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# ANTIOXIDANT POTENTIAL OF *NYCTANTHES ARBOR TRISTIS*

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## ABSTRACT

*Nyctanthes arbor tristis* is native to the Bengal region of India, where it is known as Shephali. Hydroalcoholic extract of Nyctanthes Arbor tristis was evaluated for antioxidant potential by nitric oxide scavenging activity and *in vitro* lipid peroxidation at various concentrations ranging from 10 to 1000 and 5 to 100 µg/ml respectively, due to wide arena of its pharmacological actions. In present studies both *In vitro* antioxidant models ofplant releaved, free radicals such as hydroxide anions which could play a vital role as probable antioxidant agent thereby prove its utility in inflammation.

## INTRODUCTION

Herbal therapy provides rational means for the treatment of many internal diseases, some of which are considered obstinate and incurable in other systems of medicine. Herbal medicine has come of age. Quietly, behind the scenes, it has been an ongoing 'appliance of science'[1]. Ayurveda is the oldest healing system of medicine. Major formulations used in Ayurveda are based on herbs. The medicinal herbs described in Ayurveda are used as decoctions, infusions, tinctures, and powders. Ayurvedic treatise Charaka and Sushruta have included special chapters on Ayurvedic preparations in chapters on 'Kalp-Sthana'. It deals with methods of preparation and dose of formulations.When two or more herbs are used in formulations, they are known as polyherbal formulations. Some time herbs are combined with mineral preparations also. The concept of polyherbalism is peculiar to Ayurveda although it is difficult to explain in term of modern parameters. Sanghar Samhita highlights the concept of synergism behind polyherbal formulations[2].

Nyctanthes arbor tristis belonging to Oleaceae, native to the Bengal region of India, where it is known as *Shephali* or *Parijat.* The tree is sometimes called the tree of sorrow because the flowers lose their brightness during daytime; the taxonomic name *arbortristis* also means "sad tree". The flowers can be used as a source of yellow dye for clothing. The flower is the official flower of the state of West Bengal, India, as well as the Kanchanaburi Province, Thailand. Anti-inflammatory activity in leaves of NAT supports its use in various inflammatory conditions [3].

Since antioxidant and membrane stabilizing activity have not been systematically evaluated in the NAT, therefore *in vitro* antioxidant studies were undertaken to enlighten the probable the major mechanism which could be involved in wide range of the protective effects of the healing utility.

## MATERIALS AND METHODS

### *Polyherbal formulation collection and extraction:*

Parijat (NAT) was procured as a gift sample from Herb Pharmaceuticals, India. The NAT was extracted with hydroalcoholic solvent (80% methanol) using Soxhlet apparatus for 48 hrs at 50- 60°C. The extract was filtered and evaporated under reduced pressure to give a dry powder. The powder was stored in an airtight ambered colored glass container and further used for the *in vitro* models of antioxidant potential.

### *Experimental animals:*

Albino mice of Swiss strain (20-25kg) were purchased from Bharat Serum and Vaccines, Thane. The animals were housed in polypropylene cages and maintained under standard conditions (12 hours light/12 hours dark cycle; 25 + 3○C; humidity 35-60 %).They were fed with Amrut brand pelleted standard diet manufactured by Nav

Maharashtra Chakan oils, Ltd., Maharashtra and drinking water *ad libitum*. The animals had free access to water all the time and were allowed to adapt to the animal house conditions by keeping them for a period of 8-10 days prior to using them for the experiments. The study was conducted after seeking clearance from the Institutional animal ethical committee.

***Chemicals and reagents:***

Acetic acid, Ascorbic acid, ferrous sulphate, Potassium chloride, Sodium bicarbonate,

Sodium carboxyl methyl cellulose, Sodium hydroxide, Sodium laurel sulphate, Sodium

niroprusside, Sulphuric acid and Tris-HCl buffer were procured from Sd Fine Chem Ltd, Mumbai**.**

Glacial Acetic Acid, n-Butanol, Pyridine was obtained from Sisco Labs., Mumbai.

Sodium Chloride, Ethanol was procured from Merck ltd., Mumbai.

Sulphanilamide, Phosphoric acid, 0.1% naphthylethylenediamine dihydrochloride From Hi-Media, Mumbai.

***Instruments:***

UV-Visible Spectrophotometer (Shimadazu) for carrying antioxidant studies. Homogenizer to homogenize liver for *invitro* lipid peroxidation studies.

***In-vitro antioxidant activity models***

*Nitric oxide scavenging activity*

1.5 ml, 10 mM sodium nitroprusside in phosphate buffer saline pH 7.4 was mixed with 0.5ml various concentrations (10 to 1000 µg/ml) of NAT and the mixture was incubated at 25º C for 150 min. During which sodium nitroprusside spontaneously generates nitric oxide. After the incubation 1.5 ml Griess reagent (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride) was added. The reaction mixture incubated at room temperature for 30 min. The absorbance was measured with UVspectrophotometer at 540 nm [4].The activity of NAT was compared with ascorbic acid which was used as a standard antioxidant .The nitric oxide scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = \frac{(A0 - A1) \times 100}{(A0)}$$

Where,

A0 is the absorbance of the control (blank, without NAT)

A1 is the absorbance in the presence of the NAT or the standard ascorbic acid.

*In vitro lipid peroxidation*

*Preparation of liver homogenate:*

The liver that was excised from the mice was weighed and chilled in ice cold saline. After washing with ice cold saline tissue homogenates was prepared in a ratio of 1g of wet tissue to 9ml of KCl.

*Assay Procedure:*

The reaction mixture contained 0.2 ml, 10% w/v mice liver homogenate in 0.2 ml, 0.15

M Potassium Chloride, 0.4 ml Tris buffer pH 7.5 and various concentrations (5-100µg/ml) of MECA. Lipid peroxidation was initiated by addition of 0.1 ml, 10 µM Ferrous Sulphate and 0.1 ml, 100µM Ascorbic Acid. The reaction mixture was incubated at 37º C for 1h. After the incubation, reaction was terminated by adding 2 ml 0.8% w/v thiobarbituric acid. The contents were heated at 95º C for 15 min for development of colored complex. The tubes were cooled and centrifuged at 4000 rpm for 10 min and supernatant were removed and its color intensity was

measured at 532 nm [5]. The activity of NAT was compared with curcumin which was used as a standard in lipid peroxidation. The inhibition of lipid peroxidation was calculated according to the following equation:

**% inhibition = (A0 –A1) X 100**

**(A0)**

Where,

A0 is the absorbance of the control (blank, without MRG)

A1 is the absorbance in the presence of the NAT or the standard curcumin.

***Statistical analysis:***

The data was analyzed using SPSS packages (version 6.0) and $IC_{50}$ values representing the concentration required to induce 50 % inhibition was determined from the linear segment of the curve obtained by plotting % inhibition on Y axis versus concentration of NAT concentration on X axis. The NAT was compared with standard antioxidant drug having $IC_{50}$ and the correlation coefficients (R2) were calculated from the graph.

**RESULTS**

*Evaluation of antioxidant activity*

*Nitric oxide scavenging activity*

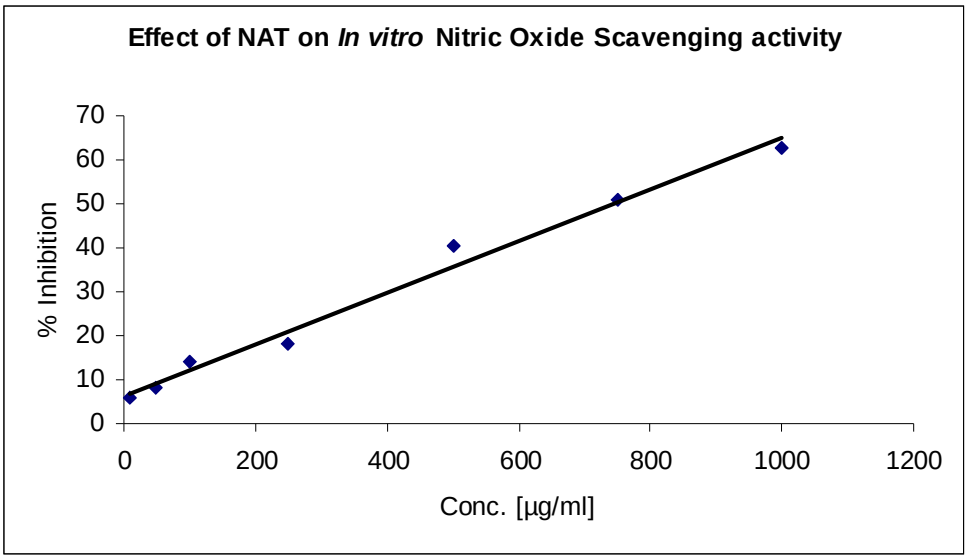
NAT showed a free radical scavenging action against nitric oxide (NO) induced release of free radicals. The $IC_{50}$ value of NAT was found to be 744.56 µg/ml. The correlation coefficient ($R^2$) was calculated from the graph and found to be 0.9930. (Table-1 and figure no.1)

**Table No 1:Effect of NAT on Nitric Oxide Scavenging Activity**

| Concentration (µg/ml ) | Nitric oxide scavenging activity of NAT | $IC_{50}$ Value of NAT |
|---|---|---|
| 10 | 5.87 | |
| 50 | 8.29 | |
| 100 | 14.02 | |
| 250 | 18.40 | 744.56 µg/ml |

| | | |
|---|---|---|
| 500 | 40.60 | |
| 750 | 50.84 | |
| 1000 | 62.60 | |

**Fig. no 1: Effect of NAT on Nitric Oxide Scavenging Activity**




Equation for the line for the linear segment of the curve

Y=0.0585x +6.4429 and R= 0.9930

$IC_{50}$ value= 744.56 µg/ml.

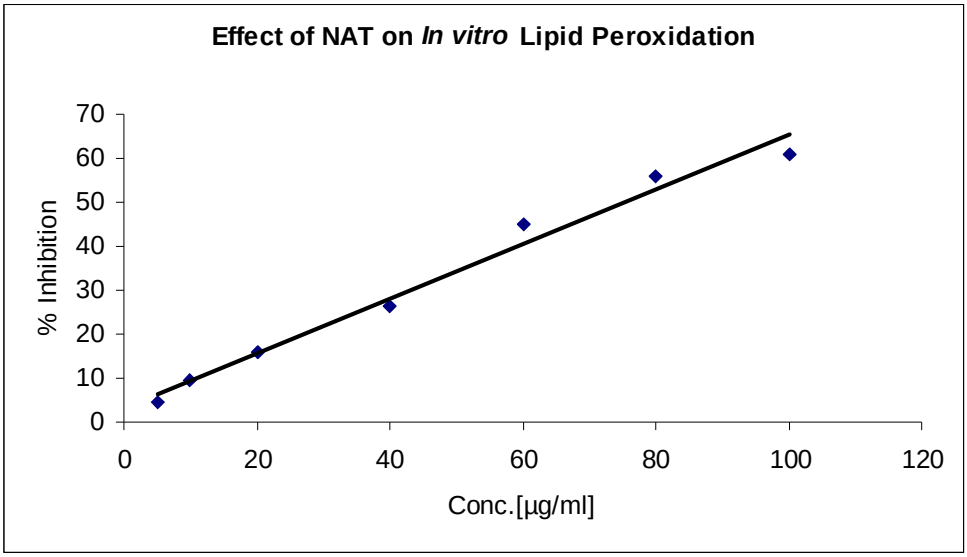
*Inhibition of lipid peroxidation*

NAT elicited concentration dependent inhibition of $FeSO_4$ induced lipid peroxidation in mice liver homogenate. The $IC_{50}$ value of NAT is 75.14 µg/ml. The correlation coefficient ($R^2$) was calculated from the graph and found to be 0.9913 (Table-2 and figure no.2).

**Table No 2: Effect of NAT on Inhibition of Lipid Peroxidation**

| Concentration (µg/ml ) | Inhibition of lipid peroxidation of NAT | $IC_{50}$ Value of NAT |
|---|---|---|
| 5 | 4.71 | 75.14µg/ml |
| 10 | 9.70 | |
| 20 | 15.87 | |
| 40 | 26.59 | |
| 60 | 45.05 | |
| 80 | 56.05 | |
| 100 | 61.03 | |

**Fig no.2: Effect of NAT on Inhibition of Lipid Peroxidation**




Equation for the line for the linear segment of the curve

Y=0.6209 +3.3456 and R= 0.9913

$IC_{50}$ value= 75.14 µg/ml

## DISCUSSION AND CONCLUSION

Sodium nitroprusside serves as a chief source of free radicals. Scavengers of nitric oxide compete with oxygen leading to reduced formation of Nitric Oxide (NO). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine is used as themarker for NO scavenging activity [4]. The chromophore formation was not complete inthe presence of NAT, which scavenges the NO thus formed from the sodiumnitroprusside and hence as the absorbance increases as the concentration of the NAT increases in a dose dependent manner.

Lipid peroxidation has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. The NAT extract exhibited protection against lipid peroxidation induced by FeSO4.Initiation of lipid peroxidation by ferrous sulphate takes place through Ferrylperferryl complex [6,7]. The NAT inhibited the FeSO4 induced lipid peroxidation in a dose dependant manner. The inhibition could be caused by the inhibition of formation of Ferryl perferryl complex. The presence of flavonoids in polyherbal formulation may be attributed for antioxidant activities.

## CONCLUSION

The data obtained in the present investigation suggests that NAT may be potential anti-inflammatory and antioxidant agent. Hence it is essential to investigate the

exact underlying molecular mechanism(s) of action of the plant.

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