Free radical scavenging activities of *Nyctanthes arbor-tristis*. L on adjuvant induced arthritis in rats

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ABSTRACT

Aims: The present study was undertaken to explore *in vivo* antioxidant potential of ethanol extracts of *Nyctanthes arbor-tristis* leaf and stem in adjuvant induced arthritic rats.

Methodology: Arthritis induced rats were administered with extract of *Nyctanthes arbor-tristis* leaf and stem. (150 mg/kg body Weight/rat/day for 30 days.

Results: A significant decrease in paw edema was observed following oral administration of the leaf and stem extracts. A significant ($p<0.05$) increase in the level of tissue TBARS, GPx and catalase was seen in arthritis induced rats (group II) and NAT treated rats (group III and group IV) showed a significant decrease in lipid peroxides, GPx and catalase level to near normalcy. The activity of total tissue SOD was found significantly ($p<0.05$) low in arthritis induced rats (group II) while a substantial increase in the activity to near normal level was noticed in NAT administered rats. The alterations in hematological and other biochemical parameters were restored to near normal levels after a treatment period of 30 days. The structural changes of the tissues shows the therapeutic ability of *Nyctanthes arbor-tristis* stem and leaf in experimental animals which were further evidenced by histological observations made on the hind limb tissue.

Conclusion: As *Nyctanthes arbor-tristis* is of natural origin, it is a safe and effective intervention for free radical mediated diseases.

Key words: Anti-inflammatory, Arthritis, *In vivo* antioxidant, lipid peroxidation, Superoxide dismutase.

1. INTRODUCTION

Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. *Nyctanthes arbor-tristis* L. commonly known as Night flowering Jasmine) a shrub or a small tree growing to 10 m tall, with flaky grey bark. The seeds, flowers and leaves possess immunostimulant, hepatoprotective [1], antileishmanial, antiviral and antifungal activities [2]. The decoctions of leaves are extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative [2, 3]. The water soluble portion of the alcoholic extract of leaves of *N. arbor tristis* (NAT) has been reported to possess anti-inflammatory activity in a variety of experimental models. In addition, analgesics, antipyretic along with ulcerogenic potency have also been observed [3]. Our previous study exhibits the *in vitro* anti-oxidant activity of *N. arbor tristis* leaf extract [4, 5]. The arbortristoside A isolated from the seeds found to have antitumor activity [6].

Adjuvant induced arthritis (AIA) in experimental rats; a chronic inflammatory disease characterized by infiltration of the synovial membrane and associated with destruction of the joints resembles closely to the human rheumatoid arthritis [7]. The role of reactive oxygen species in the pathogenesis of degenerative joint disease has already been documented. Both steroidal and non-steroidal anti-inflammatory drugs currently used for the amelioration of the symptoms of the disease offer only temporary relief and often cause severe side effects like peptic ulcer and renal failure [8]. Therefore, new drugs without side effects are being studied all over the world as an alternate to NSAIDs and opiates [9]. Hence, the present study aims to investigate the *in vivo* antioxidant property and tissue defence mechanism of the leaf and stem extracts of *Nycranthes arbor-tristis* in AIA by histopathological studies (20X).

2. MATERIALS AND METHODS

2.1 Plant Materials: Collection

Nyctanthes arbor-tristis (NAT-L (leaf) and NAT- S (stem)) were collected from Coimbatore district Tamilnadu (India) and authenticated (Voucher No- KU/2009/025/12) by Dr. V.S. Ramachandran, Bharathiar University, Coimbatore, India.

2.2 Preparation of the plant extracts

Collected materials were washed thoroughly; shade dried, powdered coarsely, and named it as NAT-L (leaf) and NAT- S (stem). The powder obtained (25 g) were extracted with ethanol (250 mL) in a Soxhlet extractor for 18-20 hrs. The extracts were concentrated using rotary flash evaporator and preserved at 4^0C in air tight container. The yield from the extract was 12% for leaf and 10% for stem.

2.3 Test animals

Male albino rats of Wister strain weighing around 160–180 g were procured from College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala for the present study. The rats were fed with commercial rat diet (Hindustan Lever Limited, Mumbai, India) and water *ad libitum*. The experiments were designed and conducted in accordance with the ethical norms. Once arthritis developed, food was served on the bottom of the cages as severely arthritic rats have difficulty in feeding from the cage top.

2.4 Induction of arthritis

Arthritis was induced by a single intradermal injection of 0.1 ml of Freund's complete adjuvant (FCA) containing 10 mg/ml dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil (Difco Laboratories, USA) into a foot pad of the left hind paw of male rats [10].

2.5 Experimental set up

The optimum dosage of NAT S and NAT L were fixed as 150mg/kg based on previous toxicity studies. Animals were divided into four groups of six animals in each group as follows:

Group I - Control rats

Group II - Adjuvant induced arthritic rats

Group III - Arthritis induced rats administered with extract of NAT leaf. (150 mg/kg Body Weight/rat/day for 30 days by intubations starting 10 days after adjuvant Injection)

Group IV - Arthritis induced rats administered with extract of NAT stem. (150 mg/kg body Weight/rat/day for 30 days by intubations starting 10 days after adjuvant Injection)

Group V- Control rats administered with extract of NAT leaf (150 mg/kg body Weight/rat/day for 30 days).

Group VI- Control rats administered with extract of NAT stem (150 mg/kg body Weight/rat/day for 30 days).

2.6 Measurement of paw volume

The paw volume was measured using plethysmometer. During the experimental period, scoring of paw was restricted to once in a week to avoid excessive handling of the animals as this can reduce the severity or incidence of arthritis after adjuvant injection and the paw edema was calculated.

2.7 Collection of Blood and Tissue and preparation of tissue homogenate

Animals were sacrificed on the day 30 by cervical decapitation; blood was collected with and without EDTA for plasma and serum separation. Joint cartilage tissue is removed immediately and these samples were used for further investigations. Joint cartilage tissue samples were homogenized in a solution of ice-cold 0.1 M Tris-HCl buffer (pH 7.4) at 4◦C and centrifuged for 10 min at 15,000 *g* in 4 ◦C [11].

2.8 Biochemical assays

The hematological parameters like hemoglobin, RBC, WBC, Platelets, ESR, and PCV [12] were determined by usual standardized laboratory method. The biochemical parameters, blood glucose [13], urea [14], uric acid [15], creatinine [16], total protein [17], were also determined in serum. Marker enzymes such as glutamate oxaloacetate transaminase/aspartate aminotransferase (GOT/AST), and glutamate pyruvate transaminase/alanine aminotransferase (GPT/ALT), [18] ALP [19] and LDH [20] were analyzed.

2.9 In vivo antioxidant assays

Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich [21]. A 0.1 ml of tissue homogenate was added to the tube containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, added 0.5 ml of EDTA solution and 1 ml of buffer. The reaction was initiated by the addition of 0.5 ml of epinephrine and the increase in absorbance at 480 nm was measured in in UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 117, India). The enzyme activity was expressed as 50% inhibition of epinephrine auto oxidation/min. Glutathione peroxidase (GPx) was assayed by following Rotruck *et al.,* method [22]. The reaction mixture consisted of 0.2 ml of EDTA, 0.1 ml of sodium azide, 0.1 ml of H_2O_2, 0.2 ml of reduced glutathione, 0.4 ml of phosphate buffer, and 0.2 ml of tissue homogenate was incubated at 37 ◦C for 10 min. The reaction was

arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged at 2000 rpm. To the supernatant, 3ml of disodium hydrogen phosphate and 1.0 ml of DTNB were added and the colour developed was read at 420 nm immediately. The enzyme activity was expressed as micromoles of glutathione oxidized/min/mg protein. The Catalase activity was measured by the method of Takahara *et al.,* [23]. To 1.2 ml of phosphate buffer, 0.2 ml of the tissue homogenate was added and the enzyme reaction was started by the addition of 1.0 ml of H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30 sec intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity is expressed as micromoles of H_2O_2 decomposed/min/mg protein. The level of Lipid peroxides was estimated using thiobarbituric acid reactive substances by the method of Ohkawa *et al.* [24]. To 0.2 ml of plasma, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was diluted to 4 ml with water and then heated in a water bath at 95 ◦C for 60 min using glass ball as a condenser. After cooling, 1ml of water and 5ml of *n*-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured in UV spectrophotometer. 1, 1, 3, 3-tetramethoxypropane was used as standard. The level of lipid peroxides was expressed as n moles of TBA /100 g of tissue. The tissue homogenates were used for these studies.

2.10 Histological studies

Hind limbs were removed and fixed in 10% buffered formalin. The tissue was sectioned to 4 µm thickness and subsequently stained with haematoxylin eosin for histological examination [25].

2.11 Statistical analysis

All the grouped data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. *P* values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± S.D. for five experiments in each.

3. RESULTS AND DISCUSSION

Figure 1 depicts the anti-arthritic effect of NAT-L and NAT-S on the changes in paw edema of control and experimental animals. Swelling and redness developed over a 24 h period in the foot injected with adjuvant. There was an appreciable increase in paw volume in group II rats. This inflammation reaction was then increased at that time when disseminated arthritis appeared and remains constant by the end of 3 weeks. A significant reduction in paw volume was observed in NAT-treated rats (group III and group IV) when compared with the arthritis-induced group (group II). Paw swelling is one of the major factors in evaluating the degree of inflammation and therapeutic efficacy of the drugs. Reduction of paw swelling from the third week onwards may be due to immunological protection rendered by the plant extract [26].

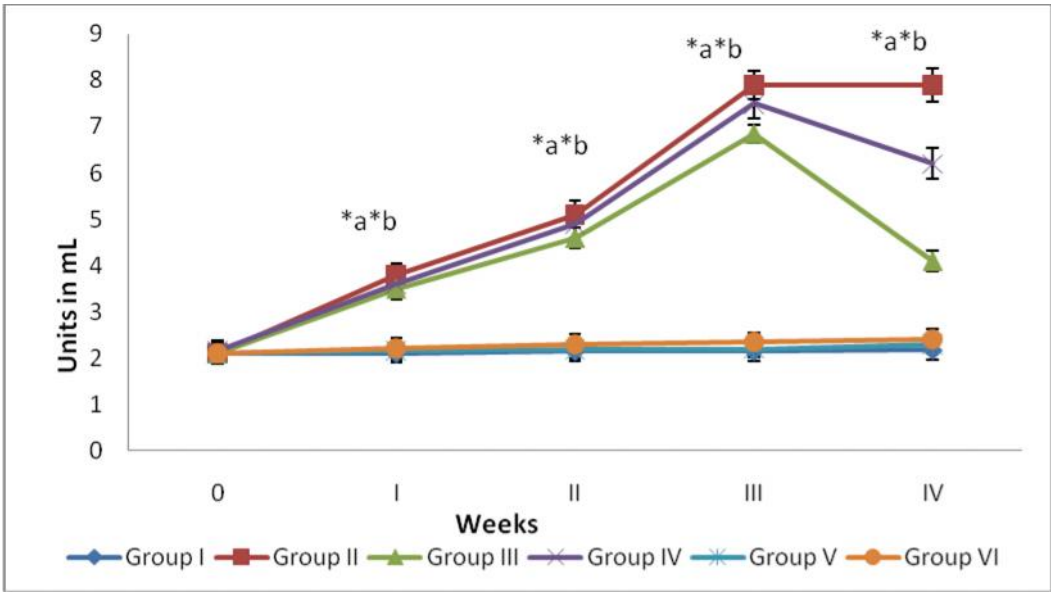

Figure 1- Paw volume changes in normal. Experimental rats. Y axis – Thickness of paw volume in mL, X axis - Weeks. Each point represents the mean ± S.D. for six animals in each group. Values are statistically significant at $*p < 0.05$; statistical significance was compared within the groups as follows: *a Arthritic rats compared with normal rats. *b NAT leaf and stem treated arthritic rats were compared with arthritic rats.

It was observed that there was no significant change in hematological parameters such as RBC, WBC, Hb, ESR, platelets, PCV and the changes in the induced group were reverted back to near normal levels on treating with NAT leaf and stem extracts (Table 1).

Table 1- Effect of NAT-L and NAT-S on hematological parameters in adjuvant induced arthritic rats

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Hb (g/dL)	14.2±0.28	9.7±0.24*a	14.01±0.2*b	12.7±0.25*b	14.2±0.13	14.3±0.3
RBC (x 10^{-6} /mm^3)	5.1±0.258	3.6±0.24*a	4.8±0.155*b	4.2±0.158NS	5.2±0.21	5.3±0.20
WBC(x10^3 /mm^3)	7.6±020	12.3±0.24*a	7.9±0.14*b	9.0±0.19*b	7.6±0.19	7.7±0.22
PLT (x10^3 cells/µL)	2.7±0.24	3.9±0.196*a	2.8±0.243*b	3.2±0.22*b	2.7±0.04	2.7±0.1
PCV%	45±2.02	25±1.975*a	43±1.984*b	33±2.08NS	46±0.18	47±0.2
ESR 30 Min	2.0±0.203	7.6±0.193*a	2.2±0.188*b	2.8±0.216*b	2.2±0.24	2.2±0.3
ESR 60min	4.2±0.193	12.8±0.23*a	3.8±0.24*b	4.0±0.223*b	4.1±0.04	4.2±0.1

Values are expressed as mean±S.D. for six animals. Comparisons are made between: *a, Group I vs. Groups II; *b, Group II vs. Groups III and IV. The letters *a and *b represent the statistical significance at $p < 0.05$. NS- Non significant. In all the experiments there were no significant changes found in extract alone administered rats (Group V and Group VI).

The levels of blood glucose, urea, uric acid and creatinine of normal and experimental groups of rats are presented in Table – 2. A significant ($p<0.05$) increase in the levels of Glucose, urea and creatinine and decrease in uric acid level were observed in arthritis-induced rats (group II) when compared with normal rats. The increase in blood glucose in arthritic rats might be due to the decreased glycolytic and increased gluconeogenic enzyme activities, since impaired hepatic biosynthetic activities were reported in AIA condition [27]. The decrease in plasma uric acid in arthritic animals might be due to its continuous utilization by the system during free radical quenching reaction [28, 29]. Renal dysfunction might be the cause of raised blood urea and creatinine levels in AIA rats.

Table-2 Effect of NAT-L and NAT-S on biochemical parameters in FCA induced experimental rats

Parameters (mg/dL)	Group I	Group II	Group III	Group IV	Group V	Group VI
Blood glucose	84.2 ± 6.47	102.8±7.7*a	87.5 ± 6.8*b	90.4 ± 9.8*b	85±3.2	86±1.6
Blood urea	17.3 ± 0.15	29.3 ±0.24*a	20.5 ±0.18*b	22.7 ± 0.1*b	17.6±0.2	18±1.4
Uric acid	1.94 ± 0.13	1.34 ±0.14*a	1.83 ±0.14*b	1.62 ± 0.18 *b	1.9±0.5	1.83±2
Creatinine	0.62 ± 0.02	1.82 ± 0.05*a	0.93 ± 0.06*b	0.92 ± 0.05 *b	0.63±0.4	0.64±0.1

Values are expressed as mean ± S.D. for six animals. Comparisons are made between: *a, Group I vs. Groups II; *b, Group II vs. Groups III and IV. The letters *a and *b represent the Statistical significance at $p < 0.05$. In all the experiments there were no significant changes found in extract alone administered rats (Group V and Group VI).

A marked increase in the activity of membrane marker enzymes (AST, ALP, and LDH) and decrease in ALT were observed in the serum of arthritic rats (group II) when compared to control rats (Group I). Arthritic rats treated with NAT-leaf extract showed a significant ($p<0.05$) change in the activity of membrane marker enzymes. Compared to NAT-S, NAT-L showed greater activity (Table 3). Assessment of the levels of AST, ALT, and ALP provides an excellent and simple tool to measure the anti-arthritic activity of the target drug. The activity of ALP was significantly increased in arthritic rats, since it is good index of liver and kidney impairment which is also considered a feature of adjuvant arthritis [30].

Table 3- Activities of membrane marker enzymes in the serum of control and experimental rats

Parameters (U/L)	Group I	Group II	Group III	Group IV	Group V	Group VI
AST	0.38±0.022	0.83±0.02*a	0.41±0.03*b	0.47±0.04*b	0.39±0.01	0.39±0.24
ALT	0.45±0.03	0.012±0.002*a	0.41±0.04*b	0.33±0.04*b	0.44±0.23	0.45±0.37
ALP	2.62±0.03	4.8±0.53*a	2.64±0.07*b	3.35±0.04*b	2.62±0.01	2.61±0.4
LDH	9.4±0.24	17.9±0.29*a	9.8±0.25*b	11.1±1.62*b	9.5±0.22	9.4±0.31

Values are expressed as mean ± S.D. for six animals. Comparisons are made between: *a, Group I vs. Groups II; *b, Group II vs. Groups III and IV. The letters *a and *b represent the statistical significance at $p < 0.05$. In all the experiments there were no significant changes found in extract alone administered rats (Group V and Group VI).

The activity of total tissue SOD was found significantly ($p<0.05$) low in arthritis induced rats (group II) than in control rats and a substantial increase in the activity to near normal level was noticed in NAT administered rats (Fig. 2). Activity was found to be higher in leaf extract (group III) than stem extract (group IV). The increased enzyme activity in NAT administered rats suggests a response of the animals against possible damage caused by oxygen free radicals. The superoxide radicals are the first product of molecular oxygen reduction. It acts as a catalyst for dismutations of superoxide radicals into H_2O_2 and into molecular oxygen to protect cells and tissues from superoxide radicals and other peroxides such as lipid peroxides *in vivo* [31].

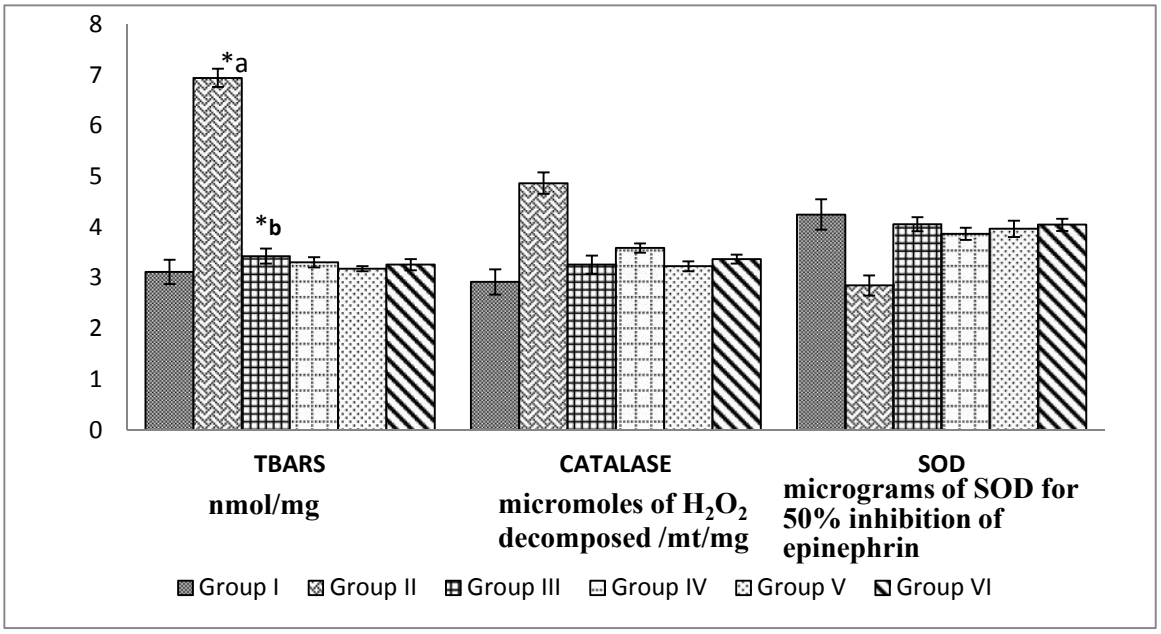

Figure 2- Effect of NAT-L and NAT-S extract on the antioxidant activity in normal and experimental rats. Values are expressed as mean ± S.D. for six animals. Comparisons are made between: *a, Group I vs. Groups II; *b, Group II vs. Groups III and IV. The letters *a and *b represent the Statistical significance at $p < 0.05$

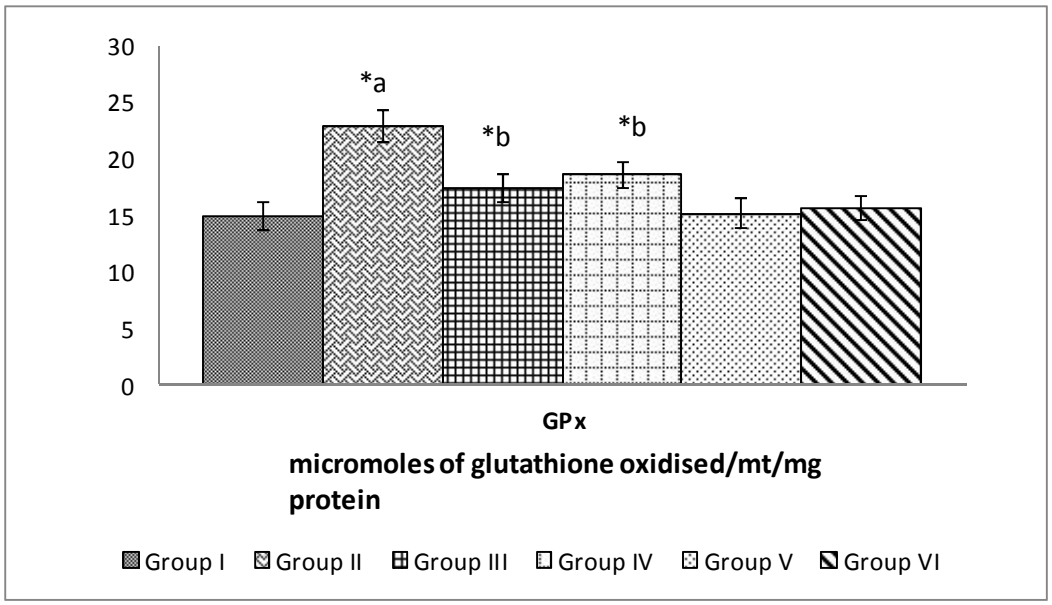

Figure 3- Effect of NAT-L and NAT-S extract on the GPx activity in normal and experimental rats. Values are expressed as mean ± S.D. for six animals. Comparisons are made between: *a, Group I vs. Groups II; *b, Group II vs. Groups III and IV. The letters *a and *b represent the Statistical significance at $p < 0.05$

Figure 2 elucidates the significant ($p<0.05$) increase in the level of tissue TBARS, GPx and catalase in arthritis induced rats (group II) when compared to control rats (group I). The NAT treated rats (group III and group IV) showed a significant decrease in lipid peroxides, GPx and catalase level to near normalcy. Lipid peroxidation is considered as a critical mechanism of the injury that occurs during RA. Oxygen free radicals are potent lipid peroxidation-inducing agents that cause the depletion of unsaturated fatty acids of the cell membrane, thus inducing loss of cell integrity and functional alteration of cell receptors and enzymes, in many diseases ,especially rheumatoid arthritis, membrane damage often occurs in some organ or tissue, which provokes and accelerates the disorder structurally and functionally. The lack of antioxidant defense leads to an increase in lipid peroxidation and subsequent deleterious effects. In the present study, the increased lipid peroxides may be due to poor antioxidant defense system. Catalase, which decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals and also inhibits other long-chain peroxides. A significant increase in catalase activity in arthritis induced group as compared to the normal rats signifies over production of hydrogen peroxide. For a given concentration of catalase, the initial rate of hydrogen peroxide removal is proportional to the hydrogen peroxide concentration. Glutathione peroxidase (GPx) is localized in the cytoplasm and mitochondria, which catalyses the degradation of various peroxides by oxidizing glutathione with the formation of its conjugates. GPx has more affinity than catalase for H_2O_2. GPx is essential for the conversion of glutathione to oxidized glutathione during which H_2O_2 is converted to water. The observed increase in the activity of GPx in joint tissue in arthritic rats indicate the increased H_2O_2 concentration and may help understanding the pathogenesis associated with arthritis [32-35]. In the previous study phytochemical

screening of the extract of the leaves and stems of Nyctanthes arbor-tristis revealed the presence of flavonoids, tannins, saponins, glycosides, alkaloids, steroids, and phenolic compounds which act as free radical scavengers [4] to combat the disease.

Histopathological examinations revealed that marked infiltration of leukocytes and eosinophilic inflammatory exudates in the synovial membrane (Plate 1). Plate 1 shows joint tissue of control rats with synovial lining and normal joint space in between two articular cartilages (group I). Plate 2a, 2b, and 2c illustrates Section of joint tissue of arthritis rats showing proliferation with granulation tissue adjacent to the damaged articular cartilage (group II). Sections studied show structure of skin. Dermis and deep tissues show dense lympho-plasmacytic infiltrates, vascular proliferation, vasculopathy (vessel wall damage), marked synovial hyperplasia with villiform appearance, well formed granulomas, occasional foreign body giant cells and sub synovial spindle cell hyperplasia.

Figure 4: Histopathological examination of Normal, arthritis induced and *Nyctanthes arbor-tristis* treated rats.

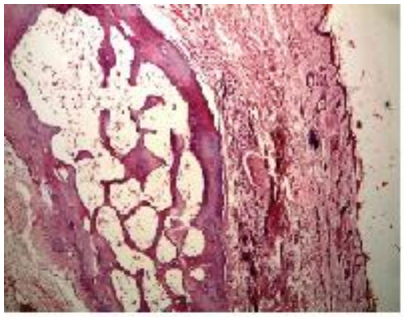

Plate 1. Normal skin and deeper tissues

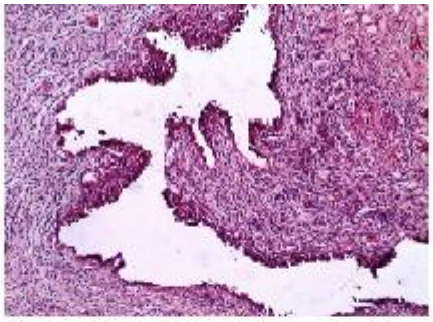

Plate 2a. Synovial hyperplasia – villiform in Adjuant induced Arthritic rats

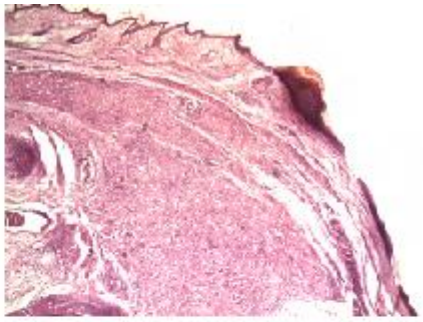

Plate 2b. Heavy lympho-plasmacytic infiltration of dermis and deeper tissues in AIA

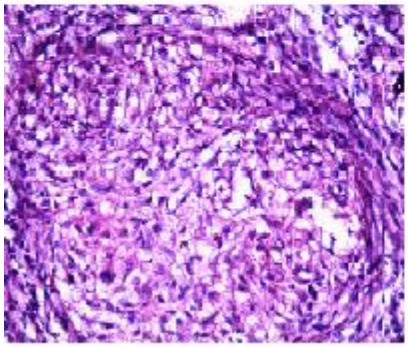

Plate 2c. Well formed granuloma in Adjuvant induced arthritic rats

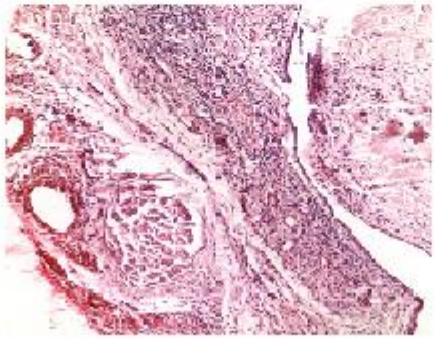

Plate 3. Less significant lympho-plasmacytic infiltration in AIA treated with NAT-L

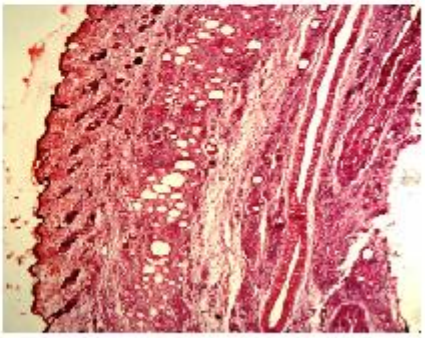

Plate 4. Moderately intense lympho-plasmacytic infiltration in AIA treated with NAT-S

Plate 3 and Plate 4 show significant regeneration of synoviocytes and disappearance of inflammatory exudates, mild focal infiltration of cells in synovial region, few cuboidal cells lining the synovial membrane in NAT leaf and stem extract treated rat (group III and group IV respectively). In all the experiments there

were no significant changes found in extract alone administered rats (Group V and Group VI).

4. CONCLUSION

Based on the results of the present investigation, it can be concluded that, ethanolic extract of *Nyctanthes arbor tristis* possess powerful *in vivo* antioxidant activity. Rats administered with NAT stem and leaf extract resulted in a significant improvement of oxidative status. The free radical scavenging activity of NAT-L was significant when compared to NAT-S. As NAT is of natural origin, it is a safe and effective intervention for free radical mediated diseases. Further, the isolation of the compounds responsible for the activity has to be taken up which may result in a modern drug from this plant.

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Conflict of Interest: None declared

5. REFERENCES

1. Tuntiwachwuttikul P, Rayanil K, Taylor WC. Chemical Constituents from the Flower of Nyctanthes arbor-tristis. Sci Asia. 2003; 29:21-30.
2. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immuno stimulant activity of Nyctanthes arbor-tristis L. J Ethnopharmacol.1994; 42(1):31–7.
3. Saxena RS, Gupta B, Saxena KK, Srivastava VK, Prasad DN. Analgesic, antipyretic, and ulcerogenic activity of Nyctanthes arbor tristis leaf extracts. J Ethnopharmacol. 1987; 19:193-200.
4. Narendhirakannan RT, Smeera T. In vitro anti-oxidant studies on ethanolic extracts of leaves and stems of nyctanthes arbor-tristis, L (night-flowering jasmine). Int J Biol Med Res. 2010; 1(4):188-92.
5. Tisdaie MJ, Mohrnoud MB. Activities of free radical metabolizing enzymes in tumors, Br J Cancer, 1983, 47,809-12.
6. Susan T, Muzaffer A, Purushothaman KK. Inhibitory activity of arbortristoside A on fibrosarcoma in albino rats. Arogya. 1986; 12:122-130.
7. Watlz DT, Drmartino JJ, Misher A. Adjuvant induced arthritis in rats, II Drug effects on physiologic, biochemical and immunologic parameters. J Pharmacol Exp Ther. 1971; 178:223-31.
8. Begum VH, Sadique J. Long term effect of Withania somnifera on adjuvant induced arthritis in rats. Indian J Exp Biol. 1988; 26:877–82.
9. Narendhirakannan RT, Subramanian S, Kandaswamy M. Antiinflammatory activity of Cleome gynandra, L on hematological and cellular constituents in adjuvant induced arthritic rats. J Med Food. 2005a; 8:93–99.
10. Mizushima Y, Tsukada W, Akimoto T. A modification of rat adjuvant arthritis for testing anti-rheumatic drugs. J Pharm Pharmacol. 1972; 24:781–85.
11. Campo GM, Angela A, Campo S, Ferlazzo AM, Altavilla DC. Efficacy of treatment with glycosaminoglycans on experimental collagen-induced arthritis in rats. Arth Res Ther. 2003; 5:122–31.
12. Docie JV. Practical Haemotology. London, J&A Churchill Ltd, 1958: 38-42.
13. Sasaki T, Matsui S. Effect of acetic acid concentration on the color reaction in the o-toludine boric acid for blood glucose determination. RinshoKagaku. 1972;1:346–53.
14. Natelson S, Scott ML, Beffa C. A rapid method for the estimation of urea in biological fluid by means of the reaction between diacetely and urea. Am J Clin Pathol. 1951; 21:275–81.
15. Caraway WT. In: Seligson D, ed. Standard Methods of Clinical Chemistry. New York: Academic Press, 1963; 4:239-47.

16. Owen JA, Iggo TB, Scandrett FJ, Stemart IP. Determination of creatinine in plasma (or) serum and in urine, Biochem J. 1954; 58:426–37.

17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265-275.

18. King J. The transferase – alanine and aspartate transaminase. In: Van, D (Ed), Practical Clinical Enzymology, Norstand Company Limited, London, 1965a;121–38.

19. Walter K, Schutt C. Acid and alkaline phosphatase in serum (two point method). In: Bergmeyer, H.U. (Ed.), In: Methods in Enzymatic Analysis, vol. 2. Academic Press, london. 1974.

20. King J. The dehydrogenase or oxide reductase – lactate dehydrogenase, In: Van, D (Ed), Practical Clinical Enzymology, Norstand company Limited, London, 1965b; 83–93.

21. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247:3170–175.

22. Rotruck JT, Pope AL, Gasther HE, Hafeman DG, Hoekstra WG. Selenium-biochemical role as a component of glutathione peroxidase. Sci. 1973; 179: 588–90.

23. Ohkawa H, Oshishi N, Yag K. Assay of lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem.1979; 95: 351–358.

24. Takahara S, Hamilton BH, Nell JV, Ogura Y, Nishimura ET. Hypocatalasemia, a new genetic carrier states. J Clin Invest. 1960; 29: 610–19.

25. Durie FH, Fava RA, Foy TM, Aruffo A, Ledbetter JA, Noelle RJ. Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. Sci. 1993; 26:1328–330.

26. Narendhirakannan RT, Subramanian S, Kandaswamy M. Free radical scavenging activity of Cleome gynandra L, leaves on adjuvant induced arthritis in rats. Mol Cell Biochem. 2005b; 276:71–80.

27. Cawthorne, M.A., Palmer, E.D., Green, J. Adjuvant-induced arthritis and drug-metabolizing enzymes. Biochem Pharmacol. 1976, 25, 2683–2688.

28. Whiteman M, Ketsawatsakul U, Halliwell B. A reassessment of the peroxynitrite scavenging activity of uric acid. Ann NY Acad Sci. 2002; 962:242–59.

29. Grootveld M, Halliwell B. Measurement of allantoin and uric acid in human body fluids, A potential index of free-radical reactions in vivo. Biochem J. 1987; 243: 803–05.

30. Rehman Q, Lane NE, Bone loss. Therapeutic approaches for preventing bone loss in inflammatory arthritis. Arthritis Res. 2001; 3:221–27.

31. Okabe T, Hamaguchi K, Inafuku T, Hara M. Aging and superoxide dismutase activity on

cerebrospinal fluid. J Neurol Sci. 1996; 141:100–04.

32. Alessio HM, Blasi ER. Physical activity as a natural antioxidant booster and its effect on healthy life style. Res Quart Exer Sport. 1997; 68:292-02.

33. Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. Bri J Nutr. 2001; 85:251-69.

34. Bowles DK, Torgan CE, Ebner S, Kehrer JP, Ivy JL, Starnes JW, Effect of acute, sub maximal exercise on skeletal muscle vit E, Free Radic Res, 1991, 14,139-43.

35. Scott MD, Eaton JW, Kuypers FA, Chiu DT, Lubin BH. Enhancement of erythrocyte SOD activity effects on cellular oxidant defence. Blood. 1989; 74:2542-49.

Figure Legends

Figure 1- Paw volume changes in normal, experimental rats. Y axis – Thickness of paw volume in mL and X axis – Weeks. Each point represents the mean ± S.D. for six animals in each group. Values are statistically significant at $*p < 0.05$; statistical significance was compared within the groups as follows: [*a]Arthritic rats compared with normal rats. [*b] NAT leaf and stem treated arthritic rats were compared with arthritic rats.

Figure 2- Effect of NAT-L and NAT-S extract on the antioxidant activity in normal and experimental rats. Values are expressed as mean ± S.D. for six animals. Comparisons are made between: *a, Group I vs. Groups II; *b, Group II vs. Groups III and IV. The letters *a and *b represent the Statistical significance at $p < 0.05$.

Plate 1. Normal skin and deeper tissues

Plate 2a. Synovial hyperplasia – villiform in Adjuant induced Arthritic rats

Plate 2b. Heavy lympho-plasmacytic infiltration of dermis and deeper tissues in AIA

Plate 2c. Well formed granuloma in Adjuvant induced arthritic rats

Plate 3. Less significant lympho-plasmacytic infiltration in AIA treated with NAT-L

Plate 4. Moderately intense lympho-plasmacytic infiltration in AIA treated with NAT-S

Abbreviations

RA Rheumatoid arthritis

AIA Adjuvant induced arthritis

NAT-L - *Nyctanthes arbor-tristis* leaf

NAT-S *Nyctanthes arbor-tristis* stem

ROS Reactive oxygen species

GPx Glutathione peroxidase

SOD Superoxide dismutase

NSAID-Non-steroidal anti-inflammatory drug

RBC -Red blood cell

WBC -White blood cell

ESR Erythrocyte sedimentation rate

ALP Alkaline phosphatase

LDH Lactate dehydrogenase