Antistress Activity of *Nyctanthes arbor-tristis* fruits in rats

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**Abstract**

*Nyctanthes arbor-tristis*, (Fam. Oleaceae) is commonly known as Parijatham, Harsinghar and Night Jasmine. 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. They have also been investigated for various pharmacological actions. Water soluble fraction of ethanolic extract of fruits of *Nyctanthes arbor-tristis* Linn (NATF) was pharmacologically validated for its antistress properties in normal and stress induced experimental animals. Extract was given orally at different dose levels (250 and 500 mg/kg p.o.) once daily for seven consecutive days. For the evaluation of antistress activity, groups of rats (n = 6) were subjected to forced swim stress one hour after daily treatment of NATF. Urinary vanillylmandelic acid (VMA) and ascorbic acid were selected as non-invasive biomarkers to assess the antistress activity. The 24 h urinary excretion of vanillylmandelic acid (VMA) and ascorbic acid were determined by spectrophotometric methods in all groups under normal and stressed conditions. The NATF observed under above parameters showed positive antistress activity.

**Keywords:** Antistress, *Nyctanthes arbor-tristis*, Harsingar

**Introduction**

Stress is the body's reaction to a change that requires a physical, mental or emotional adjustment or response. Stress can come from any situation or thought that makes you feel frustrated, angry, nervous, or anxious. Stress is caused by an existing stress-causing factor or "stressor" (Angela, 2011) the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs (Kumara, 2001). *Nyctanthes arbor-tristis*, (Fam.Oleaceae) is commonly known as Parijatham, Harsinghar and Night Jasmine. The leaves, flowers, seeds and bark of *Nyctanthes arbor-tristis* are widely used in traditional remedies and folkloric medicines in India. Widely distributed throughout India and also cultivated in gardens for its fragrant flowers.
(Kirtikar and Basu, 1935; Singh et al, 1995). The 50% ethanolic extract of the seeds, leaves, roots, flowers and stem of the plant has been proved to possess' antiamoebic (Chitravanshi et al, 1992) and anti allergic properties (Gupta et al, 1995). Many iridoid glycosides have been isolated from the leaves and seeds of the plant. These include arborside A, arborside B and arborside C (Srivastava et al, 1990) Earlier, we have reported the anxiolytic activity (Tripathi et al, 2010a) and antidepressant activity (Tripathi et al, 2010b) of leaf extract of Nyctanthes arbor-tristis. In the present investigation, the antistress activity in fruits of Nyctanthes arbor-tristis linn was evaluated in-vivo, in normal and stress induced rats following a biochemical approach.

**Experimental**

**Preparation of extract**

The fruits of Nyctanthes arbor-tristis were collected from the local garden of Lucknow, India in the month of November. The plant material was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow. A voucher specimen (LWG accessions No. 94392) of the collected sample was deposited in the institutional herbarium for future reference. The powdered fruits of Nyctanthes arbor-tristis (2 kg) were passed through S.S. sieve (20 meshes) before extraction. Plant material was successively extracted with ethanol (50%) in soxhlet apparatus. The crude extract obtained was concentrated in a rotary evaporator under reduced pressure and freeze dried to yield 10.6% w/w.Water soluble fraction of this extract (NATF) was taken for the study.

**Drug treatment**

In the acute toxicity study no deaths were observed during the period at the doses tested up to 2000 mg/kg. Hence, the NATF was administered orally at two different dose levels (250 and 500 mg/kg) once daily for seven consecutive days. Control group of animals received suspension of 1% CMC in distilled water.

**Animals**

Adult albino rats (150-180g) of either sex were obtained form the Animal House of the Institute and were randomly distributed into different experimental groups. The rats were housed in groups of six in polypropylene cages at an ambient temperature of 25±10\(^0\)C and 45-55% RH with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals.

**Antistress activity**

Rats of either sex weighing between 150-180 gm were divided into three groups each containing six animals. The 24 h urine sample from each group was collected into two different beakers, one containing 5 ml of 10% oxalic acid for the spectrophotometric determination of ascorbic acid at 550 nm (Roe, 1993) and the other containing 0.5 ml of 6 N hydroch-
loric acid for the determination of vanillylmandellic acid (VMA) spectrophotometrically at 360 nm (Pisano and Crout, 1962). The experimental protocol was divided into four phases. In the first phase of the experiment, 24 h urine samples were collected in all the three groups and subjected to analysis for both VMA and ascorbic acid and the normal values were recorded for seven consecutive days. In the second phase, the animals in each group were subjected to fresh water swimming stress individually. In this method, rats were forced to swim until exhausted (three to four minutes) in a cylindrical vessel of 60 cm height and 45 cm diameter containing water at room temperature (28°C). Water depth was always maintained at 40 cm. The 24 h urinary levels of VMA and ascorbic acid under stressed conditions were determined again as described above daily for seven consecutive days. The third phase of the experiment consists of administration of *Nyctanthes arbor tristis linn* extract to the same groups of animals after having recovered completely to normal condition. Groups II and III were administered orally with *Nyctanthes arbor tristis linn* extract (suspended in 2% gum acacia) at daily doses of 250 and 500 mg/kg body weight respectively for seven consecutive days while group I serving as control. The 24 h urine samples were collected and the levels of both VMA and ascorbic acid were determined. The final phase of the experiment consisted of administration of *Nyctanthes arbor tristis linn* extract to the same groups of animals after a recovery period of one week. Groups II and III were administered orally with extract of *Nyctanthes arbor tristis linn* at doses of 250 and 500 mg/kg body weight respectively, one hour prior to the daily induction of stress for three consecutive days while group I serving as control. The 24 h urine samples were collected and analyzed for VMA and ascorbic acid for seven consecutive days to study the influence of the extract on the stress induced biochemical changes (Satyanarayana et al, 2005).

**Statistical analysis**

The values were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman–Keuls test using Prism Pad software for the determination of level of significance.

**Results and Discussion**

Figure 1 and figure 2 reveals the excretion data of VMA and ascorbic acid in different phases of the experiment. It was observed that forced swim stress produced a significant increase in VMA and decrease in ascorbic acid excretion as compared to their respective basal excretion in normal condition. Normal level for both the parameter was recovered within three to four days after withdrawing the stress. It was also noticed that under normal condition *Nyctanthes arbor tristis linn* extract could not alter the excretion of VMA and Ascorbic Acid however daily administration of the same one hour prior to the induction of stress significantly inhibited the increase in VMA and decrease in ascorbic acid excretion manifested during the stress only. The activity was found to be significant at both the doses level in a dose dependent manner.

The reason for stress induced disorders cannot obscure the simple fact that the exhaustion of energy supply still forms the basis that triggers the disorders and collapse of energy metabolism following glucose deprivation in circulation (Aloe et al, 2002). It is reported that
noradrenaline is released during stressful conditions (Ion, 1969) and metabolized to vanillyl mandelic acid (VMA) peripherally and 3-methoxy 4-hydroxyphenyl glycol (MOPEG) centrally. Several studies also showed the decreased tissue levels of ascorbic acid on application of stress (Kutla and Forbes, 1993). Ascorbic acid being a free radical scavenger (Jose and
Kutan, 1995), it is more likely utilized in scavenging the free radicals involved in stress resulting in its decreased urinary concentration and also it has role in the biosynthesis of noradrenaline (Kalner, 1983) i.e., as a cofactor in the conversion of dopamine to noradrenaline (Goodman, 2001). In the present study, the increase in the urinary VMA excretion during stress was used as a non-invasive biochemical marker to study the antistress activity of fruit extract of Nyctanthes arbor tristis linn while ascorbic acid excretion in urine was taken as an indirect biochemical index to indicate the influence of stress on catecholamine synthesis in experimental rats.

Treatment with Nyctanthes arbor tristis linn extract along with stress reversed the stress induced biochemical changes i.e., increase in urinary VMA levels and decrease in urinary ascorbic acid levels, in a dose dependent manner. The present study provides scientific support for the antistress (adaptogenic), activities of water soluble fraction of ethanolic extract of Nyctanthes arbor tristis fruits.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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