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**ORIGINAL ARTICLE**

# Effect of *Clome viscosa* extract on Adjuvant Induced Arthritis in Rats

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**ABSTRACT**
*A study was undertaken to assess the effect of Cleome viscosa extract on adjuvant induced arthritis. Twenty four Wistar rats were randomly divided into four groups. Group I served as normal control. Group II served as arthritic control. Group III and IV received 2% Cleome viscosa extract ointment and Diclofenac ointment respectively along with Freund`s Complete Adjuvant (FCA) for induction of arthritis. Measurement of hind paw thickness and estimation of C-reactive protein were done apart from haematological and biochemical parameters. Results suggest that Cleome viscosa extract is effective at 2% concentration on external application against adjuvant induce arthritis, but not as effective as diclofenac.*
*Keywords: Clome viscosa, Arthritis, diclofena*

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## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic disorder that is characterized by a symmetric polyarthritis finally leading to progressive joint destruction. Conventional drug therapy has failed to control long term morbidity associated with arthritis. Corticosteroids and Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed therapeutic agents for the treatment of rheumatic diseases. But the drawbacks of long term use of these drugs such as immune suppression and gastritis have led to the search for better alternatives. *Cleome viscosa* extract has been reported to have anti-inflammatory properties [1]. Hence this work was designed to study the effect of C.viscosa whole plant extract on adjuvant induced arthritis (AIA) in rats and compare its effect with standard anti-inflammatory agent, Diclofenac.

## MATERIALS AND METHODS

### Experimental animals

Wistar rats of either sex, weighing approximately 140-200g supplied by the Department of Laboratory Animal Medicine, Tamil Nadu Veterinary and Animal Sciences University, Chennai, were used in this study. All the experiments in the study were approved by institutional animal ethical committee and CPCSEA. All the animals were maintained under normal condition with free access to feed and water.

### Plants

Plant samples of *Cleome viscosa* were collected from in and around the campus of Veterinary College and Research Institute, Namakkal and were authenticated by a botanist of a local Arts and Science College.

### Drug

The standard drug Diclofenac gel of Novartis Company, available in the market was used in the study for comparision.

### Freund`s Complete Adjuvant (FCA)

Freund`s Adjuvant was purchased from Sigma Chemical Co, USA and it contains heat killed *Mycobacterium tuberculosis* suspended in paraffin oil at a final concentration of 1 mg per ml.

### Preparation of 2% Cleome viscosa extract ointment

Two grams of dried extract of the plant *Cleome viscosa* was mixed well with 98 gm of white soft paraffin in mortar and pestle to prepare a 2 percent ointment.

**Experimental design**

Twenty four rats were randomly divided into four groups. Group I served as normal control and received only liquid paraffin. Group II served as arthritic control and received only FCA. Group III received FCA and 2% *Cleome viscosa* extract ointment. Group IV served as positive control for comparison and received FCA and Diclofenac ointment.

**Induction of Adjuvant arthritis in rats**

On day 0, arthritis was induced in animals belonging to group II, III and IV by injecting 0.1 ml of Freund`s Complete Adjuvant below the plantar aponeurosis of the right hind paw of the rats [2]. In control group, animals received 0.1 ml of liquid paraffin instead of FCA.

**Measurement of paw swelling**

From day 1 onwards, the test drugs were applied to the animals of respective groups up to 29 days. Their paw thickness was measured on all the days by using Digital Vernier Caliper [3].

**Animal sacrifice**

All the animals were put on fasting over night before sacrifice. On 29$^{th}$ day, they were sacrificed under deep ether anaesthesia. Blood was collected in anti coagulant coated vials for studying haematological parameters. Simultaneously blood smears were made for haemogram studies. Blood was also collected in test tubes, centrifuged and serum was separated for biochemical estimations and immunological assessment.

**Haematology**

About 2 ml of blood was collected from each animal in sterilized test tubes containing one drop of 10 percent solution of EDTA as an anticoagulant and was used to find out the Erythrocyte Sedimentation Rate (Wintrobe method) (4).

**Enumeration of WBCs**

Total WBC counts were recorded using a haemocytometer and Nambiyar`s fluid [4] as the diluting reagent.

**Differential count**

Blood smears were stained by modified copper peroxidise method of Sato and Sakiya [4]. Around 125 cells were counted from each blood smear and the percentage of different WBCs was calculated and the average of two counts was taken.

**Serum Biochemistry**

The following biochemical estimations were done in serum:

1) Alanine amino transferase (ALT) [5]
2) Aspartate amino transferase (AST) [5]
3) Acid Phosphatase [6]
4) Alkaline phosphatase [6]

**Immunological study - Estimation of C-Reactive Protein (CRP)**

The C-Reactive Protein in serum was estimated [7] to assess the extent of inflammation that had taken place.

**Statistical analysis**

The data were analysed by ANOVA followed by Students 't' test for significance [8].

## RESULTS AND DISCUSSION

**Effect of** *Cleome viscosa* extract **on paw thickness**

**At the end of first week**

The mean values of right hind paw thickness at the end of first week in all the four groups were 3.15±0.112, 6.84±0.193, 6.82±0.012 and 6.81±0.020 respectively. Mean values of left hind paw thickness were 3.12±0.098, 3.83±0.123, 3.84±0.011 and 3.81±0.027 respectively. There was no significant difference for both right and left hind paws between the treatment groups and arthritic control group during first week of treatment, while a highly significant difference ($P \leq 0.01$) was noticed between the arthritic control group and the normal control group

**At the end of second week**

The mean values of right hind paw thickness at the end of second week in all the four groups were 2.82±0.048, 7.81±0.564, 6.83±0.026 and 6.13±0.020 respectively. Mean values of left hind paw thickness were 2.70±0.057, 4.32±0.101, 3.67±0.028 and 3.65±0.007 respectively. A highly significant difference was observed for right hind paw between the Diclofenac treatment and the arthritic control group. The right hind paw of *cleome viscosa* treatment showed no significant difference from arthritic control group. A

highly significant difference (P≤ 0.01) was noticed for left hind paw between the arthritic control group and the treatment groups.

**At the end of third week**

The mean values of right hind paw thickness at the end of third week in all the four groups were 2.81±0.050, 8.37±0.710, 5.23±0.223 and 3.68±0.012 respectively. Mean values of left hind paw thickness were 2.76±0.051, 4.59±0.524, 2.99±0.062, and 2.90±0.012 respectively. A highly significant difference (P≤ 0.01) was noticed for both right and left hind paws between the arthritic control group and all other groups.

**At the end of fourth week**

The mean values of right hind paw thickness at the end of fourth week in all the four groups were 2.89±0.034, 8.76±0.628, 4.89±0.155 and 2.91±0.017 respectively, while the values of left hind paw thickness were 2.83±0.048, 5.32±0.584, 3.12±0.063, and 2.85±0.014 respectively. A highly significant difference (P≤ 0.01) was noticed for both right and left hind paws between the arthritic control group and all other groups.

During the first week of treatment, significant improvement couldn`t be observed in the treatment groups for both right and left hind paws. During second week, a highly significant reduction in right hind paw thickness was observed in diclofenac group compared to arthritic control group. The right hind paw of *cleome viscosa* treatment showed no significant difference from arthritic control group. A highly significant reduction in left hind paw thickness of both the treatment groups was observed compared to arthritic control group. During the third and fourth week, a highly significant reduction of right and left hind paw thickness was observed with both the treatment groups compared to arthritic control.

The results are in agreement with findings of early report of Narendhira Kannan *et.al.*,[9]. The beneficial effect of *Cleome viscosa* extract may be attributed to the anti-inflammatory properties.

**Haematology**

**Total leukocyte count (TLC)**

The mean values of TLC for the four groups were 7678±128, 10007±91, 8527±443 and 7648±687 respectively. A highly significant (P≤ 0.01) increase in TLC was noticed in arthritic control when compared to all other groups.

**Differential count of leukocytes**

**Neutrophils**

The mean values of neutrophil count for the four groups were 65.17±3.0, 26.50±0.9, 61.50±3.5 and 63.25±1.1 respectively. A highly significant ((P≤ 0.01) decrease in neutrophil count was observed in arthritic control (Group II) when compared to all other groups.

**Lymphocytes**

The mean values of lymphocyte count for the four groups were 34.50±3.0, 71.17±0.3, 35.33±3.0, and 38.75±3.3 respectively. A highly significant ((P≤ 0.01) increase in lymphocyte count was observed in arthritic control when compared to all other groups.

There was no significant difference between the groups in eosinophil and monocyte counts. Basophils were absent in all the blood samples.

**Erythrocyte Sedimentation Rate (ESR)**

The mean values of ESR at 30 minutes for the four groups were 8.00±0.97, 21.33±3.19, 16.50±5.79 and 8.75±2.93 respectively. The mean values of ESR at 1 hour were 19.33±2.40, 48.33±4.16, 26.33±6.34 and 20.25±6.37 respectively. A highly significant (P≤ 0.01) increase in ESR value at 30 minutes and 1 hour was observed in arthritic control group (Group II) when compared to all other groups.

Increase in ESR is an indication of active but obscure disease processes [10]. The results of the study concur with those of Narendhira Kannan *et.al* and thus suggestive of the anti-inflammatory activity of cleome viscosa extract [11].

**Serum biochemistry**

**Aspartate aminotransferase (AST)**

The mean values of AST for the four groups were 104.50±5.60, 259.64±15.44, 176.40±15.31 and 110.05±12.79 respectively. A significant (P≤ 0.05) increase in AST value was observed in arthritic control as well as *Cleome viscosa* extract treatment group, when compared to normal control.

**Alanine aminotransferase (ALT)**

The mean values of ALT for the four groups were 21.47±2.99, 46.09±1.89, 15.12±2.96 and 14.18±3.79 respectively. Compared to normal control, the arthritic control group recorded a significant increase while the treatment groups recorded a significant decrease in ALT value.

**Alkaline phosphatase**

The mean values of alkaline phosphatase for the four groups were 191.67±16.78, 451.80±30.36, 278.13±15.09 and 230.50±19.36 respectively. A significant (P≤ 0.05) increase in alkaline phosphatase value was observed in arthritic control as well as in the treatment groups, when compared to the normal control. But treatment groups showed a significant decrease in alkaline phosphatase when compared to arthritic control group.

**Acid phosphatase**

The mean values of acid phosphatase for the four groups 1.95±0.27, 3.48±0.41, 2.56±0.32 and 1.81±0.14 respectively. A significant ((P≤ 0.05) increase in acid phosphatase value was observed in arthritic control group when compared to normal control and treatment groups. Treatment groups did not differ significantly from normal control group.

The arthritic control group showed significantly higher values of AST, ALT, alkaline and acid phosphatases when compared to normal control group (Table 4). The treatment groups recorded appreciable reversal of the effects. Except for the higher value shown by *Cleome viscosa* treatment in AST the treatment groups showed values closer to normal control group. Rajkapoor *et al*. [12] and Narayanaraju, [13] have reported similar findings earlier.

The elevated levels of serum marker enzymes such as AST, ALT, and ALP are indicative of liver damage since liver impairment is also a feature of adjuvant induced arthritis [14]. In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane [15], thereby causing an increased enzyme level in serum. The reversal of these effects is indicative of the anti-inflammatory effects of *Cleome viscosa* [16].

It has already been observed that lysosomal enzyme acid phosphatase plays a major role in joint destruction in erosive synovitis and in other degenerative joint diseases [17]. This lysosomal enzyme could have been released from cells of the inflamed synovium or from polymorphs and escaped into the joint spaces resulting in the cartilage erosion which underlies the inflammatory pannus of rheumatoid joints. There is indirect evidence that lysosomes mediate tissue injury induced by immune reactions. Most clinically active NSAIDs are proved to inhibit the release of lysosomal enzyme in cultured polymorphonuclear leucocytes (PMNLs) [18].

In this study, arthritic rats treated with *Cleome viscosa* extract as well as diclofenac showed reduction in acid phosphatase level and shows that the anti-inflammatory activity of these agents could be partially due to inhibition of release of lysosomal enzymes or enhancing lysosomal membrane stability.

**Immunological study**

The mean CRP values of arthritic control, *cleome viscosa* treatment and diclofenac treatment are 16±2.02, 9.9±2.03 and 2.8±0.4 respectively. Samples from normal control did not produce any agglutination, indicating that the CRP concentration is within the normal range (0.6 mg /dl).

A highly significant increase in mean CRP value of arthritic control group was observed when compared to diclofenac treatment group. However the *cleome viscosa* treatment group though showed a decrease, did not differ significantly from arthritic control group.C-reactive protein concentration is elevated in response to stress or inflammation that occurs after infection, injury, surgery and tissue damage. So in arthritic condition, CRP level is generally elevated [19]. The reduction in CRP level, in this study is indicative of the anti-inflammatory potential of the agents used for treatment.

## SUMMARY AND CONCLUSION

A study was undertaken to assess the effect of *Cleome viscosa* extract on adjuvant induced arthritis in Wistar rats, with diclofenac as the standard drug for comparison. *Cleome viscosa* extract at 2% concentration on external application was found to be effective against adjuvant induced arthritis, but not as effective as diclofenac. However *Cleome viscosa* extract is a promising drug for use against adjuvant induced arthritis, with further studies to elucidate the most effective dose to obtain its fullest antiarthritic effect.

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