Antiarthritic Evaluation of *Crateva religiosa* Extracts

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**ABSTRACT**

The anti-arthritic potential of *Crateva religiosa* hook & frost belonging to family Capparidaceae were evaluated by taking complete Freund’s adjuvant (CFA) induced model. Arthritis was induced by injecting 0.1ml of complete Freund’s adjuvant below the plantar aponeurosis of the right hind paw. Treatment with the extracts and standard started on the day of induction of CFA and continue up to 28 days. In this study both the alcoholic and aqueous extracts significantly (p<0.01) decrease the paw edema on 28\(^{th}\) day. They also significantly rectified the deranged hematological parameters and biochemical parameters which is observed from the studies. the alcoholic extracts found to show more effect than aqueous extracts in the terms of % of inhibition. The study gives a scientific rational to the use of this plant in arthritis and related conditions.

**Keywords**: *Crateva religiosa*, complete Freund’s adjuvant, Hematological parameter, Biochemical parameters

**INTRODUCTION**

Rheumatoid arthritis (RA) is a kind of chronic inflammatory autoimmune disease\(^1\) of joints that results in joint pain, swelling and destruction. It affects an estimated 1% of the adult population throughout the world. RA progresses in three stages first stage is the swelling of synovial lining causing pain, warmth, stiffness, redness and swelling around the joints, second is the rapid division and growth of cells or pannus which causes the synovium to thicken, in the third stage the inflamed cells releases enzyme that may digest bone and cartilage, often causing the involved joint to lose its shape and alignment, resulting pain and loss of movement\(^2\). Although a number of drugs (non-steroidal or steroidal anti-inflammatory agents and immune-suppressants used in the treatment of RA have been developed over the past few decades, there is still an urgent need for more effective drugs with lower side effects\(^3\). The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists worldwide\(^4\). This is corroborated by World Health Organization in its quest to bring primary health care to the people. The plant kingdom has long serve as a prolific source of useful drugs, food, additives, flavoring agents,
colorants, binders and lubricants. As a matter of fact, it has been estimated that about 25% of all prescribed medicines today are substances derived from plants. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Medicinal plants play a key role in health care in man and many plants are claimed to possess anti-arthritic activity.

The adjuvant arthritic model represents a systemic inflammatory disease with common patho-logical features like joint swelling associated with cellular and pannus invasion of the joint space and bone resorption in rats as RA in human. Strong bone loss after intense arthritis is induced when adjuvant is injected into the footpad. Crateva religiosa hook & frost belonging to family Capparidaceae (cappaceae) is a tree usually found in the vicinity of temples of central and eastern India. It is popularly known as pasugandha in Sanskrit, three legs capper in English, varuna in hindi Ethanomedically the plant used as diuretic, laxative, lethinotriptic, antirheumatic, tonic antiperiodic etc. In folklore the bark is specially used in urinary disorders including kidney and bladder stone, caliculi effection, anti-emetic etc. The present study is an attempt to give a scientific proof for the use of this plant against inflammatory conditions taking rat as animal model.

MATERIALS AND METHODS

Plant material

The plant was collected from rural belt of Bhubaneswar, Orissa. The plant was identified and authenticated in Regional Research Laboratory, Bhubaneswar, Orissa, India. The voucher specimen bearing no.9995 was deposited at the herbarium of Regional Research Laboratory Bhubaneswar for further reference. The bark was collected in bulk and washed with tap water to remove the soil and dirt particles and then shad dried. The dried plant materials were milled into coarse powder by a mechanical grinder and sieved in sieve 20. The course powder was extracted successively using soxhlet apparatus with Ethanol (95%) and water for 72 hour. The extracts were concentrated by distilling off the solvent under reduced pressure and kept inside desiccators. The % of yield was calculated separately for each extract with respect to the air dried weight.

Experimental animals

Adult male albino mice 20-25gm and rats 150-200gms were used for the study. Animals were kept in the animal house of university department of pharmaceutical sciences, Bhubhaneswar, maintained under standard husbandry condition with free access to food and water ad libitum. The animals were acclimatized for 14 days to the laboratory conditions before doing experiments. All the experiments in this study were approved by institutional animal ethical committee with CPCSEA registration number IAEC/999/UDPS, Utka University.

Acute toxicity studies

Oral acute toxicity studies were carried out with Albino mice weighing 20-25gm. The extracts were administered as per the staircase method. The mice were fed with alcoholic and aqueous extracts of Crateva religiosa separately suspended in 5% w/v normal saline at dose 500, 1000, 1500, 2000, 2500 mg/kg bodyweight. The animals were observed continuously for 2 hours for the gross behavioral changes and then intermittently once in every 2 hours and finally at the end of 24 and 72 hours to note for any signs of toxicity including death.

Induction of arthritis

Freund’s adjuvant induced Arthritis model was used to access the anti-arthritic
activity in albino rats. Animals were randomly divided into six groups of six animals each (n=6). Group I served as control received 5% of normal saline, Group II received Diclofenac sodium (10mg/kg p.o) served as reference standard. Group III and IV received the alcoholic extracts at a conc. of 250 and 500mg/kg. Group V and VI receive aqueous extracts at conc. of 250 and 500mg/kg. Arthritis was induced by injecting 0.05ml (0.5%w/v) of freund’s adjuvant into the left hind paw. treatment with standard and the test drug were continue up to 28 days. Left paw is marked with ink at the level of lateral malleolus; basal paw volume is measured plethysmographically by volume displacement method using Plethysmometer by immersing the paw till the level of lateral malleolus. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0day), 30 minutes before adjuvant injection and continued till 28th day. Paw thickness was measured on 1st, 2nd, 4th, 8th, 12th, 16th, 20th, 24th, 28th days by plethesmograph. The percent inhibition of the mean increase in the paw edema of each group was calculated by the formula:

% Inhibition = \( \frac{V_c - V_t}{V_c} \times 100 \)

Vt and Vc edema volume in the drug treated and control groups respectively in respective days.

The difference in the paw edema from the control group to that of treated group is taken as the anti-arthritis potential of the extracts.

On 28th day the rats were anaesthetized under light ether anesthesia and blood was collected by retroorbital and cardiac puncture for biochemical and haematological estimation. RBC and WBC count were estimated according to the method of Chesbrough and McArther in an improved neubauer chamber. ESR was estimated by the method of westergren.

Assay of SGPT and SGOT activities was modified by the International Federation of Clinical Chemistry (IFCC) and become the recommended methods for measurement of SGPT and SGOT activities in serums. Alkaline phosphatase (ALP) was estimated by the p-nitrophenyl phosphate (P-NPP) method using the recommendations of German society for Clinical Chemistry (optimized IFCC, P NPP-AMP method) [IFCC/AACC]

**Statistical analysis**

The statistical significance was assessed by using one-way analysis of variance (ANOVA) and followed by dunnet’s comparisons test. All the data are presented as mean ± SEM and p<0.05 was considered as significant.

**RESULTS & DISCUSSION**

The present study was carried out to evaluate the efficiency of Indian herbal source against a chronic inflammatory disease, that is, arthritis. In the present study, rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid diseases. The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs.

The effect of *Crateva religiosa* aqueous extracts and diclofenac sodium on adjuvant arthritis is shown in Table 1. In the control group, maximum swelling was seen on the 8th day and thereafter there was gradual decline in the swelling up to 12th day. The swelling was found to increase again and become maximum on the 20th day. In addition to this, a swelling was also noticed in the uninfected paw as well as in the fore paws on 9th day. Furthermore, inflammatory lesions were also observed in the tail in the form of nodules which often ulcerated, and in the ears as dilated capillaries. These secondary lesions
were graded as mild, moderate and severe. *Crateva religiosa* and diclofenac sodium significantly inhibited both the primary and secondary phases of adjuvant arthritis. The nature of the secondary lesions was also modified from severe degree to moderate by *Crateva religiosa* extracts. The sudden alteration of physical parameters observed in our results at 12th day, namely the sharp increase in paw circumference and volume and the decrease of body weight, lack of mobility suggest that day 12 may be the beginning of a second stage where the secondary reactions began. The decrease of body weight and paw volume and circumference, after day 12 until day 20, suggests that the second stage is established within that period of time. The test drugs significantly decrease the paw volume in a dose dependent manner in both the phases. Standard diclofenac and *Crateva religiosa* aqueous extracts significantly decrease the paw edema significantly from the 1st day. At 250mg/kg the extract is found to show effect only in acute phase rather than in secondary phase. At a dose of 500mg/kg it suppresses both the phases of inflammation. Standard diclofenac suppress the inflammation from the first day of study.

Assessment of the levels of SGOT, SGPT and ALP provides an excellent and simple tool to measure the antiarthritic activity of the target drug. The activities of aminotransferases and ALP were significantly increased in arthritic rats, since these are good indices of liver and kidney impairment, which are also considered the features of adjuvant arthritis. Serum SGOT and SGPT have been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process. Also, Niino-Nanke *et al.* confirmed a positive correlation between the increased activity of serum ALP and the disease activity in RA. Elevated levels of serum ALP in arthritis induced rats can be due to increase in the liver and bone fraction or due to an increase of both isoenzymes. This in turn implicates a localized bone loss in the form of bone erosion and periarticular osteopenia, as the enzyme is released into circulation in the course of bone formation and resorption. The decreased enzyme levels on treatment of cratevareligiosa alcoholic and aqueous extract emphasizes the decreased bone loss and organ protective role of it against adjuvant induced arthritic rats, since treatment of NSAIDs which are hepatotoxic result in elevated levels of SGOT and SGPT in RA. From this it may be suggested that the anti-inflammatory effect is maybe due the rectification of biochemical parameters. The significant protection of these parameter is might be due to the combined effect of the phytochemical and lupol present in them.

Upon induction of freunds adjuvant the level of SGPT, SGOT and ALP rise to 158.4, 56.3 and 486.00 units/ml. Treatment with the *crateva religiosa* extracts significantly rectify the deranged parameters in a dose dependent manner as shown in table 2. Alcoholic extracts found to be more effective than the aqueous extracts. At a dose of 500 mg/kg the alcoholic extracts rectify the levels to 66.3, 24.4 and 226.32 for SGPT, SGOT and ALP respectively whereas the aqueous extracts at same dose level rectify it to 76.2, 30.3 and 256.58. The value of SGPT, SGOT and ALP values are 86.4, 38.6, 332.74 and 76.2, 30.3, 256.58 when they are treated with 250 mg/kg of *crateva religiosa* aqueous extracts. Standard diclofenac though shows significant anti-inflammatory effect they does not have any protective role for these biochemical parameters.

In the present study, the arthritic rats exhibited a reduced RBC count, reduced Hb level and an increased ESR. All these indicate the anemic condition, which is a common diagnostic feature in patients with chronic arthritis. Our extracts significantly
(p<0.05) rectify these condition. The RBC count increased to 5.6, 5.3, 6.6, 4.8 and 5.4 with the treatment of standard diclofenac, 250 and 500mg/kg of alcohol and aqueous extract respectively. Which indicate the usefulness of these extracts in alleviating the anemic conditions induced by freund’s adjuvant. Erythrocyte sedimentation rate (ESR) is an estimate of the suspension stability of RBC’s in plasma. It is related to the number and size of the red blood cells and to the relative concentration of plasma proteins, especially fibrinogen, α and β globulins. Increase in the rate is an indication of active but obscure disease processes. The rise in ESR responds to stress or inflammation like injection, injury, surgery and tissue necrosis. The treatment with the crateva religiosa extracts improved the ESR to a near normal level indicating the significant recovery from the arthritis progress thus justifying its significant role in arthritic conditions. White blood cells (WBC) are a major component of the body’s immune system. Indications for a WBC count include infectious and inflammatory diseases. In arthritis condition there is a mild to moderate rise in WBC count due to release of IL-IB. IL-IB increases the production of both granulocyte and macrophages colony stimulating factor. WBC count was increased in arthritic group. The migration of leukocytes is significantly suppressed in extract treated groups as seen from the significant decrease in the WBC count. All the rectification of hematological parameters supports the antiarthritic effect of crateva religiosa.

CONCLUSION

In the light of the above results it might be concluded that crateva religiosa exhibited a potent antiarthritic effect by reducing the pathological lesions via decreasing the paw volume in of the test animals and modifying the deranged hematological and biochemical parameters. The effect of crateva religiosa can be co related with the presence of phytoconstituents such as flavonoids, tannins, saponisin it. This work gives a scientific rational to the use of this plant in arthritis and related conditions.

REFERENCES

Table 1. Effect of *Crateva religiosa* aqueous extracts and diclofenac (standard drug) on CFA induced paw edema in rats

All values represent the in Avg. ± S.E.M of 6 rats for each group.
Each value in parenthesis indicates the percentage inhibition rate
Statistically significant from control *p<0.01 for control untreated Vs treatment (Dunnett’s t-test)
Table 2. Effect of *Crateva religiosa* extract treatment on biochemical parameter during Freund’s adjuvant induced arthritis

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT</th>
<th>SGPT</th>
<th>Alkaline phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline 5ml/kg p.o.</td>
<td>158.4±8.6</td>
<td>56.3±4.8</td>
<td>486.00 ± 9.59</td>
</tr>
<tr>
<td>Ethanolic extract (250mg/kg)</td>
<td>86.4±4.8(45.45)*</td>
<td>38.6±2.4(32.22)</td>
<td>332.74± 12.5(31.53)</td>
</tr>
<tr>
<td>Ethanolic extract (500mg/kg)</td>
<td>66.3±4.3(58.14)*</td>
<td>24.4±1.4(56.66)*</td>
<td>226.32± 6.42(53.4)*</td>
</tr>
<tr>
<td>Aqueous extract(250mg/kg)</td>
<td>98.8±6.2(37.62)*</td>
<td>39.2±2.6(30.37)</td>
<td>353.36± 16.4(27.36)</td>
</tr>
<tr>
<td>Aqueous extract(500mg/kg)</td>
<td>76.2±5.8(51.89)*</td>
<td>30.3±1.8(46.18)*</td>
<td>256.58± 11.3(47.32)*</td>
</tr>
</tbody>
</table>

All values represent the inAvg±S.E.M of 6 rats for each group. Each value in parenthesis indicates the percentage rectification as compared to control Statistically significant from control *p<0.01 for control untreated Vs treatment (Dunnett’s t-test)

Table 3. Effect of *Crateva religiosa* stem extracts on various hematological and biochemical parameters in FCA induced poly arthritis in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (normal saline 5ml/kg)</th>
<th>Standard (diclofenac 10mg/kg)</th>
<th>Alcoholic extract (250mg/kg)</th>
<th>Alcoholic extract (500mg/kg)</th>
<th>Aqueous extract (250mg/kg)</th>
<th>Aqueous extract (500mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm) Total</td>
<td>8.12±1.4</td>
<td>2.86±0.4</td>
<td>6.2±2.3</td>
<td>4.8±1.2</td>
<td>5.6±1.8</td>
<td>3.8±1.4</td>
</tr>
<tr>
<td>Hb%</td>
<td>8.4±1.6</td>
<td>12.3±0.8</td>
<td>10.4±1.2</td>
<td>12.6±1.6</td>
<td>11.2±1.4</td>
<td>12.2±1.2</td>
</tr>
<tr>
<td>WBC (cmm)</td>
<td>10560±465</td>
<td>7050±340</td>
<td>8625±425</td>
<td>8218±385</td>
<td>7992±486</td>
<td>7348±363</td>
</tr>
<tr>
<td>Lymphocytes(%)</td>
<td>69±5.8</td>
<td>32±3.2</td>
<td>58±6.4</td>
<td>53±4.3</td>
<td>47±4.4</td>
<td>42±3.6</td>
</tr>
<tr>
<td>RBC (million/cmm)</td>
<td>4.2±0.64</td>
<td>5.6±0.32</td>
<td>5.4±0.46</td>
<td>6.6±0.34</td>
<td>4.8±0.42</td>
<td>5.3±0.36</td>
</tr>
</tbody>
</table>

All values represent the inAvg±S.E.M of 6 rats for each group. Statistically significant from control p<0.01 for control untreated Vs treatment (Dunnett’s t-test)