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ANTI-OXIDATIVE EFFECT OF *NYCTANTHES ARBORTRISTIS* EXTRACT IN MOUSE MODEL OF ARTHRITIS

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ABSTRACT: Rheumatoid arthritis is a chronic immuno-inflammatory systemic disease, affecting 1-2% population worldwide. The disease is very common among women rather men. Mostly the victims of this disease belong to the age group 45 onwards, while juvenile cases are also reported. The exact pathogenesis of the disease is still a question. Reactive oxygen species have been implicated as mediators of tissue damage in arthritic patients. Various herbal plant preparations are reported to scavenge free radicals and improve different human diseases such as diabetes, Alzheimer's disease, atherosclerosis, *etc*. The present investigation was designed to evaluate the effectiveness of *Nyctanthes arbortristis* towards disturbed antioxidant status in inflamed tissue. *Nyctanthes arbortristis* has been known for medicinal uses such as anti-helmenthic, anti-bacterial activity, *etc*. Water-soluble ethanolic extract of *Nyctanthes* seed (NSE) and leaf (NLE) was administered orally to adjuvant-induced arthritic mice (AIA) at the dose of 23.72 mg/kg body weight for 47 days. Enzymatic and non-enzymatic anti-oxidants were assessed on day 2, 14 and 47. On the one hand, daily administration of NLE significantly elevated catalase activity and albumin levels, on the other hand, lowered the malondialdehyde, uric acid and protein levels as compared with control animals. NLE was observed more effective as compared to NSE. All these results demonstrate the efficacy of NLE as an anti-arthritic agent for the treatment of rheumatoid arthritis. However, further studies are needed to elucidate the absolute mechanism of NLE for scavenging free radicals.

INTRODUCTION: Rheumatoid arthritis (RA) is the most common inflammatory disease affecting approximately 1-2% of the population worldwide [1]. RA results in the inflammation in diarthrodial joint tissue [2], followed by progressive destruction of bone and cartilage [3].

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The exact etiology of RA remains still unknown. It has been reported that either a foreign agent or some alteration in control of cellular responses is involved in the synovial inflammation [4]. Free radicals are species capable of independent existence containing one or more unpaired electron(s) which makes them para-magnetic and highly reactive.

The formation and scavenging of free radicals and other oxygen-derived species in the biological system have received much attention. Free radicals are reported to play an important role in various human diseases, such as Alzheimer's disease, male

infertility, diabetes, atherosclerosis, Parkinson's *etc* [5, 6]. There is the number of reports implicating these highly reactive oxygen products in the pathogenesis of RA [7]. Moreover, there are also reports that the disturbed anti-oxidant levels in rheumatoid arthritis may be due to the acceleration of some cellular reactions or insufficiency of the antioxidant defense system [8]. During the last few years, alternative medicine has been practiced as an effective tool for therapeutic purposes [9, 10]. Among alternative medicine methods, herbal remedies are now widely accepted therapy to arthritis [11]. Recent studies from our laboratory have revealed the repair of oxidative stressed state using anti-oxidants [12], and the control of this stress using herbal preparations [13]. Also, *Nyctanthes arbortristis* extract has proven to reduce inflammatory cytokines in adjuvant-induced arthritis [14]. In the present study, we have tried to assess different extracts of the *Nyctanthes arbortristis* for their anti-oxidant property. This study will help in understanding the mechanism of antioxidant potential of *Nyctanthes arbortristis* and open up new avenues for treatment of other human diseases linked to oxidative stress.

MATERIALS AND METHODS:

Preparation of *Nyctanthes arbortristis* Extracts: For our experiment, seeds and leaves of *Nyctanthes arbortristis* (NAT) were collected in January and February, from the tree growing in the premises of the Institute. A sample specimen (Bnp101) of the plant was deposited to National Botanical Research Institute herbarium (LWG), Lucknow, India for species authentication. Further, seeds and leaves were dried in the shade and powdered. The powder was macerated with 95% ethanol, the extract was filtered, and the solvent was evaporated using lyophilizer. The residue was stirred vigorously with distilled water; the mixture was allowed to stand for 30 min and filtered. The filtrate was again lyophilized. The yield of extract from seeds and leaves was 22.34 and 6.28% respectively. The stock solution was appropriately diluted in sterile distilled water and administered an oral dose of 23.72 mg/kg body weight to each mouse. The dose was selected as effective one from our previous study [14].

Experimental Animals: Female Balb/c mice weighing 25-30 g were used throughout the studies. Prior permission for the experiment was sought from the Institutional Animal Ethics Committee. Animals were kept in separate cages under standard conditions of the animal house, and fed pellet diet and water *ad libitum*. Mice were divided into 5 groups of 15 animals each. The group I comprised of normal mice, group II comprised of arthritic mice receiving distilled water, group III and IV comprised arthritic mice receiving seed and leaf extract daily till day 47. The NAT extracts were administered orally. Five animals from each group were used for drawing blood on day 2, 14 and 47.

Induction of Arthritis: 10 µl Freund's Complete Adjuvant (FCA) (Sigma, USA: Lot # 8048808) containing heat-killed *Mycobacterium tuberculosis* (H37Ra, ATCC, 25177) was injected in the sub planter surface of the right hind paw of mice to induce arthritis. A booster dose of 10 µl FCA was given to animals in sub planter surface of the same hind paw on the 12th day. Thus, adjuvant-induced arthritis animals were prepared.

Treatment: NAT seed and leaf extract (NSE, NLE) treatment were started on the day 0, orally (23.72 mg/kg body weight/day), simultaneous with the FCA injection. On day 2, 14 and 47 blood and plasma was collected to determine different anti-oxidant enzyme and biochemical parameters. These three-time points were selected as day 2 represents the peak time point showing primary edema, day 14 represents the progression phase of secondary edema and the day 47 represent the well-defined arthritis phase.

Blood Collection: Blood was drawn from the retro-orbital sinus using the capillary tube and divided in two sterile tubes, one containing heparin to separate plasma and another one as plain to separate serum. Whole blood was kept at room temperature for 2 h. Plasma was collected as supernatant after centrifugation at 2500 rpm for 5 min. and the pellet was collected in a separate tube for the preparation of lysate.

RBC Lysate Preparation: Plasma was separated from the blood collected in EDTA vials, by centrifugation at 2500 RPM for 15 minutes at room temperature. Plasma was then transferred to separate sterile tubes for biochemical analysis. The RBC pellet was found intact in the bottom of the tube, which was then washed at least twice with

normal saline (0.9%) to remove the buffy coat. Further, chilled distilled water was added equally to the amount of plasma separated. After that, it was centrifuged at 8000-10,000 rpm for 20 min and the supernatant (lysate) was collected and the pellet (cell debris) was discarded.

Quantitative Determination of Biochemical Parameters and Anti-oxidant Activity: Total proteins and uric acid levels were measured in serum with the help of Biochemical Autoanalyser (Chemwell, USA) using Spinreact Diagnostic reagents. Catalase, superoxide dismutase and lipid peroxides were estimated following the standard methodology [15, 16, 17].

Statistical Analysis: Student 't' test was performed to evaluate the significance of the difference in the mean values of extract treated and untreated group. $P<0.05$ was considered significant.

RESULTS:

Effect of NAT Extracts on Superoxide Dismutase (SOD) Activity: In AIA animals, significant ($P<0.01$) reduction in SOD activity was observed as compared with normal mice. *Nyctanthes arbortristis* leaf extract (NLE) and *Nyctanthes arbortristis* seed extract (NSE) treated animals exhibited high SOD activity on 14th and 47th day as compared to AIA animals. NLE showed maximum change as compared with NSE **Table 1**.

TABLE 1: EFFECT OF NSE AND NLE ON SUPEROXIDE DISMUTASE ACTIVITY (U/mg protein)

Group	Day 2	Day 14	Day 47
Normal	84.31 ± 3.43	84.76 ± 4.18	84.78 ± 2.72
AIA	73.26 ± 2.71 [a]	66.43 ± 1.19 [a]	53.12 ± 3.31 [a]
AIA+NSE	72.86 ± 3.82	68.26 ± 1.48	59.27 ± 2.72
AIA+NLE	72.87 ± 1.81 [b]	70.23 ± 3.04 [b]	77.96 ± 2.20 [b]

[a] significant ($P<0.01$) in comparison to normal mice at the respective time point; [b] significant ($P<0.05$) in comparison to arthritic mice on the respective time point

Effect of NAT Extracts on Catalase (CAT) activity Low: CAT activities were recorded in AIA animals as compared with normal animals, while NLE and NSE treated animals showed a marginal increase in CAT activity on day 2, but statistically significant on day 14 and 47. NLE was found efficient in increasing CAT activity than NSE **Table 2**.

Effect of NAT Extracts on Lipid Peroxides: A significant increase in lipid peroxide levels in AIA animals reflects the disturbed antioxidant status during disease. This increase in lipid peroxide was much comparable to normal mice. Regular administration of NLE and NSE tried to bring down the lipid peroxide levels in diseased animals. The maximum effect was observed at a dose of 23.72 mg/kg b.w. of NLE **Table 3**.

Effect of NAT Extracts on Total Protein Level: Total protein levels in AIA animals showed a significant rise on all the three-time points as compared with normal mice, while none of the extract was found effective after 2 day treatment; significant improvement was recorded on 14 and 47 days **Table 4**.

TABLE 2: EFFECT OF NSE AND NLE ON CATALASE ACTIVITY (U/mg protein)

Group	Day 2	Day 14	Day 47
Normal	0.491 ± 0.07	0.501 ± 0.09	0.521 ± 0.05
AIA	0.212 ± 0.02 [a]	0.200 ± 0.03 [a]	0.183 ± 0.01 [a]
AIA+NSE	0.233 ± 0.03	0.243 ± 0.03	0.256 ± 0.02
AIA+NLE	0.336 ± 0.03 [b]	0.374 ± 0.02 [b]	0.401 ± 0.02 [b]

[a]significant ($P<0.01$) in comparison to normal mice at the respective time point; [b]significant ($P<0.05$) in comparison to arthritic mice at the respective time point

TABLE 3: EFFECT OF NSE AND NLE ON LIPID PEROXIDES (nmole/ml)

Group	Day 2	Day 14	Day 47
Normal	3.41 ± 0.38	3.62 ± 0.26	3.82 ± 0.41
AIA	4.26 ± 0.80 [a]	5.48 ± 0.24 [a]	6.30 ± 0.90 [a]
AIA+NSE	4.78 ± 0.26	5.04 ± 0.21	5.65 ± 0.69
AIA+NLE	3.46 ± 0.14 [b]	4.81 ± 0.19 [b]	4.15 ± 0.27 [b]

[a]significant ($P<0.01$) in comparison to normal mice on the respective time point; [b]significant ($P<0.05$) in comparison to arthritic mice on the respective time point

TABLE 4: EFFECT OF NSE AND NLE ON PROTEIN LEVELS (g/dl) IN AIA MICE

Group	Day 2	Day 14	Day 47
Normal	5.14 ±0.32	5.16± 0.41	5.19 ±0.39
AIA	6.36 ±0.70[a]	6.80 ±0.52[a]	6.80 ±0.10[a]
AIA+NSE	6.36 ±0.58	5.73 ±0.15	5.66 ±0.20[b]
AIA+NLE	6.10 ±0.91	4.46 ±0.84[b]	5.43 ±0.10 [b]

[a] significant (P<0.01) in comparison to normal mice on the respective time point; [b]significant (P<0.01) in comparison to arthritic mice on the respective time point

Effect of NAT Extracts on Uric Acid Level: A sharp increase in uric acid levels were recorded in AIA animals on all the three-time points of study. This increase was found statistically significant. NLE and NSE treatment significantly brought down the uric acid levels near to the normal values. The maximum change was recorded at 23.72 mg/kg b.w. dose of NLE **Table 5**.

Effect of NAT Extracts on Albumin Level: Marginal but significant decrease in albumin levels was recorded in AIA animals. However, only momentous elevation in albumin levels in treatment receiving animals was recorded on 47 days at 23.72 mg/kg b.w. dose of NLE. NSE was not capable of producing any change **Table 6**.

TABLE 5: EFFECT OF NSE AND NLE ON URIC ACID (mg/dl) IN AIA MICE

Group	Day 2	Day 14	Day 47
Normal	4.20 ± 0.71	4.20 ± 0.34	4.40 ± 0.41
AIA	5.50 ± 0.14 [a]	6.60 ± 0.49 [a]	9.30 ± 0.10 [a]
AIA+NSE	5.49 ± 0.42	6.30 ± 0.90	8.70 ± 0.28
AIA+NLE	5.18 ± 0.75[b]	6.08 ± 0.35 [b]	8.60 ± 0.38 [b]

[a]significant (P<0.01) in comparison to normal mice on the respective time point; [b]significant (P<0.01) in comparison to arthritic mice on the respective time point

TABLE 6: EFFECT OF NSE AND NLE ON ALBUMIN (mg/dl)

Group	Day 2	Day 14	Day 47
Normal	0.56 ± 0.02	0.56 ± 0.03	0.57 ± 0.02
AIA	0.55 ± 0.01 [a]	0.47 ± 0.02 [a]	0.33 ± 0.01 [a]
AIA+NSE	0.55 ± 0.02	0.47 ± 0.02	0.37 ± 0.02
AIA+NLE	0.54 ± 0.03[b]	0.46 ± 0.04 [b]	0.44 ± 0.02 [b]

[a]significant (P<0.01) in comparison to normal mice on the respective time point; [b]significant (P<0.01) in comparison to arthritic mice on the respective time point

DISCUSSION: During arthritis, an imbalance in the enzymatic and non-enzymatic antioxidant system and hypoalbuminemia is reported [12]. Therefore, the assessment of these parameters in AIA animals and extract treated animals may help in determining the free radical scavenging property as well as anti-arthritic activity of *Nyctanthes arbortristis*.

The present study is an attempt to observe the relationship between antioxidant parameters during RA and the possible role of NLE and NSE to combat free radicals. We have assessed the biochemical parameters such as total protein, uric acid, and albumin in arthritis and during different treatment stages. There is the production of acute phase protein in inflammatory condition along with higher concentrations of uric acid. Also, there are many cases of hypoalbuminemia during arthritis.

In adjuvant-induced arthritis, there is a complex series of reactions executed by the host in an effort to prevent tissue damage and destroy the infective organism by activating the repair process that is necessary to bring an organism to normal functioning [5]. This process is commonly known as the acute phase response (APR). A sequence of events is thus initiated, leading to the release of soluble mediators that mobilize the metabolic response of the organism. Bacterial products activate macrophage, which in turn release different mediators such as TNF and IL-1. These TNF and IL-1 possess pleiotropic activity and have been implicated as major players in arthritis [2]. The release of these cytokines further enhances the inflammatory response and worsens the tissue. Thus, the APR follows a sequence of events in which macrophages and platelets are activated, and thus cytokines are released [4].

In our previous study, we have observed the down-regulation of TNF-α and 1L-1β following the oral administration of NLE [14]. Various workers have reported the anti-inflammatory activity of Chinese and Korean herbal plants in arthritis. Down-regulation of uric acid levels following the NLE and NSE treatment also proves the efficacy of the NLE and NSE.

There are several studies regarding the increased production of free radicals in the diseased RA joint. These free radicals may further perpetuate the inflammatory process in the affected synovium. Free radicals are also known to alter the cellular activities by lipid peroxidation, DNA damage, *etc*. Free radicals oxidatively deteriorate the unsaturated fatty acids, a process known as lipid peroxidation. Normally, there is a fine balance between lipid peroxidation and its inhibition by plasma anti-oxidants.

On the other hand, during diseased state, the antioxidant defense mechanism gets perturbed either due to over-production of free radicals or decreased antioxidant activity. There are numerous reports of increased production of free radicals during arthritis [18, 19], as well as altered antioxidant system [5, 20, 21]. There are also reports revealing the increased lipid peroxidation [22, 23,] and decreased SOD and CAT activities in RA [24, 25]. Simultaneously, supplementation of dietary antioxidants such as Vitamin C, and E have been reported to contain the over-production free radical during arthritis. Different herbal preparations have also been reported to combat the free radical activities [26].

Further, it is a well-established fact that in RA, changes are not limited only to the joints but also associate involvement of the organ and tissues. During RA, reduction in albumin levels has been confirmed. The albumin levels are affected by different unrelated factors. In the present study, we have tried to study whether there is any co-relation between albumin levels and the severity of the disease. Under physiological conditions, albumin also has antioxidant potential and is involved in scavenging of free radicals. The albumin concentration is the function of its rate of synthesis and degradation, along with its distribution between intravascular and extravascular compartments. Our observations also reveal the hypoalbuminemia, as reported by previous workers.

CONCLUSION: Although, RA is a disease of unknown etiology, yet it is quite well established that free radicals play an important role in the pathogenesis of the disease. Overproduction of free radicals perpetuates the progression of arthritis, due to the impaired antioxidant system in the body. Many herbal preparations have been proven to improve antioxidant status during diseased state. The present study reveals the effectiveness of NLE, an Indian herbal extract for its antioxidant potential during experimental arthritis. This may further provide evidence to elucidate the mechanism of action of *Nyctanthes arbortristis* extract for its anti-oxidant property, too. However, further studies are earnestly needed to understand the antioxidant mechanism of this herbal extract completely.

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CONFLICT OF INTEREST: Nil

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