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EVALUATION OF NOOTROPIC AND ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF *CROSSANDRA INFUNDIBULIFORMIS*

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ABSTRACT

To evaluate the Nootropic and Anticonvulsant activity of the ethanolic extract of the leaves of *crossandra infundibuliformis*. Nootropic activity was evaluated by scopolamine induced dementia animal model with elevated plus maze and object identification procedures were done. The Inflexion ratio [IR] specific for continue memory and Discrimination intex [DI], specific for selective memory were evaluated respectively from the above test. and the Anticonvulsant activity was evaluated on an adult wister rats. Two different study models such as, Maximal electroshock [MES] and Isoniazid [INH] induced convulsion method. Nootropic and Anticonvulsant activity were performed in two different concentration such as 200mg/kg, 400mg/kg of EECI. Both the activities were dose dependent. The largest concentration showed the maximum activity.The present study concluded that the ethanolic extract of *Crossandra infundibuliformis* possessed Nootropic and Anticonvulsant Action. Nootropic and Anticonvulsant activity leads some support to the use of *Crossandra infundibuliformis* for different disease in the folkloric medicine of india.

Keywords: Ethanolic extract of *crossandra infundibuliformis*, Nootropic activity, scopolamine, Anticonvulsant activity, Maximal Electricshak, Isoniazid, Wister rats.

INTRODUCTION

Crossandra (*Crossandra infundibuliformis* (L.)Nees) is commonly known as firecracker flower in English, kanaka-ambaram in Tamil, Malayalam, Telugu, Aboli in Marathi, Kanakambara in Kannada. Its flowers are very popular due to their attractive bright colour, light weight, and free flowing nature. Flowers are used for making garlands, offered to temple deities and also used for adorning women's hair. It belongs to the family Acanthaceae. It is mainly grown in open field condition and mostly grown under tropical climate. In India, Crossandra is commercially cultivated in southern states [1].

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Blooming Time: Late winter to Late Autumn. The tube-shaped blossoms are flattened into a 5-lobed disk.

Culture: *Crossandra infundibuliformis* need part shade to full sun. The compost should consist of equal parts of loam and peat moss with sand added for drainage. The compost should be kept moist but not overly wet. Fertilize weekly with a balanced fertilizer dilute to ½ the strength recommended from March to October. The temperature should never drop below 55 degrees or the leaves will turn black. While this doesn't seem to harm the plant, it does make it unsightly. Trim the plant often to keep a desired form. Reporting should be done in February [2].

Propagation: *Crossandra infundibuliformis* are easily propagated by cutting taken in March or by seed.

Phytochemical screening of various solvent extracts of *C.infundibuliformis* revealed the presence of carbohydrates, flavonoids, alkaloids, saponins, tannins, terpenoids, and steroids [3]. The different parts used include root, stem,

flower, fruit, twigs exudates and modified plant organs. While some of the raw drugs are collected in smaller quantities by the local communities and folk healers for local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. *C.infundibuliformis* is one of the perennial crops which occupies in the same rhizosphere soil for more than years and remove most of the available nutrients particularly phosphours [4].

MATERIALS AND METHODS

Plant Collection

The leaves *Crossandra infundibuliformis* were collected from the north western district of the state of Tamil Nadu in the month of march. The plant *crossandra infundibuliformis* was identified and confirmed by the botanist Dr. M. Kannan, Head, Department of Botany, Vivekanandha college of Arts and Science for Women, Tiruchengode, Namakkal Dt., Tamil Nadu.

Preparation of plant Extract

The plant leaves were washed thoroughly using normal water before drying it completely under shadow for 12 days. The dried leaves were grinded using grinder machine to increase its surface area. About each 500gm of leaves powder was packed in soxhlet extraction unit and exhaustively extracted using 1000ml of solvents such as petroleum ether, ethanol and water respectively at 60ºC for 12 hours. The extract was completely dried using water bath at 40ºC and subsequent stored at 4ºC [5].

Experimental Animals

Healthy adult wister rats weighing between 150g and 200g were procured and maintained in polypropylene cages at an environment temperature of 22 ± 1°C and relative humidity of 50–60% with a 12 hours light and dark cycle in registered animal house. The animal experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) proposal under the number (JKKMMRFCP/IAEC 2020/005). India and approved by the Institutional Animal Ethics Committe (IAEC). Throughout the experimental study, the animals were feeded with standard pellet diet and water *ad libitum.*

NOOTROPIC ACTIVITY

Scopolamine (SCP) Induced Model of Dementia

The animals were divided into five groups, each group comprised six rats. The animals are fasted overnight prior to the test but water was supplied *ad libitum*. Group I maintained as Normal control was given with 0.5% of CMC(10ml/kg ,p.o) only for daily for 7 days. Group II (Negative Control) was injected with scopolamine alone (1mg/kg,i.p.) only on 7th day, Group III (Positive control) with piracetam (50 mg/kg,p.o) which served as standard, Groups IV,V Animals were treated with different doses of EECI 200 and 400(mg/kg,p.o)respectively once daily for 7 days [6]. On 7th day 90 min after administration of the last dose for all groups III,IV,V were given with Scopolamine (1mg/kg,i.p).

All groups were treated accordingly as mentioned above for a period of 7days and Scopolamine was given (1mg/kg,i.p).90 min after last dose of standard and different doses of EECI to induce impairment of memory through muscarinic system. Transfer latency (TL) was recorded using Elevated plus maze(EPM) 45 min and 24 hrs after injection of scopolamine .The apparatus used in this model consists of two open arms (50 cm ×10 cm) and two closed arms (50 cm×10 cm×40cm), extended from a central platform (10 cm ×10 cm) and the maze is elevated to a height of 50 cm from the floor.

On the 8th day, 90 min after above treatment, each rats was placed at the end of an open arm of EPM facing away from the Middle platform. TL (Transfer latency) was recorded i.e. the time taken by rats to move into one of the enclosed arms with all its four legs. If the animal did not enter into one of the enclosed arms within 90(s),it was gently pushed into one of the two enclosed arms and the transfer latency was assigned as 90(s).The rats was allowed to explore the maze for next 10(s) and then returned to its home cage. Retention of this learned-task was examined 24 h after the 8th day trial. The inflexion ratio was calculated by the formula as follows [7].

Inflexion ratio **(IR) = (L0 − Lt)/ L0**
where L0- Initial Transfer latency on 8th day
and Lt- Transfer Latency on the 9th day.

ANTICONVULSANT ACTIVITY

Isoniazid (INH) Induced Convulsion

Wister rats of animals were divided into four groups, each group comprised six rats. Group I was maintained as control which was given with 0.5% of CMC, (10 ml/kg p.o.) once daily for 7 days on 7th day 60 min after administration(0.5% of CMC) INH (300mg/kg,i.p) was administered. Group II was administered diazepam (5 mg/kg i.p.) alone on 1st day only, 30 min after administration (diazepam) INH was administered [8].

Groups III and IV were treated low dose (200mg/kg,p.o) and high dose (400mg/kg,p.o) of EECI respectively once daily for 7 days. On 7th day 60 min after extract administration the INH was administered. The rats was placed in an isolated chamber, during the next 120 min for observing the occurrence of clonic seizure. The following parameters were recorded during test session of initial, 30min and up to 24 h [9].

- Latency (onset of clonus)
- Onset of tonic convulsion

Maximal Electro Shock (MES) Induced Convulsions

Animals were divided in four groups,each group comprising six animals.Group I was maintained as Control which was given with 0.5% of CMC. Group II was administered with standard drug phenytoin(20mg/kg i.p),this group was considered as standard [10].

Remaining Two groups of animals were administered with the EECI, designated as Group III and Group IV the dose was in increasing order as(200,400mg/kg p.o) respectively.

On the 7th day, seizures are induced to all the groups by using Superamaximal electrical stimulus of 150mA for 0.2s through the corneal electrodes on cornea (10). A drop of electrolyte solution (0.9% Nacl) with lignocaine was applied to the rats. This increase the contact and reduces the incidence of fatalities.(Nirmala M *et al.*, 2014).

The animals were observed for various phases of MES seizures i.e.,Tonic Hind limb flexion of 1 to 2 seconds, Follwed by a Tonic Hind Extension of roughly 10 to 12 seconds,and finally generalized clonic movements,stupor for 12 seconds. The total duration of the seizure is approximately 25 Seconds [11].

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests [12]. Data were analyzed using one and two way ANOVA followed by pairwise comparison. The results of the studies were expressed as MEAN$\pm$ SEM (Standard error of mean).n=6, ***P<0.001 is considered as highly significant. The statistical analysis was done using the software Graphpad PRISM version no: 5.04. and Graphpad INSTAT version no: 3.10.[13]. Results were presented as tables and Figures.

Fig: Elevated plus maze

Fig: Chamber

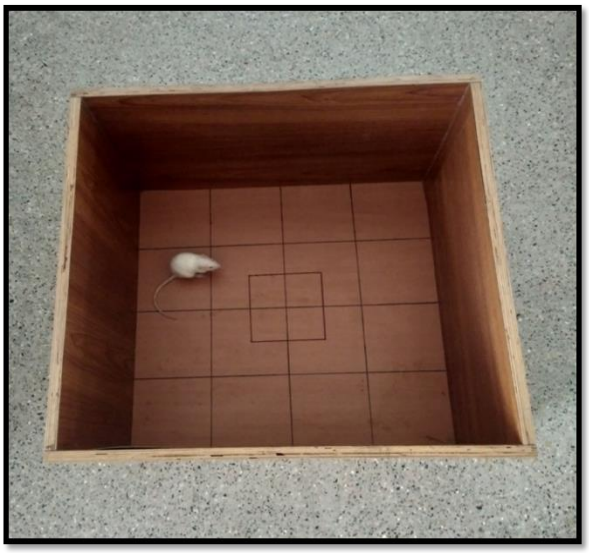


Fig: Electro convulsiometer

Table 1.Effect of EECI on inflexion ratio in Scopolamine-induced amnesic model in Rats (mean±SEM). (Interoceptive behavior model)

Treatment	Dose/kg	Inflexion ratio (mean±SEM)
Normal control	0.5% of cmc,10 ml p.o.	0.407±0.065
Scopolamine (Negative Control)	1 mg/kg, i.p.	0.262±0.046
Piracetam + Scopolamine	50 mg/kg, p.o. + 1mg i.p.	0.711±0.033**
EECI + Scopolamine	200 mg p.o.+ 1 mg i.p.	0.509±0.041**
EECI + Scopolamine	400 mg p.o. + 1 mg i.p.	0.608±0.028**

All Values are expressed as mean +SEM.(n=6) Comparison between control v/s all the other groups. Statistical test done by One - way ANOVA,Tukey kramer followed by multiple comparison test,*p<0.05, **p<0.01,***p<0.001,****p<0.0001

Table 2.Effects of EECI On Latency of Tonic& Clonic Phase In (Min) Among Various Groups In INH Model.

Group No.	Treatment	Latency of Tonic convulsion (min)	Latency of Clonic convulsion (min)
1.	Control (0.5% CMC)+ Isoniazid (300mg/kg)	33.22±0.789	33.22±0.789
2.	Diazepam (5 mg/kg) + Isoniazid (300 mg/kg)	62.12±0.354***	71.64±0.256***
3.	EECI (200mg/kg) + Isoniazid (300 mg/kg)	38.35±0.496**	51.34±0.355**
4.	EECI (400mg/kg) + Isoniazid (300 mg/kg)	43.85±0.426**	53.42±0.361**

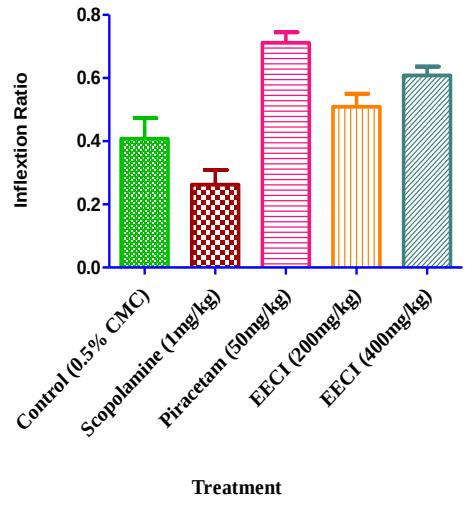
Values are expressed as mean + SEM. Comparison between control v/s all the other groups. Statistical test done by Two - way ANOVA followed by multiple comparison test,*p<0.05, **p<0.01,***p<0.001,****p<0.0001.

Table3.Effect of Ethanolic extract of leaves of *Crossandra Infundubuliformis* on MES induced convulsion in rats.

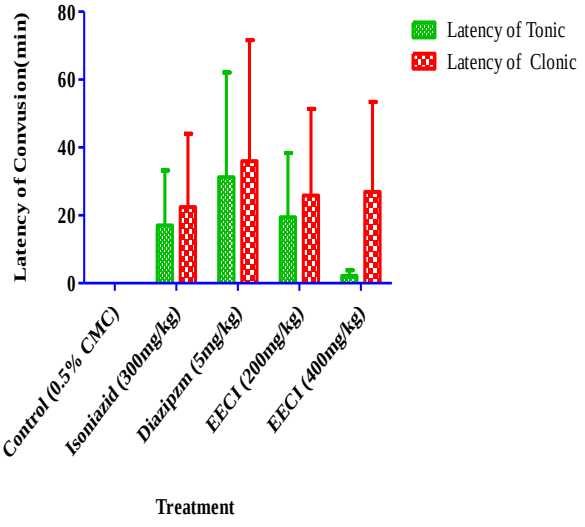
Group	Duration Of Convulsions (Sec)			
	Flexion	Extension	Clonus	Stupor
Control	8.700±1.085	7.266±0.950	19.916±0.504	286.5±48.358
Standard phenytoin (20mg/kg i.p)	3.883±0.278**	Not Detected	10.983±2.820**	93.67±3.639**
Plant extract (200mg/kg p.o)	4.600±0.543**	6.100±0.399**	12.716±0.865**	113.5±5.506**
Plant extract (400 mg/kg p.o)	4.233±0.470**	3.733±0.642**	11.6±0.3804**	105.7±3.518**

Values are expressed as mean + SEM. Comparison between control v/s all the other groups. Statistical test done by Two - way ANOVA followed by multiple comparison test,*p<0.05, **p<0.01,***p<0.001,****p<0.0001.

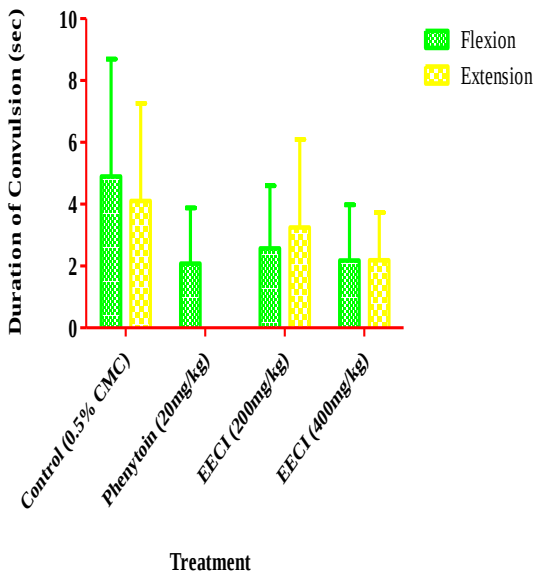
Bar diagram 1: Effect EECI on inflextion ratio in scopolamine-induced amnesia model in rats (mean ± SEM). (Introceptive behavior Model)



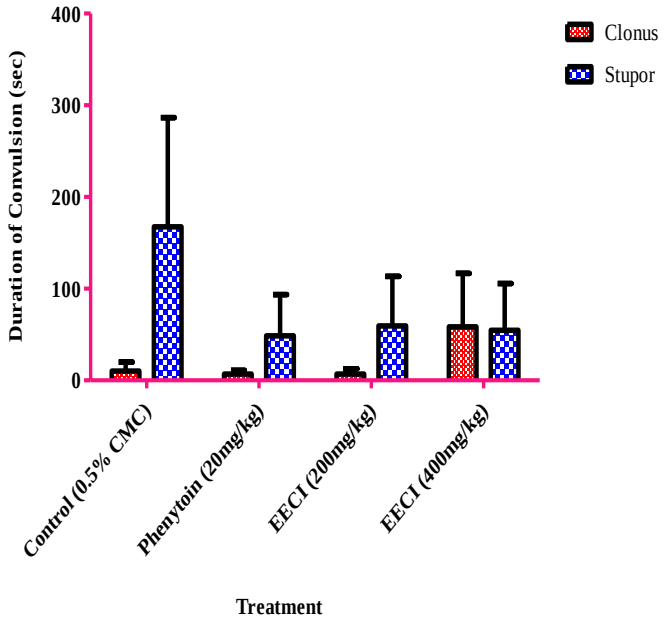
Bar diagram 2: Mean Tonic & Clonic Seizure Latency Duration (min) of various groups in INH Seizure Model.



Bar diagram 3: Mean duration of Tonic Hind Limb Flexion & Extension (sec) of various groups in MES Seizure Model.



Bar diagram 4: Mean Clonus & Stupor duration (sec) of various groups in MES Seizure Model.



RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening ethanolic extract of *Crossandra infundibuliformis* reveals the presence of steroids,Glycosides, Alkaloids, Tannins,Terpenoids,flavanoids.

Nootropic Activity

Scopolamine Induced model

Scopolamine treated group exhibited with impairment of memory and has shown decrease in IR as compared to normal control group which indicates the induction of amnesia. When compared to IR of normal control group 0.407± 0.065, scopolamine treated group was noted with impairment of memory as depicted by decrease in IR 0.262 ± 0.046. Piracetam, EECI with Low dose and high dose treated groups have shown significant increase in the IR as recorded by 0.711 ± 0.033 and 0.509 ± 0.041, and 0.608 ± 0.028 respectively. The results are tabulated in the table 1 and fig.1.

Anticonvulsant Activity

Assessment of anticonvulsant activity by INH

The anticonvulsant property of ethanolic extracts of *Crossandra Infundibuliformis* is assessed by its ability to delay the Tonic and clonic convulsion. The results of anticonvulsant effects of *Crossandra Infundibuliformis* ethanolic extract against INH induced convulsion are shown in table No 2. And Fig.2. Ethanolic extracts of *Crossandra Infundibuliformis* at doses of 200 and 400 mg/kg p.o showed onset of convulsions after 38.35±0.496 and 43.85±0.426sec respectively which is significant ($p<0.01$) when compared to control 33.22± 0.789sec.

Assessment of anticonvulsant activity by MES

In MES model, the duration of tonic extension of hind limb is used as an end point i.e. the protective action. The result of anticonvulsant effects of *Crossandra Infundibuliformis* plant against MES induced convulsion are shown in table No 3. and fig.3. The data showed that the extract reduced the hind limb extension in a dose dependent manner. Ethanolic extracts of *Crossandra Infundibuliformis* 200 and 400 mg/kg decreases the duration of hind limb extensor in 6.100±0.399 and 3.733±0.642sec respectively which is most significant as compared to control 7.266±0.950sec.

CONCLUSION

It was concluded that ethanolic extract of *crossandra infundibuliformis* leaves wild promising herb for the patients of Alzheimer's disease and other cognitive deficit states.

In the present study anticonvulsant activity of ethanolic extracts of *Crossandra Infundibuliformis* leaves was investigated by means of INH and MES models. The oral administration of the extract of *Crossandra Infundibuliformis* showed delayed tonic of convulsions in INH model while reduction in tonic hind limb extension in MES model, indicating its potent anticonvulsant activity. Higher protection was observed with higher dose i.e. 400mg/kg b.w orally. The results confirms the anticonvulsant activity (both MES and INH models) of ethanolic extract of the leaves of *Crossandra Infundibuliformis* in rats. Isolation and characterization of phytoconstituents from ethanolic extract may produce natural antiepileptic drugs.

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