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PHYTOCHEMICAL AND ANTI-CONVULSANT STUDY OF *NYCTANTHES ARBOR-TRISTIS* EXTRACT

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ABSTRACT: The drug is obtained from the dried fruit of *Nyctanthes arbor-tristis* of family Oleaceae. It is also called Coral Jasmine, Parijat, and Harsingar, etc. To confirm the veracity of the claim above, the anticonvulsant effect of the ethanolic extract has been evaluated. In this investigation, the rats were pretreated with different dose of *Nyctanthes arbor-tristis* ethanolic extract (100, 200 mg/kg) for 14th days and then, they were subjected to maximal electroshock-seizures, the dose of 100 and 200 mg/kg significantly reduced the duration of hind limb extension, and both the doses were statistically found to be equipotent. The reference, standard, Phenytoin (30 mg/kg) provided complete protection. Thus, the present study revealed the anticonvulsant effect of *Nyctanthes arbor-tristis* against maximal electroshock-induced convulsion in the rat.

INTRODUCTION: *Nyctanthes arbour - tristis* commonly called as coral jasmine. It is a tree growing to 10 m tall, with flaky grey bark. The leaves are simple, opposite, 6 - 12 cm long and 2 - 6.5 cm broad, with an entire margin. The flowers are fragrant, with a five to eight-lobed white corolla with an orange-red center. The calyx is 6 - 8 cm long. The fruit is a flat brown heart-shaped to round capsule 2 cm diameter, with two sections each containing a single seed¹. The plant is found in different parts of India, including Himalayan region, Western Uttar Pradesh, Punjab. It is also distributed in Bhutan, Nepal, Burma, and Bangladesh².

Different parts of *Nyctanthes arbor-tristis* are known to possess various ailments by rural mainly tribal people of India along with its use in Ayurveda, Siddha and Unani systems of medicines. Juice of the plant leaves is used as antimicrobial activity³, anxiolytic activity⁴, and hepatoprotective activity⁵. In the traditional system of medicine, it is also used in nervous disorders so *Nyctanthes arbour - tristis* is recommended for the treatment of epilepsy⁶.

Epilepsy is a common neurological disorder which is manifested by recurrent unprovoked seizures. It is affecting about 5% of the world population. A seizure is an abnormal function of ion channels and neural networks which results in rapid, synchronous, and uncontrolled spread of electrical activity in brain⁷. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "Green Medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse effects⁸.



Nyctanthes arbor-tristis belongs to the family Oleaceae and has chemicals constituents such as flavonoids⁹, polyphenolic compounds, etc. It is used for the treatment of convulsions¹⁰, aggression¹¹, diabetes¹² and inflammation¹³ in Indian indigenous medical system. However, there is no scientific report available in support of anticonvulsant activity of fruit of *Nyctanthes arbor-tristis* in mice. Therefore, to justify the traditional claims we have assessed the anticonvulsant activity of fruit of *Nyctanthes arbor-tristis* in the rat.

MATERIAL AND METHOD:

Preparation of the Extract: The dried fruit of the plant were collected and powdered mechanically to a coarse powder. This powdered drug was kept in airtight container until the time of use. About 1kg of the powder material was subjected to Soxhlet extraction using 70% ethanol for 48 h. The solvent was distilled off at low temperature under reduced pressure and evaporate at 40 °C. The yield of the product was approximately 5% w/w. The ethanolic extract of *Nyctanthes arbor-tristis* (NAE) was stored in a refrigerator and reconstituted in water for injection just before use.

Phytochemical Screening: Qualitative test for the presence of secondary plant metabolites such as alkaloids, carbohydrates, saponins, glycosides, flavonoids, protein and amino acid, fixed oils, gum, and mucilage were carried out on the ethanolic extract using standard procedure.

Experimental Animals: Wister rat (8 - 10 weeks) of both sexes were fed with standard diet before and after during the experiment. After randomization into various groups and before initiation of experiment, the rats were acclimatized for 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h *ad libitum*. The present study was conducted after getting experimental protocol approval from the Institutional animal

ethics committee (SDCOP& VS/CPCSEA/IAEC/014/M.PHARM/2015).

Standard Drug: Phenytoin injection (Sun Pharmaceuticals Pvt. Ltd.) was used as a standard drug and was administered intraperitoneally.

Acute Toxicity Studies: Acute oral toxicity study of ethanolic extract of *Nyctanthes arbor-tristis* was carried out according to OECD guidelines 423. Acute toxicity studies were conducted by using Wister rat of either sex weighing 150 - 200 gm. The animals fasted overnight before the experimental procedure. The method of Up and down or staircase was used to determine the dose. The median lethal dose of the extracts having anticonvulsant activity was determined by administering 100, 200, 2000 mg/kg dose and percent mortality was observed 24 h later.

Assessment of Anticonvulsant Activity:

Maximum Electroshock Induced Seizures (MES): The electrical shock applied through ear-clip electrodes separately to each mouse. The stimulus duration was 0.2 s and the current frequency 150 mA. Each group containing five rats which was administered with extract and standard drug (phenytoin 30 mg/kg I.P.) for seven days and on an experimental day, the test was started 30 min after drug administration. The animals were observed for the occurrence of tonic hind limb extension and mortality for a duration of 15 min. The NAE was administered to Group III, and IV (100 and 200 mg/kg body P.O) whereas group I and II received 1% of Tween 80 and Phenytoin 30 mg/ kg I.P. respectively.

Statistical Analysis: All the data were expressed in mean \pm SEM. The significance of the difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Significance of difference between normal and control group was considered $P < 0.05$.

RESULT AND DISCUSSION:

Preliminary Phytochemical Studies:

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE ETHANOLIC EXTRACT OF NYCTANTHES ARBOR-TRISTIS SHOWS THE PRESENCE OF VARIOUS FUNCTIONAL GROUPS

Category	Name of the test	Pet. Ether	Chloroform	Ethanol	Distilled water
Alkaloids	Mayer's test	-	+	-	-
	Wagner's test	+	+	+	+

	Hager's test	-	+	+	+
	Dragendorff's Test	-	-	+	-
Carbohydrates and Glycosides	Molisch test	+	+	+	+
	Fehling test	-	-	+	-
	Barfoed's test	-	-	-	-
	Benedict's test	+	+	+	+
	Borntrager's test	-	-	+	+
	Legal test	+	+	+	+
Saponins	Foam test	+	+	+	+
Protein and Amino acid	Millon's reagent	-	+	+	-
	Biuret test	-	+	+	-
Fixed oils and Fats	Spot test	-	-	-	-
	Saponification test	+	+	-	-
Phenolic compound and Flavonoids	FeCl ₃ test	+	+	+	+
	Gelatin test	+	+	-	-
	Lead acetate	+	+	+	+
	Alkaline reagent	+	+	+	+
Gum and Mucilage	Test for gum and mucilage	-	-	-	-

Acute Toxicity Studies: There was neither change in the behavioral pattern or any sign of toxicity during the observations up to 24 h for mortality. The extracts were safe up to a maximum dose of 2000 mg/kg. The biological evaluation was carried out at doses of 100 and 200 mg/kg.

Assessment of Anticonvulsant Activity:

Effect on Maximum Electroshock Induced Seizures: NAE showed significant anticonvulsant activity by significantly lowering the duration of

hind limb tonic extension (HLTE) induced by maximal electroshock. NAE at a dose of 100 mg/kg P.O. not showed significant reduction HLTE and reduced the mortality to 40%. NAE dose of 200 mg/kg P.O. significantly reduced the duration of HLTE ($p < 0.05$) and no mortality was found. Phenytoin (30 mg/kg I.P.) significantly reduced the duration of MES-induced HLTE ($p < 0.01$) and completely prevented the various phases of convulsion induced by MES.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF NYCTANTHES ARBOR-TRISTIS AGAINST MES INDUCED SEIZURES IN RATS

Group	Time taken (sec)				
	Flexion	Extensor	Stupor	Recovery	% protection
Control 1% Tween 80 (2 mL/kg, P.O)	8.80 ± 0.37	13.20 ± 0.37	39.60 ± 0.50	65.20 ± 0.86	1/5 (20%)
Standard Phenytoin sodium (30 mg/kg, I.P.)	2.60 ± 0.40**	2.8 ± 0.52**	16.80 ± 0.58**	19.20 ± 0.58**	5/5 (100%)
NAE (100 mg/kg, P.O.)	6.0 ± 0.54 *	6.8 ± 0.37*	31.60 ± 0.50*	53.20 ± 0.58*	3/5 (60%)
NAE (200 mg/kg, P.O.)	4.20 ± 0.66*	5.20 ± 0.58*	24.40 ± 0.50**	47.0 ± 0.44**	5/5 (100%)

All the results were expressed as mean ± SEM (n = 5), for each experimental group. The statistical analysis was carried out using one way ANOVA method. Significant after analysis of variance (ANOVA) followed by Dunnett's test. * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$; when compared to control group.

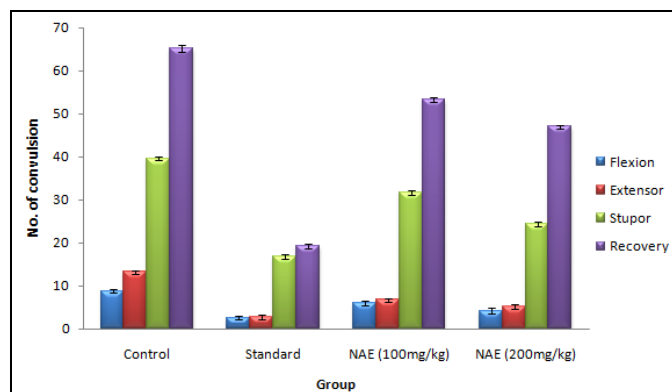


FIG. 1: EFFECT OF ETHANOLIC EXTRACT OF NYCTANTHES ARBOR-TRISTIS ON MAXIMAL ELECTRO CONVULSION SHOCK (MES) INDUCED SEIZURE IN RATS

CONCLUSION: The present study revealed that *Nyctanthes arbor-tristis* ethanolic extract possesses significant anticonvulsant activity against MES induced convulsions. In conclusion, the ethanolic fruit extract of *Nyctanthes arbour-tristis* demonstrated possess anticonvulsant properties and less toxicity in experimental animals at the doses used. However, further studies still needed to be carried on the exposure of the extract to human and its use in folk medicine for seizures control.

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CONFLICT OF INTEREST: Nil

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