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COMPARATIVE EVALUATION OF ANTIDEPRESSANT ACTIVITY OF VARIOUS EXTRACTS OF *NYCTANTHES ARBOR-TRISTIS*

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Nyctanthes arbor-tristis, Immobility
time, Locomotor activity, Depression

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ABSTRACT: The present study evaluates the antidepressant activity of *Nyctanthes arbor-tristis* (NT). In the present study antidepressant effects of hydroalcoholic leaf extract (250 & 500 mg/kg) (NTL), flower extract (250 & 500 mg/kg) (NTF) and combination extract (leaf+flower extract in equal ratio) (250 & 500 mg/kg) (NTC) of *Nyctanthes arbor-tristis* were evaluated by the use of forced swim test. The animals were divided into different groups and were given daily dosing for seven successive days. On the seventh day, the immobility time and locomotor activity were noted down. The locomotor activity was taken before and after dosing. Initial phytochemical investigations showed the presence of some active constituents which were important for the antidepressant activity. All extracts of NT & imipramine treated groups demonstrated a significant reduction of immobility time. There were also statistically remarkable differences in immobility time in groups treated with combination extract compared to groups treated with leaf and flower extract alone. All extracts of NT also showed slight increment in the locomotor activity but were not statistically significant. Therefore, it was concluded that the hydroalcoholic extracts of NT show remarkable antidepressant effect.

INTRODUCTION: There are associations of low mood levels and reduced level of activity with depression; this alters the person's impression, thinking, perceptions, and attitude^{1, 2}. Depression involves many symptoms, and the person feels guilty, sad, anxious, inferior, helpless, and uneasy. People who have been diagnosed with depression are not able to carry out their tasks in a proper manner; this leads to a reduction in their appetite, poor concentration, inability to make decisions, forgetfulness, a decrease of motor function, and withdrawal from society^{3, 4, 5}. They are prone to suicidal thoughts and even commit suicide.

Alterations occur in day to day activities like changes in sleep patterns. Also, aches, tiredness, perceptions of pain, problems in digestion, and low energy level form characteristic features of depression^{6, 7, 8}. Depression is a very common disease in different ages, genders, different societies and instances of depression are also found in some animals^{9, 10, 11}.

Depression is a neurometabolic type disorder, alteration in the levels of various hormones like serotonin, dopamine, and noradrenaline (neurotransmitters) is the hallmark of depression^{12, 13, 14, 15}. The treatment approaches towards depression involves the use of psychotherapy, antidepressant drugs, stress management methods, and some complementary drugs (phytotherapy)¹⁶. The drug therapy of depression by allopathic drugs carries the risk of unwanted side effects, and in most cases, the drugs therapy proceeds lifelong^{17, 18, 19}. Therefore, to explore new dimensions of

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antidepressant drug therapy, the focus needs to be diverted towards herbal antidepressant drugs, which can be developed as safer alternatives for allopathic drugs^{20, 21, 22}.

Nyctanthes arbor-tristis (Greek words, 'Nykhta'-Night and 'anthos'-flower) is a well known herbal medicinal plant and belongs to the family Oleaceae^{23, 24}. It is characterized as a type of small sacred ornamental tree of India and distributed from wild sub-Himalayan areas to Godavari^{25, 26, 27}. Commonly this medicinal plant is referred to as 'Harsinghar' or 'Night Jasmine' because flowers of this plant provide a pleasant and strong fragrance during night time^{28, 29, 30}.

This plant has been used in the Indian system of medicine and different parts of this plant are used for various pharmacological actions like antiamebic activity, antipyretic activity, antiulcer activity, antihistaminic activity, anti-inflammatory activity immunostimulant activity, antiallergenic properties, antimalarial activity, analgesic activity, tranquilizing activity and purgative activity^{31, 32, 33, 34, 35}. The active phytochemical constituents of this plant are friedelin, oleanolic acid, arborside A, arborside B and arborside C, Nyctanthic acid, β -monogentiobioside of α -crocin or crocin 3, triterpenoids lupeol, β -monogentiobioside- β -D-monoglucoside ester of α -crocin or crocin 2 and β -digiobioside of α -crocin or crocin 1.

These phytochemicals are present in different parts. The present study was undertaken for evaluation of the antidepressant activity of leaf, flower extract, and a combination of leaf and flower extract in an equal ratio of *Nyctanthes arbor-tristis*^{33, 34, 35}.

Nyctanthes arbor-tristis is a plant with numerous pharmacological activity and uses. It has been extensively studied for pharmacological activities. Therefore the study of antidepressant effects of various extracts of NT can prove to be beneficial in finding effective herbal treatment for depression. According to various studies, the safety margin of the plant was good, and there were no side effects related to plant^{36, 37, 38}.

MATERIAL AND METHODS:

Chemicals: Imipramine hydrochloride (Yarrow chem. Products, Mumbai) was used in the study. Imipramine hydrochloride (Standard) and extracts

were dissolved in distilled water and administered either intraperitoneally (i.p) or orally (p.o). Distilled water was used as the vehicle.

Preparation of Plant Extract: The plant material was taken from the local garden of Haldwani (Uttarakhand) India. The material was authenticated by Late Dr. K. S. Negi, Principal scientist, ICAR- National Bureau of Plant Genetic Resources Regional Station, Niglat, Bhowali, Nainital, Uttarakhand (Specimen no. A.G.1). The collected plant material (leaves and flowers) were dried in the shade and grounded with mixer grinder.

The powdered crude drug was extracted with ethanol 50% (hydro-alcoholic) using soxhlet apparatus with a sufficient amount of solvent for 48 h^{39, 40, 41}. The concentration of the crude plant extract was performed with the use of rotary vacuum evaporator and dried at 20 °C temperature for 72 h to yield 17.06% w/w (NTL) and 15.4% w/w (NTF).

Animals: Wistar albino rats of either sex weighing (50-100 g) were taken for the experimental work. The animal was maintained under standard condition husbandry, room temperature $26 \pm 2^\circ\text{C}$, the relative humidity of 45-55%, 12 h light/ dark cycle, in animal house approved by CPCSEA, which is Committee for control & supervision of experiment on the animal. The animal had free access to diet and water & housed in the polypropylene cages. The use of animals was approved by IAEC of Department of Pharmaceutical Sciences, Bhimtal campus, Kumaun University (Nainital) for the present work.

Drug Treatment: Acute toxicity studies have been performed on NT, and according to literature survey, two different doses were selected for NT, one was 250 mg/kg, and the other was 500 mg/kg. Also the combination extract (leaf + flower extract in equal ratio) was given in a dose of 250 mg/kg and 500 mg/kg^{41, 42}. The dosing was performed once in a day for a total duration of seven days. Distilled water was received by animals which were distributed into the control group. Imipramine hydrochloride was used as the standard drug.

Approval of IAEC (Institutional Animal Ethics Committee): The animal were obtained &

approved by IAEC of Department of Pharmaceutical Sciences, Bhimtal campus, Kumaun University, Nainital for the present research work. The IAEC approval No for the present study was KUDOPS/39.

Phytochemical Studies: Hydroalcoholic extracts of the plant *Nyctanthes arbor-tristis* were subjected to phytochemical tests for the identification of their active constituents like alkaloids, flavonoids, phenols, tannins, glycosides, carbohydrates and proteins^{33,42}.

Assessment of Antidepressant Activity:

Forced Swim Test: Forced swim test (FST) is a commonly used behavioral model for screening antidepressant activity in rats and mice. In the present study, rats were made for swimming in an enclosed space from which escape was impossible, and thus a characteristic behavior of immobility was induced in them, which served as a sign of behavioral despair. Reduction in immobility time is associated with the antidepressant effect. Wistar albino rats were used in the study. The rats were taken to the laboratory one day before the experiment & were kept separately in the cages with free access to food & water. Cylindrical perspex tank (40 cm height, 18 cm diameter), filled up to 15 cm with water (22 ± 5 °C) was used for evaluating antidepressant activity. FST was done in two phases, the induction phase (15 min) and the test phase (6 min). After 24 h of the induction phase, the rats were placed in tanks for the test phase. In the test phase, the duration of immobility (sec) was measured.

Doses were given once daily for 7 days to the rats. On the 6th day, rats were subjected to 15 min induction phase. Then rats were removed and allowed to dry in a heated enclosure. After 24 h, they were administered either the test extracts (one hour before the test) or standard drug (thirty minutes before test) according to their respective groups, and the total duration of immobility was measured during a 6 min test^{43,44}.

Spontaneous Locomotor Activity: The degree of depression was determined using the actophotometer. Spontaneous locomotor activity of plant extracts was measured automatically by using Actophotometer (Medicraft photo Actometer INCO, model no: 600M-4D, S. no: PA-0129,

India). The units of the activity counts were based on the beam crossed by the movement of the animal.

The locomotor activity was recorded individually for 10 min. Basal activity score was noted before drug administration. The level of activity was again measured after 30 min of administration of Imipramine hydrochloride and 60 minutes after NTL, NTF, and NTC. The difference in the activity was measured, and finally, a percentage decrease in locomotor activity was calculated⁴⁴.

Statistical Analysis: The data was represented as Mean \pm SEM. One-way analysis of variance (ANOVA) was performed on the data, and after that, multiple t-tests was used. All statistical analysis of the data was performed by using Graph Pad Prism, Version 6.01 software.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Studies: The preliminary phytochemical screening showed that the hydro-alcoholic leaf extract of *Nyctanthes arbor-tristis* contained steroids, carbohydrates, flavonoids, tannins, phenols, terpenoids, saponins, and alkaloids however flower extract of *Nyctanthes arbor-tristis* contained flavonoids, carbohydrates, phenols, terpenoids, saponins, and alkaloids. Thus from the above results, we can say that hydro-alcoholic extract of a combination of leaf and flower of *Nyctanthes arbor-tristis* also contains the chemical mentioned above compounds. The phytochemicals detected in the various tests could be partially or fully responsible for the reduction in the immobility time and showed antidepressant-like activity.

Assessment of Antidepressant Activity:

Forced Swim Test: After seven days treatment of rats with hydro-alcoholic extracts of leaf, flower and their combination (leaf + flower), it was seen that all extracts of *Nyctanthes arbor-tristis* at both high (500 mg/kg) and low (250 mg/kg) dose, and also the standard drug-treated group showed significant reduction in immobility time ($p < 0.0001$) compared to control group. **Table 1** shows the antidepressant activity of all extracts and standard drug when compared to the control group.

There was no statistically significant difference between the antidepressant activity of high and low

dose of leaf and flower extract when compared to the standard, but the combination extract (leaf + flower) at both high and low dose showed statistically significant greater activity compared to standard imipramine treated group ($p < 0.005$).

When the individual extract of leaves and flowers of NT were compared with combination extract (leaf + flower) concerning antidepressant activity, it was found that both high and low dose of individual leaf and flower extract showed significantly more immobility time as compared to the combined extract (leaf + flower).

Thus, the combination showed significantly greater reduction in the immobility time compared to individual extracts of leaf and flower ($p < 0.001$ and $p < 0.005$ respectively). The flower extract showed slight greater activity compared to leaf extract at both low and high dose, but these effects were not found to be statistically significant. **Table 2** shows the comparison between the antidepressant activity of leaf, flower, and combination extract. **Fig. 1**, shows the effect of control, hydroalcoholic extracts of NT, and standard drug on immobility time in FST.

TABLE 1: EFFECT OF HYDROALCOHOLIC EXTRACTS OF NT AND STANDARD DRUG-ON IMMOBILITY TIME IN FST

| S. no. | Treatment group | Dose | Immobility time (seconds) [n=6] |
|--------|---------------------------------|-----------|---------------------------------|
| 1 | Control Group (Distilled water) | 10 ml/kg | 197.34 ± 9.912 |
| 2 | Standard Group (Imipramine HCl) | 15 mg/kg | 100.667 ± 9.959* |
| 3 | Test Group A (NTL) | 250 mg/kg | 129.34 ± 8.293* |
| 4 | Test Group B (NTL) | 500 mg/kg | 112.0 ± 9.294* |
| 5 | Test Group C (NTF) | 250 mg/kg | 109.667 ± 12.792* |
| 6 | Test Group D (NTF) | 500 mg/kg | 104.167 ± 11.791* |
| 7 | Test Group E (NTC) | 250 mg/kg | 60.667 ± 7.279*# |
| 8 | Test Group F (NTC) | 500 mg/kg | 53.333 ± 9.149*# |

* $p < 0.0001$ statistical significance in comparison to control group. # $p < 0.005$ statistical significance in comparison to standard group.

TABLE 2: COMPARATIVE EFFECT OF HYDRO-ALCOHOLIC EXTRACTS OF NTL (LEAF EXTRACT), NTF (FLOWER EXTRACT) AND NTC (LEAF + FLOWER EXTRACT) ON IMMOBILITY TIME IN FST

| S. no. | Treatment group | Dose | Immobility time (seconds) [n=6] |
|--------|--------------------|-----------|---------------------------------|
| 1 | Test Group A (NTL) | 250 mg/kg | 129.34 ± 8.293*# |
| 2 | Test Group B (NTL) | 500 mg/kg | 112.0 ± 9.294*## |
| 3 | Test Group C (NTF) | 250 mg/kg | 109.667 ± 12.792*### |
| 4 | Test Group D (NTF) | 500 mg/kg | 104.167 ± 11.791*### |
| 5 | Test Group E (NTC) | 250 mg/kg | 60.667 ± 7.279 |
| 6 | Test Group F (NTC) | 500 mg/kg | 53.333 ± 9.149 |

* $p < 0.001$, ** $p < 0.007$ & *** $p < 0.10$ statistical significance in comparison to combination extract at low dose (250mg/kg). # $p < 0.0001$, ## $p < 0.001$ & ### $p < 0.005$ statistical significance in comparison to combination extract at high dose (500 mg/kg).

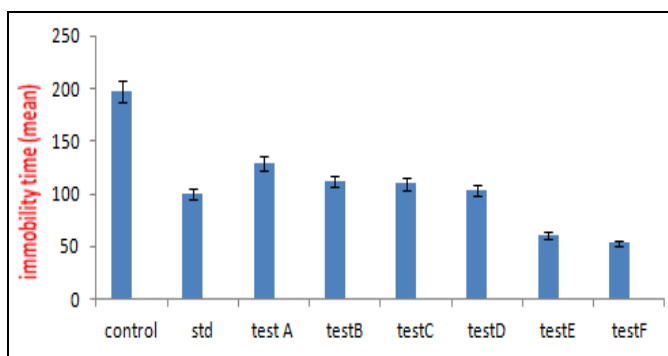


FIG. 1: EFFECT OF HYDROALCOHOLIC EXTRACTS OF NT AND STANDARD DRUG ON IMMOBILITY TIME IN FST

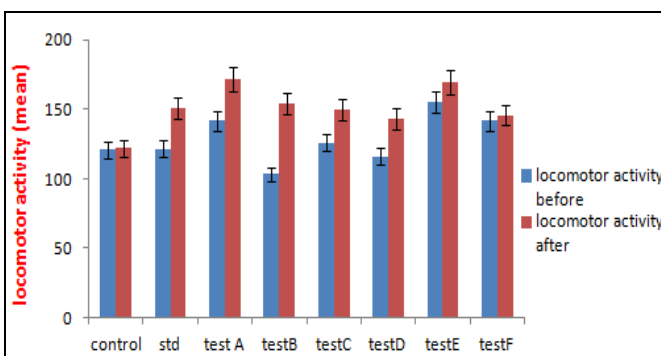


FIG. 2: EFFECT OF HYDROALCOHOLIC EXTRACTS OF NYCTANTHES ARBOR-TRISTIS AND STANDARD DRUG ON LOCOMOTOR ACTIVITY

Spontaneous Locomotor Activity: Seven days treatment of rats with hydroalcoholic extracts of leaf, flower, combination (leaf + flower) at both low and high doses and standard drug (imipramine

HCl) showed an increase in the locomotor activity after drug treatment as compared to before drug treatment. Increase in the locomotor activity is considered to be due to an increase in alertness and

decrease in the activity indicates sedative effect or CNS depression in animals⁴⁴. **Table 3**, shows the mean score of locomotor activity of the standard group and *Nyctanthes arbor-tristis* extract treated groups. After treatment of rats with various extracts of *Nyctanthes arbor-tristis* at both low dose and

high dose, there was an increase in the locomotor activity, but the values were not statistically significant. **Fig. 2**, shows the effect of hydroalcoholic extracts of *Nyctanthes arbor-tristis* and standard drug on locomotor activity in rats.

TABLE 3: EFFECT OF HYDRO-ALCOHOLIC EXTRACTS OF NT AND STANDARD DRUG-ON LOCOMOTOR ACTIVITY

| S. no. | Treatment group | Mean score of locomotor activity | | % Increase in activity |
|--------|--------------------------------------|----------------------------------|------------------|------------------------|
| | | Before dose | After does | |
| 1 | Control (Distilled water) (10 ml/kg) | 121.167 ± 11.107 | 122.0 ± 12.503 | 0.69% |
| 2 | Standard (Imipramine HCl) (15 mg/kg) | 121.50 ± 13.764 | 151.167 ± 9.911 | 24.42% |
| 3 | Test A, NTL (250 mg/kg) | 141.833 ± 12.063 | 172.0 ± 8.422 | 21.27 % |
| 4 | Test B, NTL (500 mg/kg) | 103.333 ± 13.744 | 153.667 ± 13.071 | 48.71 % |
| 5 | Test C, NTF (250 mg/kg) | 126.167 ± 3.719 | 149.833 ± 7.743 | 18.76 % |
| 6 | Test D, NTF (500 mg/kg) | 116.167 ± 12.924 | 143.167 ± 11.771 | 23.24 % |
| 7 | Test E, NTC (250 mg/kg) | 155.167 ± 7.622 | 169.333 ± 8.597 | 9.13 % |
| 8 | Test F, NTC (500 mg/kg) | 143.667 ± 5.696 | 145.833 ± 6.519 | 1.51 % |

CONCLUSION: The hydro-alcoholic extract of leaf and flower of NT and their combination were subjected to various pharmacological studies to establish the antidepressant activity. From the present investigation, we can conclude that leaf, flower, and combination extracts of *Nyctanthes arbor-tristis* showed antidepressant action.

Also, the individual leaf and flower extracts, when compared to the combined extract of *Nyctanthes arbor-tristis*, showed significantly more immobility time in FST. This means that the combination at both high and low dose showed better activity than the leaf and flower extract. Phytochemical testing performed on various hydroalcoholic extracts of NT revealed the presence of phytochemicals like phenols, terpenoids, alkaloids, and flavonoids.

So, the antidepressant activity might be due to one or more phytochemicals present in the extract. However, the exact phytochemicals responsible for this activity need to be investigated. Based on the pattern of reduction in immobility time and % increase in locomotor activity it can be said that the leaves, flowers, and combination of leaves and flowers of NT demonstrated decent antidepressant activity in the present study.

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