

## ORIGINAL RESEARCH ARTICLE

**Comparative Evaluation of *Nyctanthes arbor-tristis* and *Alstonia scholaris* Leaves Extracts in Freund's Complete Adjuvant Induced Arthritis in Rats**Swapnil Goyal<sup>1</sup> & <sup>2\*</sup>, Navin R. Sheth<sup>3</sup>, DN Srivastava<sup>4</sup><sup>1</sup>Ph. D. Research Scholar, Uttarakhand Technical University, Dehradun, UK, India<sup>2</sup>Department of Pharmacology, Mandsaur Institute of Pharmacy, Mandsaur, MP, India<sup>3</sup>Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujrat, India<sup>4</sup>Department of Pharmacology, BR Nahata College of Pharmacy, Mandsaur, MP, India

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**ABSTRACT**

The present study was carried out to evaluate anti-arthritic potential of *Nyctanthes arbor-tristis* and *Alstonia scholaris* extracts of leaves in Complete Freund's Adjuvant (CFA)-induced arthritis in rats. The anti-arthritic activity was evaluated by adjuvant-induced arthritis at the dose of 200 and 400mg/kg body weight and the standard drug used was prednisolone in 10 mg/kg. The extracts administered in higher doses reduced the lesions to a greater extent showing a dose-dependent decrease in lesions comparable with standard drug prednisolone. The extracts of *Nyctanthes arbor-tristis* and *Alstonia scholaris* showed significant increase in body weight as compared to arthritic control group. The extracts of *Nyctanthes arbor-tristis* and *Alstonia scholaris* showed significant decrease ( $P < 0.001$ ) in WBC count, ESR, increase in hemoglobin contents, and RBC count as compared to control group. In conclusion, we demonstrate that, at 400mg/kg body weight, doses of *Nyctanthes arbor-tristis* and *Alstonia scholaris* extracts were highly effective in preventing and suppressing the development of adjuvant-induced arthritis.

**Key words:** Anti-arthritic potency, CFA, Prednisolone, *Nyctanthes arbor-tristis*, *Alstonia scholaris***INTRODUCTION**

Rheumatoid arthritis (RA) is a chronic, inflammatory, multisystem, autoimmune disorder. It commonly affects the joints in a polyarticular manner <sup>[1]</sup>. It is an autoimmune disorder of unknown etiology that is characterized by progressive joint destruction, deformity, disability and premature death in most patients. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) supplemented with steroid hormone remains the major recommended strategy for its treatment <sup>[2, 3]</sup>. Furthermore, long-term treatment with NSAIDs may result in serious side effects, such as gastrointestinal ulcerogenicity and renal morbidity. Owing to these shortcomings, a more effective and safe therapeutic strategy is desired to treat RA <sup>[4]</sup>.

Some plants such as Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Neem (*Azadiracta Indica*), Karela (*Momordica Charantia*), Methi (*Trigonella foenumgracum*), Cumin (*Cuminum cyminum*), parijat (*Nyctanthes arbor-tristis*), saptarni (*Alstonia scholaris*) Guggul. Among

them, *Nyctanthes arbor-tristis* and *Alstonia scholaris* are well documented plants & are widely used for the treatment of RA.

*N. arbor-tristis* and *A. scholaris* have been proved antioxidant, antitumor, anti-inflammatory and CNS depressants <sup>[5, 6, 7]</sup> activities. However, no report is available for anti-arthritic activity. Hence, an effort is made to investigate the anti-arthritic activity in experimentally induced RA in rats.

**MATERIAL AND METHODS****Collection of Plant Materials-**

Fresh leaves of *Nyctanthes arbor-tristis* and *Alstonia scholaris* were collected from nursery and local area of Mandsaur district in month of July-august. The leaves were identified and authenticated by Dr. Gyanendra Tiwari, botanist in KNK college of Horticulture, Mandsaur, M.P. India. The herbarium file was submitted in department of Pharmacognosy at Mandsaur Institute of Pharmacy, Mandsaur, India.

## Preparation of Extracts and Phytochemical Screening-

Fresh leaves of *Nyctanthes arbor-tristis* and *Alstonia scholaris* were collected, shade-dried and powdered mechanically. About 250 g of the leaf powder was extracted with 1000 ml of 95% ethanol by continuous hot percolation using soxhlet apparatus for 72 h. The extracts were dried at 40°C under vacuum and yields of the extracts were calculated. The extracts were screened for the presence of various active phytoconstituents i.e. steroids, fatty acids, flavonoids, alkaloids, tannins, terpenoids, phenolics, saponins, carbohydrates and glycosides [8].

## Animals

Wistar albino rats (150-200 g.) of either sex were used for the study. The animals were maintained under environmental condition and fed with standard pellet diet and water *ad libitum*. The study protocol was approved by Institutional Animal ethical Committee (IAEC). CPCSEA guidelines were adhered to during maintenance and experiment.

## Acute Toxicity Studies

Acute toxicity studies were carried out for ethanolic extract of *Nyctanthes arbor-tristis* and *Alstonia scholaris* leaves according to OECD guidelines 423. The ethanolic extracts of both plants were administered orally in dose of 2000 mg/kg body weight. The animals were observed 24 hrs for the signs of toxicity. The attention was directed on convulsion, diarrhea, coma, respiratory depression, salivation and perspiration [9].

## Induction of experimental arthritis and treatment protocol

The animals were divided into six groups of six animals each as follows:

Group I – Arthritic control, 2% (w/v) Tween 80, p.o.

Group II – Arthritic rats standard treated, 10 mg/kg prednisolone, p.o.

Group III- *N. arbor-tristis* (200 mg/kg orally, from zero day of FCA injection) treated rats

Group IV- *N. arbor-tristis* (400 mg/kg orally, from zero day of FCA injection) treated rats

Group V- *A. scholaris* (200 mg/kg orally, from zero day of FCA injection) treated rats

Group VI- *A. scholaris* (400 mg/kg orally, from zero day of FCA injection) treated rats

Arthritis was induced in all the groups by injection of 0.1 ml of Freund's Complete

Adjuvant (FCA) in the sub-plantar region of the left hind paw on day zero [10]. The treatments were given to the respective groups once daily orally from zero day of injection.

## Measurement of body weight and paw volume -

Body weight was measured of all groups at zero days before immunization and at 21<sup>st</sup> day after treatments over. Paw volume was measured by the means of plethysmometer at zero days before injection and at 4, 8, 12, 16, 21 days. The percentage of inhibition was measured by following formulas.

$$\frac{(\text{Volume of arthritic control rats} - \text{volume of treated rats}) \times 100}{\text{Volume of arthritic control rats}}$$

## Measurement of hematological parameter

On the 21<sup>st</sup> day after arthritis induction, rats were anaesthetized with ether and blood samples were collected into ethylenediamine tetra-acetic acid (EDTA)-coated tubes from retro orbital junction. The number of leukocytes from each rat was determined using a counting chamber (cell dyn-1200, Abbott Carepam). Erythrocyte sedimentation rate (ESR) was determined using the Wintrobe method [11]. RBCs and Haemoglobin were determined by routine laboratory method [12].

## Statistical analysis

The results were expressed as the mean±SEM. The significance of the difference was evaluated by one-way ANOVA followed by Dunnet's 't' test. Data were considered statistically significant if  $p < 0.05$ .

## RESULTS

### Phytochemical Screening-

Results of phytochemical investigation of *Nyctanthes arbor-tristis* and *Alstonia scholaris* leaves extracts showed presence of coumarin glycoside, fats and oils, tannins, triterpanoids and phenolic compounds, carbohydrates, and proteins.

### Acute toxicity study

The tested extracts did not exhibit any toxicity systems and mortality in all groups when given orally at dose of 2000 mg/kg. Hence, 200 and 400 mg/kg were taken as therapeutic dose.

### Effects of *N. arbor-tristis* and *A. scholaris* on paw volume-

Observations of paw volume were recorded on the 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup>, and 21<sup>st</sup> days after adjuvant injection. The CFA-induced arthritis control group showed signs of arthritis development, as seen by the increase in the paw volume and other

indications, such as a decreased body weight, also showed induction of arthritis in the CFA-treated control group rats. The assessment made on the 21st day showed that the *N. arbor-tristis* and *A. scholaris* treatments at both doses had moderately significantly and highly significantly reduced ( $p < 0.01$  &  $p < 0.001$ ) the adjuvant-induced lesions in the respective treatment groups as compared with the arthritis control group (Figure 1).

### Effects on body weight-

Animals in which arthritis had been induced gained less weight after induction, which was highly significantly lower than initial days zero to 21<sup>st</sup> day ( $p < 0.001$ ). In *N. arbor-tristis* & *A. scholaris* treated arthritic rat's weight was not decrease as such to disease control animals in dose-dependent manner (Figure 2).

### Effects on Hematological Parameters-

The CFA-induced arthritic rat's hematological parameters, such as an increase in the WBC count, a decreased RBC count, a decreased Hb count and an increased ESR were also significantly as well as highly significantly altered by *N. arbor-tristis* & *A. scholaris* treatment by both doses (Figure 3, 4, 5 & 6).

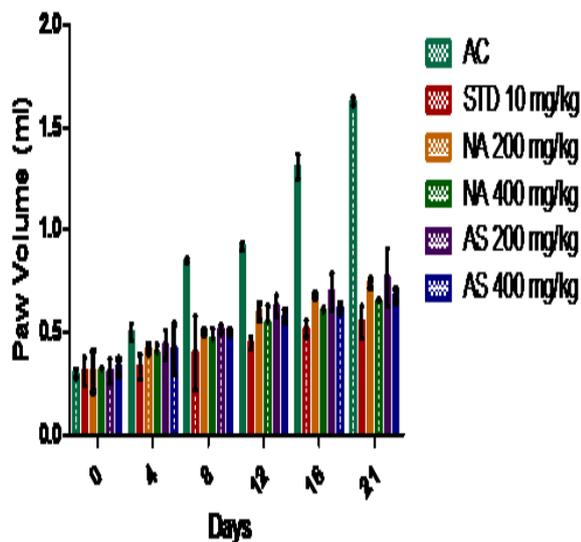


Figure 1: Effects of *N. arbor-tristis* & *A. scholaris* on paw volume in FCA induced arthritis

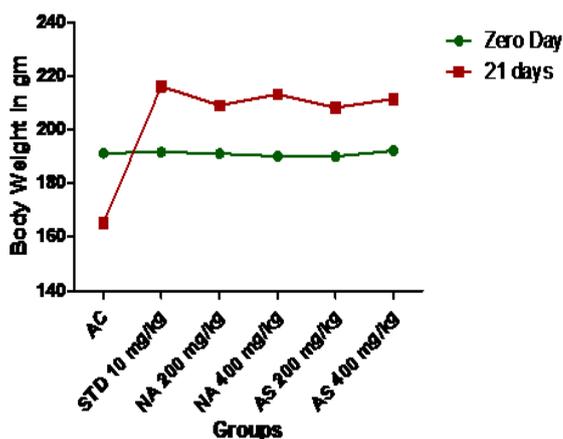


Figure 2: Effects of *N. arbor-tristis* and *A. scholaris* on body weight in FCA induced arthritis

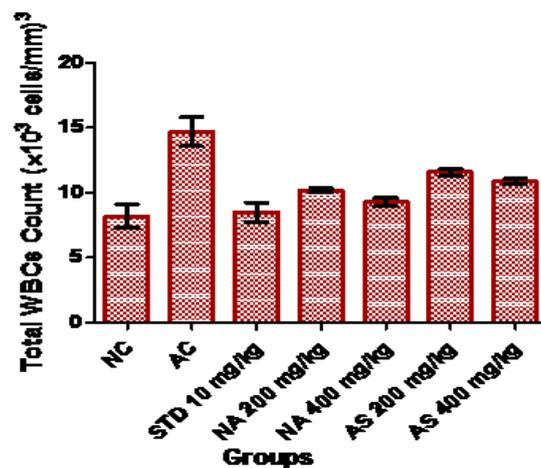


Figure 3: Effects of *N. arbor-tristis* and *A. scholaris* on WBCs count in FCA induced arthritis

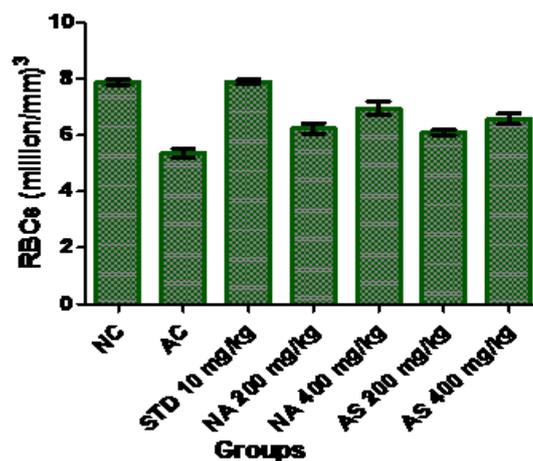


Figure 4: Effects of *N. arbor-tristis* and *A. scholaris* on RBCs count in FCA induced arthritis

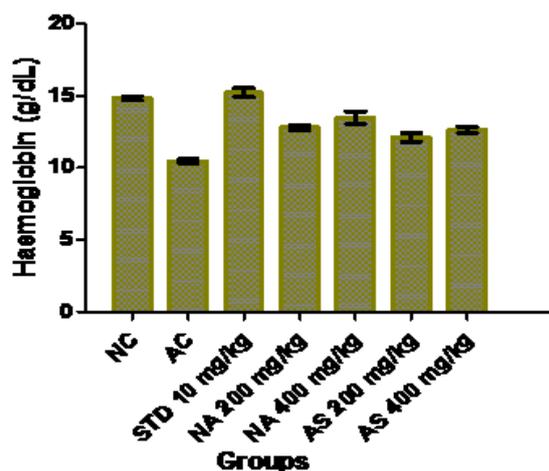


Figure 5: Effects of *N. arbor-tristis* and *A. scholaris* on Hemoglobin in FCA induced arthritis

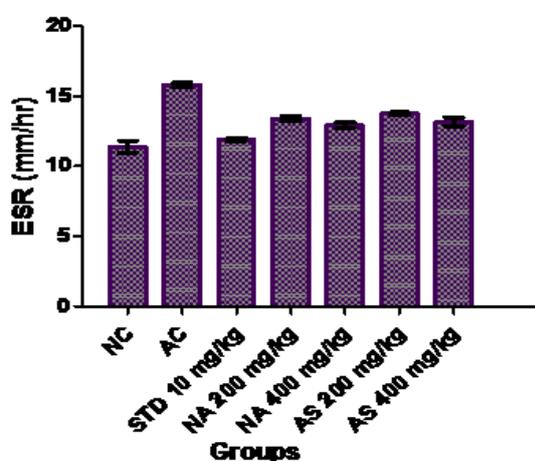


Figure 6: Effects of *N. arbor-tristis* and *A. scholaris* on ESR in FCA induced arthritis

## DISCUSSION

The model of adjuvant-induced arthritis in rats is a useful tool to study the pathophysiology of rheumatoid arthritis, especially because the experimental model and the human disease share various signs and symptoms<sup>[13]</sup>. Administration of Freund's complete adjuvant to the rat induces acute inflammation in the injected paw and chronic inflammation and arthritic lesions in the un injected paw, between 10 and 14 days. The chronic phase is accompanied by splenomegaly and lymphocyte-mediated events<sup>[14]</sup>.

The present investigations established the comparative anti-arthritic potential of *Nyctanthes arbor-tristis* and *Alstonia scholaris* extracts using adjuvant induced arthritis model rats because rats develop a chronic swelling in multiple joints, with the influence of inflammatory cells, erosion of joint cartilage, and bone destruction. It is very close to human arthritis disease.

Chronic inflammation involves the release of inflammatory mediators such as cytokines (IL-1B and TNF- $\alpha$ ), interferon and Platelet derived growth factors (PDGF). These mediators are mainly responsible for the pain, destruction of bone cartilage which can cause severe disability<sup>[15]</sup>. In FCA induced arthritis, the present study revealed that the paw volume increases with ankle stiffness in adjuvant-challenged animals. *Nyctanthes arbor-tristis* in 200 & 400 mg/kg administration delayed the onset and suppressed severity of arthritis, as demonstrated by decreased both the paw volume, while *Alstonia scholaris* have lower therapeutic effects as compared to *Nyctanthes arbor-tristis* in a dose dependent manner. The percentage inhibition of paw edema showed maximum protection on 21<sup>st</sup> day by *Nyctanthes arbor-tristis* is 60 % at 400 mg/kg while *Alstonia scholaris* causes 58 %.

Change in the body weight is also generally used to assess the course of the arthritis and to assess positive benefit obtained with the anti-inflammatory drugs. With the increase in severity of arthritis, there is moderately decrease in the body weight of the rats can occur. The decrease in body weight was attributed to reduced absorption of <sup>14</sup>C- glucose and [<sup>14</sup>C]-leucine in rat's intestine. But the body weight increases in the course of the therapy due to increase in the absorption capacity of intestine<sup>[16, 17]</sup>. In our present investigation, *N. arbor-tristis* significantly increase body weight of treated groups in dose dependent manner while there is a increment in body weight of treated groups by *A. scholaris* but less as compared to *N. arbor-tristis*.

In arthritic state, there is a mild to moderate rise in WBCs count due to the release of IL-1 inflammatory response, and IL-1 increases the production of both granulocyte and macrophages colony stimulating factors. In the present study, the migration of leucocytes into the swelled area is significantly suppressed by *N. arbor-tristis* and *A. scholaris* extracts when compared to arthritic control, as seen from the significant reduction in the total WBCs count. As a comparative effects *N. arbor-tristis* causes significantly decrease in WBCs count however, *A. scholaris* causes significantly decrease in count but less as compared to *N. arbor-tristis*.

Erythrocyte sedimentation rate is an estimation of the suspension stability of RBC's in plasma. It is associated to the number and size of the red cells

and with the relative concentration of plasma proteins, especially fibrinogen, alpha, and beta globulins. Increase in the rate, is an indication of active but obscure disease processes. The acute phase proteins in ESR and C-reactive proteins (CRP) share the property of showing elevations in the concentration in response to stress or inflammations like injection, injury, surgery, and tissue necrosis. The ESR count significantly increased in arthritic control group, whereas these counts were remarkably counteracted in the standard prednisolone and *N. arbor-tristis* and *A. scholaris* extracts groups and thus justifying its significant role in the arthritic conditions <sup>[18]</sup>.

In addition to this, other characteristic hematological alterations such as the decreased Hb and RBCs count was also significantly restored by the extracts of *N. arbor-tristis* and *A. scholaris* treatments. It is proposed that the reduction in the Hb & RBCs count during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells <sup>[19]</sup>.

In accordance with previous phytochemical studies, phytoconstituents like steroids, flavonoids, alkaloids, terpenoids and tannins have been shown to possess anti-inflammatory and analgesic activity <sup>[20, 21]</sup>. The phytochemical analysis of *N. arbor-tristis* and *A. scholaris* extracts confirmed the presence of steroids, flavonoids, tannins, glycosides, alkaloids and terpenes.

*N. arbor-tristis* are very rich source of flavonoids, phenolics and glycosides compounds while *A. scholaris* are rich sources of alkaloids and terpenes.

The *N. arbor-tristis* was found to show more anti-arthritic activity when compared with *A. scholaris*. Thus the anti-arthritic activity of *N. arbor-tristis* and *A. scholaris* leaves may be due to presence of active constituents like flavonoids and terpenes.

## CONCLUSION

From the above results it was found that *N. arbor-tristis* & *A. scholaris* have significant anti-arthritic activity. So these can be used as a potential natural source of inflammation disorders by preventing or slowing the progress of symptoms of arthritis. The study is further extended to identify and characterize the most active bioactive fractions & phytoconstituents which are

responsible for the observed significant anti-arthritic activity & to understand the mechanism of action against adjuvant-induced arthritis in rat.

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