



EVALUATION OF NEUROLOGICAL INVESTIGATION OF ETHANOLIC EXTRACT OF FLOWERS OF NYCTANTHES ARBOR-TRISTIS LINN. BY DIFFERENT PHARMACOLOGICAL MODELS

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Abstract

AIM- The aim of the present investigation is to evaluate Neurological Investigation of Ethanolic Extract of Flowers of *Nyctanthes arbor-tristis* Linn. by Different Models.

MATERIAL & METHODS- The plant material i.e. flowers were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of flower powder of *Nyctanthes arbor-tristis* were extracted using petroleum ether for the removal of fats and then different solvents were used according to polarity charts. Qualitative chemical tests of different extracts were subjected to various chemical tests to detect various phytoconstituents. The animals were under observation for their behavioral changes if any, at 30 minutes intervals in the first one hour and at the hourly intervals for the next 4 hour for the following parameters. Locomotor activity is easily measured using actophotometer which operates on photoelectric cells connected with a counter. When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally. The animals were discarded and replaced if they failed to do so. All the groups' animals were placed on the rota rod and the fall off time from the rotating rod was noted after 60 minutes of test drug and standard administration.

RESULTS- The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds. Rats treated with ethanolic extract of *Nyctanthes arbor-tristis* and were submitted to general behavioural profile studies did not show any difference in their behaviors. The ethanolic extract of *Nyctanthes arbor-tristis* in a dose level of 200 mg/kg, and 400 mg/kg, p.o did not produce statistically any significant reduction in locomotor activity as compared to the control animals receiving only the vehicle. There was statistically significant decrease in the time of falls within 3 minutes after the treatment with ethanolic extract of *Nyctanthes arbor-tristis* at dose level of 200 mg/kg and 400 mg/kg, p.o and result reveals that *Nyctanthes arbor-tristis* has no muscle relaxant property.

CONCLUSION- *Nyctanthes arbor-tristis* has been demonstrated to ameliorate cognitive processes, not only preventing amnesia induced by pharmacological treatments in elevated plus maze, but also by producing facilitation of social memory in a social learning task which demonstrates that the

extract displays memory enhancing properties.

Keywords- Neurological Investigation, Ethanolic Extract, Flowers, *Nyctanthes arbor-tristis* Linn, Muscle Relaxant Activity, Locomotor Activity

INTRODCUTION

In living systems, oxidation is a basic part of the normal metabolic process, in which reactive oxygen species (hydrogen peroxide and hypochlorous acid) and many free radicals (hydroxyl radical (OH) and superoxide anion) are generated. Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging. Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body (Pinton *et al.*, 2012).

Depending on the duration of exposure of animals to drug, toxicological studies may be of three types: acute, sub-acute and chronic studies (Baki *et al.*, 2007). Acute and chronic toxic effects differ principally from each other with respect to the amount of chemical compound involved and the time intervening before the effect is seen (Timbrell, 2002). Acute effects are normally observed soon after exposure and result from the uptake of large amounts of poison, generally as a single dose. On the other hand, chronic effects are often detected over an extended period of time during which exposure may be continuous or intermittent, though obviously at levels which are too low to produce an acute effect (Loomis and Hayes, 1996; Pascoe, 1983). In the search of new therapeutic drugs for the remedy of neurological disorder, medicinal plants are studied worldwide for promising developments in the area of herbal medicines as sources for new therapies for dementia (Heinrich & Lee Teoh, 2004). *Nyctanthes arbor-tristis* is the well documented medicinal plant that has been traditionally used to treat the various neurological disorders. In the absence of any scientific evidence, a scientific attempt was made to explore the neurological evaluation of flowers of the selected plant.

MATERIAL & METHODS

Plant materials

The flowers of *Nyctanthes arbor-tristis* were collected from local area of Indore, Gwalior and college campus of Oriental University in the month of August & September.

Authentication of Plant Materials

The plant materials were taxonomically identified by submitting herbarium specimen sheet for Identification and authentication in Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P under Voucher Specimen Number: J/Bot. CFWF-016 dated 22/06/2022.

Preparation of Total Crude Extract

The plant material i.e. flowers were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of flower powder of *Nyctanthes arbor-tristis* were extracted using petroleum ether for the removal of fats and then different solvents were used according to polarity charts i.e. DCM, Ethyl acetate, ethanol and finally water by soxhlet apparatus for 72 h. After drying, the respective extracts were weighed and percentage yield was determined (Mukherjee, 2002).

Preliminary Phytochemical Tests

Qualitative chemical tests of different extracts were subjected to various chemical tests to detect various phytoconstituents (Kokate, 2003; Khandelwal, 2006).

Selection of animals

Wistar albino rats of either sex between 2 and 3 months of age weighing 150-200 g were used which were procured from the central animal house of College of Pharmacy, India. All animals were housed in an animal room under normal condition of $25\pm 1^{\circ}\text{C}$, 12-h light and dark cycle. The animals were allowed free to access commercial rat pallet diet (Lipton India Ltd, Mumbai, India) and water *ad libitum*. The bedding materials of the cages were changed every day. All the experimental procedures were carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The study designs were approved by Institutional Animal Ethical Committee (IAEC).

NEUROPHARMACOLOGICAL EVALUATION OF EXTRACTS

General behavior studies

Evaluation of general behavioral profiles was performed by the method. Albino Wistar rats were divided in to five groups (n=6). Ethanolic extract of flowers of *Nyctanthes arbor-tristis* were administered for first three groups at dose of 200 and 400 mg/kg p.o respectively. While the last two groups were administered with diazepam (5mg/kg) as drug control and 2% v/v Tween 80 as vehicle control. The animals were under observation for their behavioral changes if any, at 30 minutes intervals in the first one hour and at the hourly intervals for the next 4 hour for the following parameters (Dixit & Varma, 1976; Murugesan *et al.*, 1999).

Locomotor activity

Locomotor activity was measured using actophotometer. Rats were divided into different groups consisting of 6 per group. Three groups received the Ethanolic extract at a dose of 200 and 400 mg/kg body weight. The other two groups received control vehicle 2% v/v Tween 80 and standard drug (Diazepam 2 mg/kg, i.p). Locomotor activity is easily measured using actophotometer which operates on photoelectric cells connected with a counter. When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally. Each rat was placed individually in the activity cage floor for 10 minutes. The activity score was recorded after 60 min of drug and standard administration (Turner RA, 1972).

Effect on motor coordination

Rats were divided into different groups consisting of 6 rats per group. Treated groups received the Ethanolic extract at a dose of 200 and 400 mg/kg body weight. The other two groups received control vehicle 2% v/v Tween 80 and standard drug (Diazepam 2 mg/kg, i.p). All the groups of rats were trained to remain on the rotarod. Only those rats which could balance themselves were taken for the study. The animals were discarded and replaced if they failed to do so. All the groups' animals were placed on the rota rod and the fall off time from the rotating rod was noted after 60 minutes of test drug and standard administration (Ozturk *et al.*, 1963).

Assessment of Anxiolytic activity in rats using the Elevated plus maze

The elevated plus maze test has been widely validated to measure anxiety in rodents (Lister RG, 1990). The elevated plus maze consists of two open arms and two closed arms with the open arm perpendicular to the closed one. Rats were divided into different groups as per the protocol of study consisting of 6 rats per groups. Treated groups received the Ethanolic extract at a dose of 200 and 400 mg/kg body weight. The other two groups received control vehicle 2% v/v Tween 80 and standard drug (Diazepam 2 mg/kg, i.p) and 30 minutes later, the animals were individually placed at the center of the plus maze and observed for 5 minutes. The number of entries and time in seconds spent by the animals in the open arm were noted and compared with the control groups.

STATISTICAL ANALYSIS

Data were expressed as the mean standard error of mean (S.E.M.) of the means and statistical analysis

was carried out employing one-way ANOVA. Differences between the data were considered significant at $P < 0.05$.

RESULTS

Extractive Value Determination

Dried flowers of *Nyctanthes arbor-tristis* were extracted using petroleum ether, DCM, Ethyl acetate, ethanol and finally water. The percentage yields of all dried extracts were determined by using the following formula.

Weight of Extract

Percentage yield = ----- x 100

Weight of powder drug Taken

Table No 1: Different extracts with their appearance and % yield (in gm)

S. No.	Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Pet. ether extracts of <i>Nyctanthes arbor-tristis</i>	Dark Brown	Sticky	11 %
2	DCM extracts of <i>Nyctanthes arbor-tristis</i>	Dark Orange	Sticky	8.3 %
3	Ethyl acetate extracts of <i>Nyctanthes arbor-tristis</i>	Dark Orange	Sticky	2.4 %
4	Ethanolic extracts of <i>Nyctanthes arbor-tristis</i>	Dark Brown	Sticky	10.4 %
5	Aqueous extracts of <i>Nyctanthes arbor-tristis</i>	Light Color	Sticky	8.6 %

Preliminary Phytochemical Screening

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds.

Table No 2: Qualitative chemical analysis of extracts by chemical tests

S. No	Phytoconstituents	Chemical Tests	DCM Extract	Ethyl acetate Extract	Ethanol Extract	Aqueous Extract
1	Alkaloids	Wagner's test	+	+	-	+
		Dragendorff's test	+	+	-	+
		Mayer's test	+	+	-	+
		Hager's test	-	-	-	+
2	Amino Acid	Millon's test	+	+	-	-
		Ninhydrine test	-	-	-	-
3	Flavonoids	Shinoda test	+	+	+	+
		Alkaline reagent test	+	+	+	+
		Zinc hydrochloride test	-	+	+	-
4	Phenolics (Tannins)	Gelatin test	+	+	+	+
		Phenazone test	-	-	-	-
		Ferric chloride test	+	+	+	+
5	Protein	Biuret test	+	+	-	-
		Hydrolysis test	+	+	+	-
		Test with trichloroacetic acid	-	-	-	-
6	Triterpenoids & Steroids	Liebermann-Burchard test	+	+	+	+
		Salkowski test	+	+	+	+
7	Carbohydrates	Benedict's test	+	+	+	+
		Fehling's test	+	+	+	+
		Molish's test	-	-	-	-
8	Anthraquinone glycosides	Borntrager's test	+	+	+	+
		Modified Borntrager's test	+	+	+	+
9	Coumarin glycosides	-----	-	-	-	-
10	Saponin glycosides	-----	+	+	+	+
11	Cardiac glycosides	Baljet's test	+	+	+	+
		Legal's test	+	+	+	+
		Keller-killiani test	+	+	+	+

Where, (-) Negative, (+) Positive

NEUROPHARMACOLOGICAL EVALUATION OF EXTRACTS

General behaviors studies

Rats treated with ethanolic extract of *Nyctanthes arbor-tristis* and were submitted to general behavioural profile studies did not show any difference in their behaviors. They were alert, with normal grooming, touch response, sound response, pain response, motor activity and grip strength were normal. The animals showed no signs of depression during the observation period. However,

the standard drug diazepam caused a significant depression of all these responses compared with the ethanolic extract of *Nyctanthes arbor-tristis*. The results are shown in Table no.3.

Table No.3: Effect of ethanolic extract of *Nyctanthes arbor-tristis* on general behavioral studies in rats

Behaviors type	Extract (200mg)	Extract (400mg)	Diazepam (5mg/kg)	2%v/v Tween 80
Spontaneous activity	–	–	++++	–
Alertness	–	–	+++	–
Sound response	–	–	+++	–
Touch response	–	–	+++	–
Pain response	–	–	+++	–
Righting reflex	–	–	+++	–
Pinna reflex	–	–	++++	–
Grip strength	–	–	++++	–

No effect (-), slight depression (+), moderate depression (++) , strong depression (+++), very strong depression (++++).

Locomotor activity

The ethanolic extract of *Nyctanthes arbor-tristis* in a dose level of 200 mg/kg, and 400 mg/kg, p.o did not produce statistically any significant reduction in locomotor activity as compared to the control animals receiving only the vehicle. Diazepam treated groups revealed a statistically significant ($p < 0.01$) decrease in Locomotor activity as compared to the control. The results are shown in Table no.4.

Table No.4: Effect of ethanolic extract of *Nyctanthes arbor-tristis* on locomotors activity in rats using actophotometer

S. No.	Treatment	Locomotor activity (scores)in 10 min	
		Before treatment	After treatment
1.	Control (vehicle, p.o)	410.11±3.67	412.45±4.75
2.	Ethanolic Extract (200 mg/kg p.o)	415.53 ± 4.69	310.44 ± 3.12**
3.	Ethanolic Extract (400 mg/kg p.o)	420.60 ± 5.93	267.67 ± 4.56*****
4.	Standard(Diazepam 2mg/kg i.p)	412.45± 6.22	128.89 ± 4.79***

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6) Values are mean ± SEM of 6 animals per groups. Comparison was made between before drug administration and after drug administration *P< 0.05 vs control

Effect on motor coordination

There was statistically significant decrease in the time of falls within 3 minutes after the treatment with ethanolic extract of *Nyctanthes arbor-tristis* at dose level of 200 mg/kg and 400 mg/kg, p.o and result reveals that *Nyctanthes arbor-tristis* has no muscle relaxant property. However diazepam treated groups showed statistically significant ($p < 0.01$) decrease in the time of falls as compared to the control. The results are shown in Figure No.1.

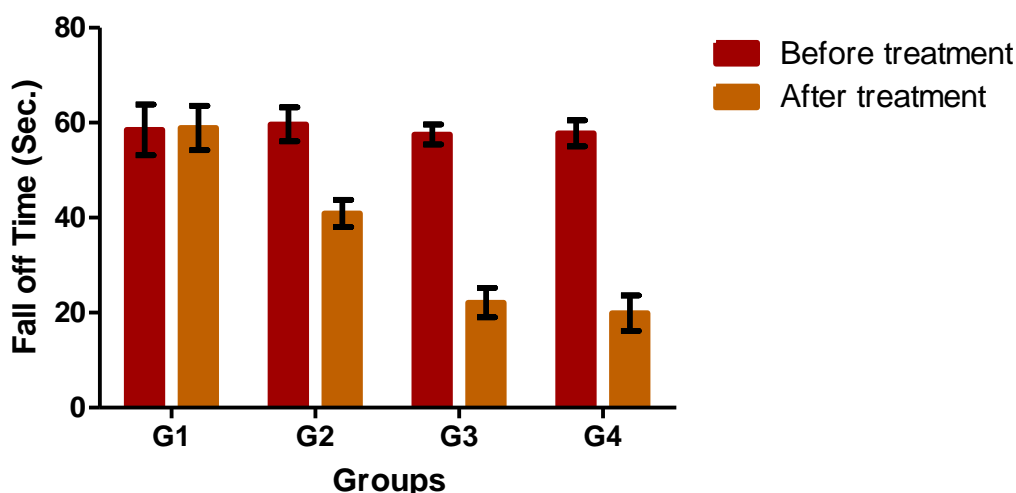


Figure No. 1: Effect of ethanolic extract on muscle relaxant activity

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6) Values are mean \pm SEM of 6 animals per groups

Assessment of Anxiolytic activity in rats using the Elevated plus maze

The results obtained indicate that the groups treated with ethanolic extract of *Nyctanthes arbor-tristis* at dose level of 200 mg/kg and 400 mg/kg, p.o did not produce significant increase in number of entries in open arm compared to the control groups. However diazepam treated groups showed statistically significant increase ($p < 0.01$) in entries in open arm. The results are shown in Table no.5.

Table No.5: Effect of ethanolic extract of *Nyctanthes arbor-tristis* on anxiety induced in rats using elevated plus maze

S. No.	Treatment	No of entries in Closed arm	No of entries in Open arm	Time spent in Open arm (secs)	Time spent in Closed arm (secs)
1.	Control (Anxiety)	17.60 \pm 0.22	1.33 \pm 0.12	10.12 \pm 3.23	210.59 \pm 3.69
2.	Ethanolic Extract (200 mg/kg p.o)	07.88 \pm 0.56***	5.61 \pm 0.16**	105.56 \pm 2.27 ***	30.50 \pm 4.11***
3.	Ethanolic Extract (400 mg/kg p.o)	03.67 \pm 0.76***	7.78 \pm 0.36***	140.61 \pm 3.76***	16.33 \pm 4.29***
4.	Diazepam(2mg/kg)	02.33 \pm 0.89***	8.98 \pm 0.33***	167.77 \pm 3.32***	18.41 \pm 3.51***

Statistical significance test was done by ANOVA followed by Dunnet's 't' test (n=6) Values are mean \pm SEM of 6 animals per groups $P < 0.05$ Vs Control

DISCUSSION

Recently, the inhibition of Acetylcholinesterase enzyme was targeted as a new approach to prevent the progression of Alzheimer disease as it is neurochemical characterized by a consistent deficit in cholinergic neurotransmission. For this reason, symptoms can be treated by the use of agents that retrieve the level of acetylcholine through inhibition of Acetylcholinesterase, AChE. In late stages of AD, levels of AChE decline by up to 85% in the brain (Ballard CG *et al.*, 2005). The most important strategy to increase cholinergic function is inhibition of acetylcholinesterase. AChE inhibitor is always the target of many Alzheimer dementia drugs (Heinrich M, & Teoh HL, 2004).

Phytotherapeutic products are often considered as safe because they are natural (Gesler WM, 1992). However these products may contain bioactive principles with potential to cause adverse effects (Bent S & Ko R., 2004). In supplement, the poor Pharmacogivilane surveillance in this field makes it difficult to determine the adverse effects caused by the utilization of phytotherapeutic products (Eisenberg DM *et al.*, 1998). Herbal remedies requires an scientific validation of their efficacies and

safety due to their increase use throughout the world. Thus all the natural products used in therapeutics must be evaluated for their efficacy and safety tests by the same methods as used for new synthetic drugs (Talalay & Talalay, 2001). In the present study no mortality was observed during acute toxicity evaluation in the various doses administered. No signs or symptoms of toxicity were observed. The results of the study reveal that the ethanolic extract of *Nyctanthes arbor-tristis* should be regarded practically as non toxic. The change in body weight has been used as an indicator of potential adverse effect of drugs and chemicals. Results from the body weight of treated groups when compared to control rats also suggest that at sub chronic administration of *Nyctanthes arbor-tristis* has revealed no effect on the normal growth of rats. The haematological system is the one of the most sensitive targets for toxic compounds and important index of physiological and pathological states in man and animals. In the present study all the haematological parameters remained under the reference range for the rats in drug treated and control groups. General behavior studies suggest that ethanolic extract does not possess any neurotoxicity The ethanolic extract of *Nyctanthes arbor-tristis* were found to have no effect on the Locomotor activity. Locomotor activity is regarded as an indicator of alertness and a decline in the activity would suggest sedative activity.

Experimental findings suggest the extract did not illustrate any effect on the muscle coordination which was indicated by the outcome with respect to the Rota rode experimental test. In our investigation, the extracts did not produce any significant change or increase in the exploratory activity of the rats in the elevated plus maze method; hence we can conclude that the extract does not possess Anxiolytic activity. Generally most of the Anxiolytic agents have an adverse effect on memory as seen with the benzodiazepines, commonly used as Anxiolytic (Murgandam *et al.*, 2000). Considering the lack of need of drugs with proven effect in improving learning, specific memory improving and antidepressant effect of *Nyctanthes arbor-tristis* can be of enormous interest for neurochemical investigation which can unravel the mechanism of action of plant drug with respect to activity.

CONCLUSION

Furthermore, the lack of effect on Locomotor activity works to the benefit of the plant illustrating nootropic activity. *Nyctanthes arbor-tristis* has been demonstrated to ameliorate cognitive processes, not only preventing amnesia induced by pharmacological treatments in elevated plus maze, but also by producing facilitation of social memory in a social learning task which demonstrates that the extract displays memory enhancing properties.

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