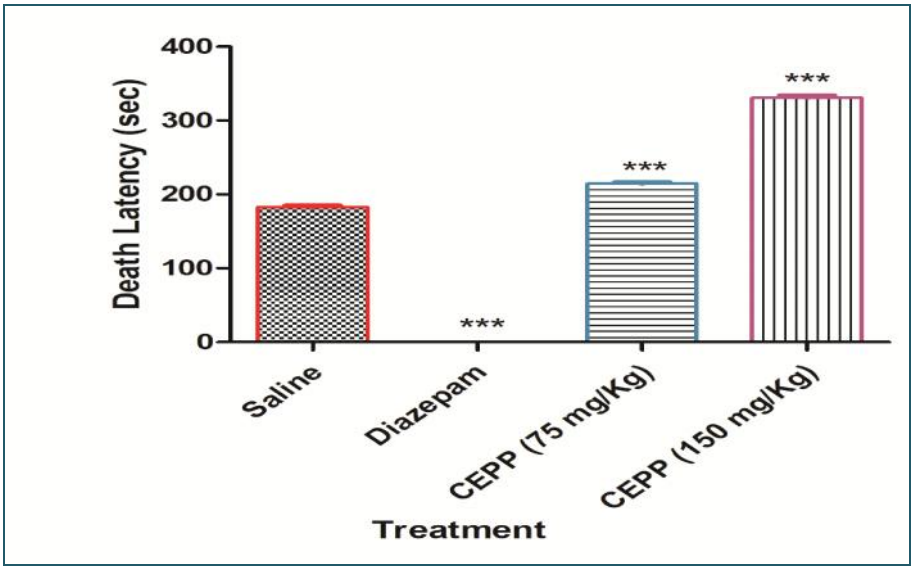

Figure 3 Statistical comparison: One way ANOVA, followed by Dunnett's multiple comparison test compared to control (Pilocarine method; Death Latency)

The maximal electroshock induced convulsion in animals represents grand mal type of epilepsy. In the present study maximal electroshock produced seizures in all the animals used. Antiepileptic drugs that block MES-induced tonic extension are known to act by blocking seizure spread. Moreover, drugs that inhibit voltage-dependent Na+ channels, such as phenytoin can prevent MES-induced tonic extension.

Pilocarpine is a nonspecific muscarinic acetylcholine receptor agonist; it has been suggested that cerebral structures with a high density of muscarinic receptors are likely to represent the sites of origin of acute pilocarpine seizures. Pilocarpine administration to rodents leads to repetitive limbic seizures and status epilepticus, replicating several features of human temporal lobe epilepsy.

The ability of the plant extract to reduce the onset of occurrence of such actions was considered as protection against convulsions.

Conclusion

The present investigation was carried out with an objective to explore the anticonvulsant potential of the common ornamental plant *Plumeria pudica*. It could be concluded from the present investigation that the anticonvulsant effects of the chloroform extract of leaves of *Plumeria pudica* may be attributed to non-specific mechanisms. Indeed, extensive studies are needed to evaluate the active phytomolecule(s) and precise mechanism(s) involved in the anticonvulsant action of the plant.

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