Free radical scavenging activities of Nyctanthes arbor-tristis. L on adjuvant induced arthritic rats

Smeera Thomas[1], Grace Nirmala J[2] and Narendhirakannan R.T*[2]

1Department of Biotechnology Engineering

Sahrdaya college of Engineering and Technology

Kodakara. Thrissur, Kerala, India

2Department of Biotechnology

School of Biotechnology and Health Sciences

Karunya University (Karunya Institute of Technology and Sciences)

Coimbatore – 641 114, Tamil Nadu, India

***Address for Correspondance:**

Dr. R.T.Narendhirakannan

Assistant Professor (SG)

Department of Biotechnology

School of Biotechnology and Health Sciences

Karunya University

Coimbatore – 641 114

Phone : 0422 - 2614300

Fax : +91- 422- 2615615

e-mail: bionaren_phd@yahoo.co.in

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. It also significantly decreased the levels of lipid peroxides and activities of catalase, glutathione peroxidase and reduced the activity of superoxide dismutase in arthritic 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.

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

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 possesses immunostimulant, hepatoprotective, antileishmanial, antiviral and antifungal activities. The decoction 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 [1, 2]. 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 [2]. Our previous study exhibits the *in vitro* anti-oxidant activity of *N. arbor tristis* leaf extract [3, 4].

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 [5]. 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 [6]. Therefore, new drugs without side effects are being studied all over the world as an alternate to NSAIDs and opiates [7]. 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 and electron microscopical studies.

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 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, then extracted using ethanol in a Soxhlet extractor. The extracts were concentrated and stored at 4^0C in air tight container.

2.3 Test animals

Male albino rats of Wister strain weighing around 160–180 g were procured from Coimbatore, 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 [8].

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 plethismometer 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 tissue is removed immediately and processed further [9].

2.8 Biochemical assays

2.9 Blood analysis

The hematological parameters like hemoglobin, RBC, WBC, Platelets, ESR, and PCV [10] were determined by usual standardized laboratory method. The biochemical parameters, blood glucose [11], urea [12], uric acid [13], creatinine [14], total protein [15], were also determined in serum. Marker enzymes such as glutamate oxaloacetate transaminase/aspartate aminotransferase (GOT/AST), and glutamate pyruvate transaminase/alanine aminotransferase (GPT/ALT), [16] ALP [17] and LDH [18] were analyzed.

2.10 In vivo antioxidant assays

Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich [19]. Glutathione peroxidase (GPx) was assayed by following Rotruck *et al.,* method [20]. Takahara *et al.,*[21] method was followed to measure catalase activity. The level of Lipid peroxides was estimated using thiobarbituric acid reactive substances by the method of Ohkawa *et al.* [22].

2.11 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 [23].

2.12 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 [24].

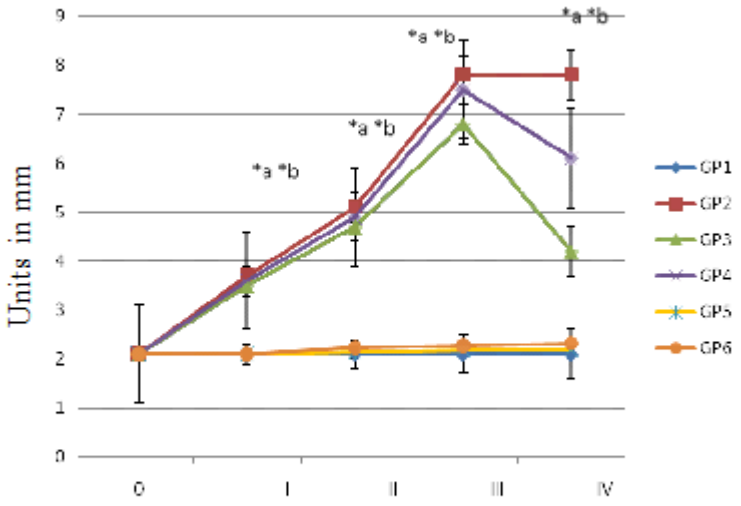

Figure 1- Paw volume changes in normal, experimental rats.

Y axis – Thickness of paw volume in mm. Each point represents the mean ± S.D. for three 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.

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

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

Parameters	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
Hbg/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(Millions/mm_3)	5.1±0.258	3.6±0.24*[a]	4.8±0.155*[b]	4.2±0.158[NS]	5.2±0.21	5.3±0.20
WBC(Thousands/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
Platelets (lakhs/ml)	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±2.08[NS]	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 three 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).

155 The levels of blood glucose, urea, uric acid and creatinine of normal and experimental groups of rats
156 are presented in Table – 2. A significant ($p<0.05$) increase in the levels of Glucose, urea and creatinine and
157 decrease in uric acid level were observed in arthritis-induced rats (group II) when compared with normal rats.
158 The increase in blood glucose in arthritic rats might be due to the decreased glycolytic and increased
159 gluconeogenic enzyme activities, since impaired hepatic biosynthetic activities were reported in AIA
160 condition. The decrease in plasma uric acid in arthritic animals might be due to its continuous utilization by

161 the system during free radical quenching reaction [25, 26]. Renal dysfunction might be the cause of raised
162 blood urea and creatinine levels in AIA rats.

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

Parameters (mg/ml)	**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 three animals. Comparisons are made between: *a, Group I vs. Groups II; *b, Group II vs. Groups III and IV. The letters * 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).

164 A marked increase in the activity of membrane marker enzymes (AST, ALP, and LDH) and decrease
165 in ALT were observed in the joint tissues of arthritic rats (group II) when compared to control rats (Group I).
166 Arthritic rats treated with NAT-leaf extract showed a significant ($p<0.05$) change in the activity of membrane
167 marker enzymes. Compared to NAT-S, NAT-L showed greater activity (Table 3). Assessment of the levels
168 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 [27].

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

Parameters (mg/dl)	**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 three 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, GPx and catalase 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 [28-30].

Figure 2 elucidates the significant ($p<0.05$) increase in the level of tissue TBARS 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 peroxide level to near normalcy. Lipid peroxidation is considered as a critical mechanism of the injury that occurs during RA [31].

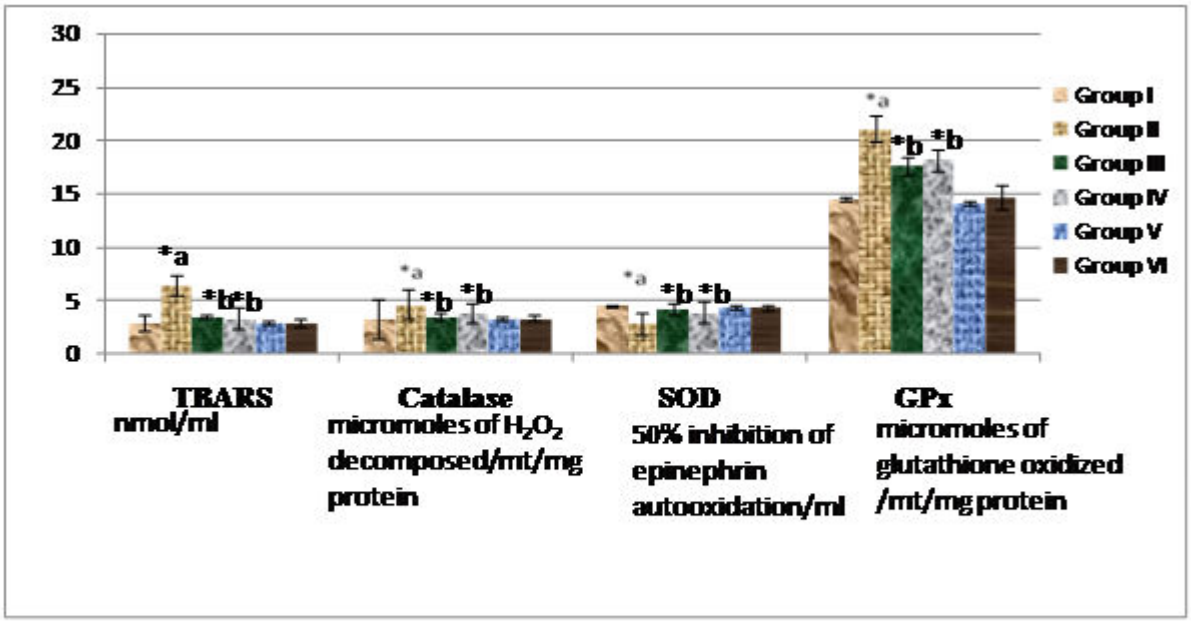

Figure 2- Effect of NAT-L and NAT-S extract on the antioxidant activity in normal and experimental rats.

Figure 2- Values are expressed as mean ± S.D. for three 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 $p < 0.05$.

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. 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).

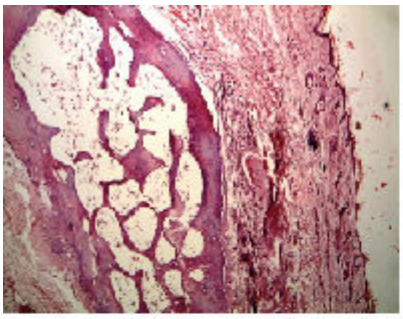

Plate 1. Normal skin and deeper tissues

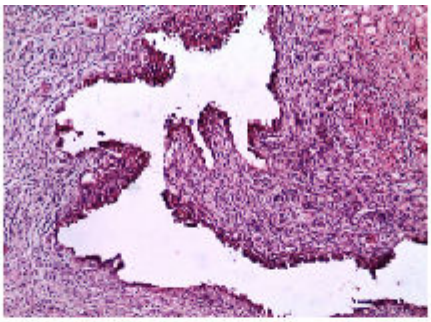

Plate 2a. Synovial hyperplasia – villiform in Adjuant induced Arthritic rats

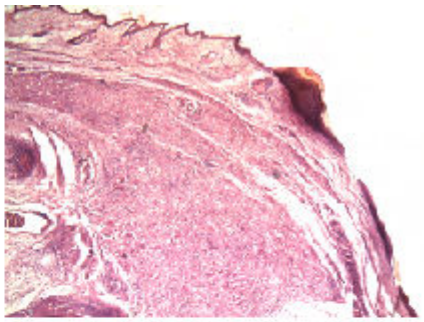

Plate 2b. Heavy lympho-plasmacytic infiltration of dermis and deeper tissues in AIA

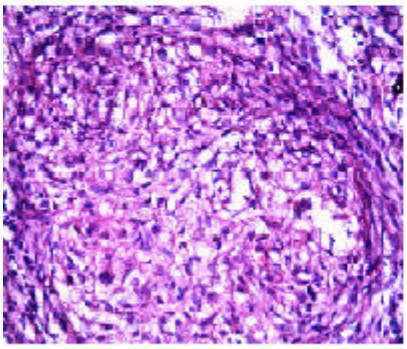

Plate 2c. Well formed granuloma in Adjuvant induced arthritic rats

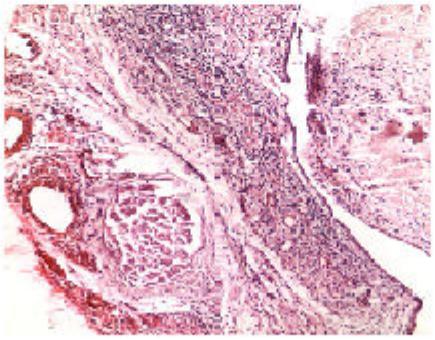

Plate 3. Less significant lympho-plasmacytic infiltration in AIA treated with NAT-L

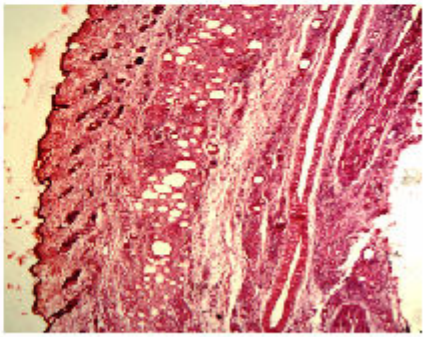

Plate 4. Moderately intense lympho-plasmacytic infiltration in AIA treated with NAT-S

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. 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.

ACKNOWLEDGMENT:

The authors expressed their gratitude to Dr. Paul Dinakaran, Chancellor, Dr. Jame EJ, Vice chancellor, Dr. J, Registrar of Karunya University for providing the necessary facilities for carrying out the experiments.

Conflict of Interest: None declared

5. REFERENCES

1. 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.
2. 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.
3. 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.
4. Tisdaie MJ, Mohrnoud MB, Activities of free radical metabolizing enzymes in tumors, Br J Cancer, 1983, 47,809-12.
5. 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.
6. Begum VH, Sadique J. Long term effect of Withania somnifera on adjuvant induced arthritis in rats. Indian J Exp Biol. 1988; 26:877–82.
7. 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.
8. Mizushima Y, Tsukada W, Akimoto T. A modification of rat adjuvant arthritis for testing anti-rheumatic drugs. J Pharm Pharmacol. 1972; 24:781–85.
9. 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.
10. Docie JV. Practical Haemotology. London, J&A Churchill Ltd, 1958: 38-42.
11. 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.

12. 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.

13. Caraway WT. In: Seligson D, ed. Standard Methods of Clinical Chemistry. New York: Academic Press, 1963; 4:239-47.

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

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

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

17. 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.

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

19. 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.

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

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

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

23. 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.

24. 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.

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

26. 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.

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

28. Okabe T, Hamaguchi K, Inafuku T, Hara M. Aging and superoxide dismutase activity on cerebrospinal fluid. J Neurol Sci. 1996; 141:100–04.

29. 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.

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

31. 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 mm. Each point represents the mean ± S.D. for three 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 three 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