INTRODUCTION

Rheumatoid Arthritis (RA) is a systemic and chronic inflammatory autoimmune disease affecting 0.5-1% of the entire human population. It has been well characterized by a symmetric polyarthritis affecting several joints, accompanying synovial hyperplasia, consequently leading to joint destruction and deformity, loss of function and reduced quality of life. Although the exact pathogenesis and etiology of the disease remain unclear, the main pathological changes have been defined, such as abnormal immunity, chronic synovitis, inflammatory cell infiltration, pannus formation, destruction of cartilage and bone erosion. The development of RA involves a complex interplay of several types of cells, including B and T lymphocytes, macrophages, fibroblasts-like synoviocytes, endothelial cells and dendritic cells. Notably, B and T cells play critical roles in the pathogenesis. Currently, the clinical need for effective treatment of RA remains unmet and more novel drugs are highly demanded.

Type II Collagen induced arthritis in rats and mice were well-known to have both clinical and histological similarities to human RA, and this model has been widely used in experiments to study RA. Rat type II Collagen arthritis results when rats were immunized against homologous or heterologous Type II Collagen. The resulting polyarthritis is characterized by marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption, periosteal proliferation and peri-articular inflammation. In Indian system of medicine, a large of drugs of either herbal or mineral origin has been advocated for various types of diseases and different unwanted conditions in humans. Ayurvedic medicines are largely based upon herbs and herbalmineral preparations and have specific diagnostic and therapeutic principles. Acalypha indica belongs to Euphorbiaceae family and can be found in plains of south India. Acalypha indica has been traditionally used as Anthelmintic, cathartic, scabies, rheumatism, anodyne. The extracts of Acalypha indica have been reported to possess anti-inflammatory, analgesic and antimicrobial properties. As there was no scientific evidence for its Anti-Arthritic activity, the present study was undertaken to evaluate the Anti-Arthritic activity of hydroalcoholic extract of whole plant Acalypha indica on Type II Collagen induced Arthritis in Wistar rats.

MATERIALS AND METHODS

Collection and Extraction

Whole plant Acalypha indica was collected in and around Kancheepuram District, Tamil Nadu, India and taxonomically identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, Chennai, India. Voucher number being PARC/2010/610. The plant was shade dried and coarsely powdered. The measured amount of powder (250g) was defatted with petroleum ether and then extracted with hydroalcohol at 45ºC for 48 h using Soxhlet apparatus. The hydroalcoholic extract of Acalypha indica (HEAI) was subjected to preliminary phytochemical screening.

Preliminary Phytochemical Screening

The hydroalcoholic extract of Acalypha indica was subjected to preliminary phytochemical screening for their presence or absence of active phytochemical constituents.

Animals

The experiment was carried out on Wistar rats weighing 150 – 200gms. The animals were procured from King’s institute, Guindy, Tamil Nadu, India. They were allowed for acclimation under controlled conditions for a week before use. They were feed with standard laboratory diet and water ad libitum and were maintained at 12 hr light / dark cycles at 25 ± 2°C and 50 ± 10% humidity. The study was approved by Institutional Animal Ethical Committee, CPCSEA with the proposal number being IAEC/105/2010. The animals were divided into 4 groups of 5 each.

Chemicals

Type II Collagen (SIGMA Ltd., India), Complete Freund’s Adjuvant (SRL Ltd., India) and methotrexate (Ranbaxy, India) were used in pharmacological studies.

Induction of Arthritis

Type II Collagen was dissolved overnight at 4ºC in 0.1mol/L acetic acid at 2.0 mg/mL, after which the solution was emulsified in an equal volume of complete Freund’s adjuvant (CFA) in an ice cold water bath. Arthritis was induced by an intradermal injection of 0.1
Results

Phytochemical screening

Preliminary phytochemical screening of the hydroalcoholic extract of *Acalypha indica* (HEAI) revealed the presence of alkaloids, flavonoids, phenol compounds, glycosides and triterpenes.

Histopathological studies

Histopathological studies of synovial joint obtained from rats were carried out on Day 21. Dissected joints were washed with normal saline and then kept in 10% formal saline. The joint then kept in Bouin’s fixative for 18-24 hr and then were washed twice with distilled water and placed in 70% alcohol. A pinch of lithium carbonate was added to remove excessive stain. The joints were washed and placed in 70% alcohol again. After that, they were transferred into 100% alcohol for 3 hr, then into xylene until they become transparent. At this point, the samples were fixed in paraffin to perform sectioning. Several sections (3 μm thickness) were taken from each joint and sections with uniform shape and size were selected for histology. Selected sections were fixed on clear glass slides and stained using Hematoxylin and Eosin (H & E) stain.

Statistical analysis

All the values were expressed as MEAN ± SEM. The data was analysed by one way ANOVA followed by Dunnet’s test. All statistical analyses were performed using Graph Pad software (San Diego, CA).

Results

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Haematological parameters estimation

The total WBC counts were remarkably increased in Negative control rats. However, methotrexate and HEAI 400 mg kg⁻¹ treated groups significantly (p<0.05) decreased the total WBC count.

The drastic increase in ESR count in Negative control has been remarkably counteracted by the standard and HEAI 400 mg kg⁻¹, has shown significant (p<0.05) decrease in ESR levels when compared to that of Negative control thus justifying their significant role in Arthritic condition. HEAI 200 mg kg⁻¹ does not show any significant change when compared to Negative control. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC (×10³ /mm³)</th>
<th>ESR (mm/h)</th>
</tr>
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<tbody>
<tr>
<td>Negative control</td>
<td>15.26±0.67</td>
<td>12.12±0.20</td>
</tr>
<tr>
<td>HEAI 2000 mg/kg</td>
<td>14.50±0.64</td>
<td>11.86±0.11</td>
</tr>
<tr>
<td>HEAI 4000 mg/kg</td>
<td>12.02±0.4**</td>
<td>7.4±0.13***</td>
</tr>
<tr>
<td>Standard 0.6 mg/kg</td>
<td>10.98±0.25**</td>
<td>3.46±0.17***</td>
</tr>
</tbody>
</table>

Values are expressed in terms of mean ± SEM, n=5 in each group. Value comparisons were made between Negative control Vs Group II, III, IV (p < 0.05). ** value is highly significant.

Measurement of inflammatory mediators in serum

The data reflects the significant increased (p<0.05) levels of rheumatoid factor (RF), cytokine IL-6 and C-reactive protein (CRP) levels when compared to Negative control. The results are shown in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>RF (IU/ mL)</th>
<th>IL-6 (pg/ mL)</th>
<th>CRP (μg/ mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>17.36±0.21</td>
<td>74.46±1.47</td>
<td>41±6.23</td>
</tr>
<tr>
<td>HEAI 2000 mg/kg</td>
<td>16.54±0.18</td>
<td>72.46±1.47</td>
<td>39.7±5.38</td>
</tr>
<tr>
<td>HEAI 4000 mg/kg</td>
<td>12.48±0.23***</td>
<td>66.36±1.10</td>
<td>28.2±8.92***</td>
</tr>
<tr>
<td>Standard 0.6 mg/kg</td>
<td>6.08±0.09***</td>
<td>5.10±2.62</td>
<td>11.9±3.80***</td>
</tr>
</tbody>
</table>

Values are expressed in terms of mean ± SEM, n=5 in each group. Value comparisons were made between Negative control Vs Group II, III, IV (p < 0.05). *** value is highly significant.

Histopathological studies

Histopathology of knee joint in Type II Collagen treated rats, revealed enhanced neutrophil infiltration, pannus formation and bone erosion, whereas in HEAI treated rats there were significant reduction in neutrophil infiltration, pannus formation and bone erosion. Figure 1, (a) is the TS of knee joint of Negative control rat as shown in Figure 1, (b). On treatment with HEAI 200 mg/kg there is very slight reduction in the neutrophil infiltration, pannus formation and bone erosion but HEAI 400 mg/ kg showed significant reduction in neutrophil infiltration, pannus formation and bone erosion, which is comparable with reference standard drug Methotrexate as shown in Figure 1, (c-e).

Discussion

Type II Collagen induced Arthritis in rats is a well established experimental model for the study of the pathophysiology of various types of human arthritis, especially Rheumatoid Arthritis (RA)¹. It results in marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption and periosteal proliferation and inflammation. Considering these advantages we utilized the Type II Collagen induced arthritic model in rats to assess the potential effects of whole plant extract of *Acalypha indica* upon inflammatory parameters.
Changes in body weight have been used to assess the course of disease and the response to therapy of standard. Arthritis is characterised by reduced weight loss and is associated with increased production of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1. Treatment with HEAI has shown significant increase in body weight when compared to Negative control.

In the present study, the Arthritic rats established increased WBC count and increased ESR levels, a common diagnostic feature in patients with chronic Arthritis. Increased WBC count indicates infectious and inflammatory condition. Increase in WBC count is mainly due to increased levels of B cells, T cells, Monocytes. Changes in ESR levels are also a useful measure of inflammation during RA and were proportional to the levels of disease severity.

Animals from the Group III and Group IV have showed decreased ESR rates and WBC count when compared to Negative control.

Rheumatoid Factor is useful as a measure for assessing the severity of Rheumatoid Arthritis. Intrasyovial B lymphocytes produce Rheumatoid Factors. RF is an antibody against the Fc portion of IgG, which is itself an antibody. It has been reported that RF can be synthesized by B-cells and Plasma cells that have infiltrated into the synovium of RA patients. These auto antibodies can belong to any of the three main Ig classes, G, A or M, but the classical rheumatoid factor is pentameric IgM. Rheumatoid factors react against IgG molecules that are abnormal in their carbohydrate moieties, a feature that probably renders them immunogenic. The resulting immune complex is likely to participate in the perpetuation of inflammatory processes.

The anti-CII antibodies produced by the immunization of heterologous CII protein and CFA bind to the CII of cartilage, and the complement system is activated. CSA, which is a cleavage product of complement component 5, is potently chemotactic for neutrophils and macrophages. The bound CII antibodies activate through CSA the recruited neutrophils and macrophages. These activated leukocytes secrete chemotoxic material and proinflammatory cytokines, such as IL-1β, TNF-α, IL-8, IL-6, nitric oxide (NO), and prostaglandins (PGE2), which activate synovial macrophages and infiltrating mononuclear cells. In ongoing inflammation, T cells, B cells, dendritic cells, and synovial macrophages proliferate and act in concert to secrete cytokines while interacting with each other. MMPs, elastase, and cathepsin G degrade cartilage. The synovial fibroblasts and macrophages progress to hyperplasia. Under restricted conditions, such as lower levels of IFN-γ and IL-4 and higher levels of TGF-β and IL-6, Th17 cells are induced and osteoclastogenesis occurs. The generated osteoclasts cause bone erosion. The T cell plays a regulatory role in the synovium and enhances or inhibits inflammation and bone erosion, depending on the prevailing conditions.

IL-6 is a pro-inflammatory cytokine that appears to play a pivotal role in inflammation of RA. It activates the production of acute phase C-reactive protein, fibrinogen and serum amyloid. IL-6 levels are significantly elevated in patients with RA and are correlated with clinical variables (e.g., morning stiffness duration and number of affected joints) and laboratory variables (e.g., erythrocyte sedimentation rate, C-reactive protein and rheumatoid factor titre). IL-6 contributes to pannus development and to the other local manifestations of RA. IL-6 can activate many cell types including immune system cells and synovial fibroblasts. Together with TGF-β and IL-1, IL-6 may be involved in the differentiation of naive lymphocytes to Th17 lymphocytes, although this remains to be confirmed in humans. Th17 cells in turn produce IL-17 which activates NF-κB and stimulates the production of several inflammatory mediators including TNF-α, IL-1, RANKL, IL-6, IL-8, PGE2.

Osteoblasts are among the cells that express the transmembrane IL-6 receptor. However, although the level of expression increases during osteoblast differentiation, it remains low, and soluble IL-6R seems required for IL-6 to exert its full effects on osteoblasts. IL-6 coupled to its soluble receptor may promote both the differentiation and the activation of osteoblasts. IL-6 can increase the expression of osteoblast differentiation markers such as alkaline phosphatase or osteocalcin.
The osteoclast is the main cell involved in bone erosions in RA. The effect of IL-6 on osteoclastogenesis is also complex but seems chiefly mediated by an indirect mechanism. Osteoclasts are cells of monocytic lineage whose differentiation to mature osteoclasts depends mainly on two cytokines, M-CSF and RANK-L. IL-6 is expressed in large amounts at sites of synovial membrane inflammation in patients with RA. Increased IL-6 levels during Arthritic condition acts indirectly on osteoclastogenesis by stimulating the release of RANK-L by bone tissue cells including osteoclasts resulting in bone resorption. This effect may be related chiefly to an indirect mechanism involving increased RANK-L release by inflammatory pannus cells or increased IL-17 production via differentiation of Th17 lymphocytes. Synovial fibroblasts also produce RANK-L after stimulation by IL-6. A highly significant correlation between IL-6 level and the severity of chronic arthritis in rats has been shown.

IL-6 is the main stimulator of C-Reactive protein that rises in the blood which indicates inflammation. IL-6 induces CRP production in the liver by activating Janus kinases. Signal transducers and activators of transcription subsequently switch on the CRP gene expression leading to production of CRP. Higher dose of extract i.e. Group III have shown significant decrease in levels of RF, IL-6 and CRP when compared to Negative control.

Our phytochemical investigation revealed the presence of Alkaloids, Flavonoids, Phenols, Carbohydrates, Proteins and amino acids, Terpenes, Gums and mucilage in the hydroalcoholic extract. Presence of wide range of constituents indicates such as Alkaloids, Flavonoids, steroids and Terpenes type of compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check Acalypha indica for possible Anti-Arthritic activity as these are known to inhibit inflammation.

From the results observed in the current investigation, it may be concluded that hydroalcoholic extract of Acalypha indica 400mg/kg possess potentially useful Anti -Arthritic activity. This study warrants the investigation to isolate and identify the active principles and to elucidate the exact mechanism of action.

ACKNOWLEDGMENT

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REFERENCES


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