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Research Article

ANTI ARTHRITIC ACTIVITY OF WHOLE PLANT *ACALYPHA INDICA* ON TYPE II COLLAGEN INDUCED ARTHRITIS IN WISTAR RATS

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ABSTRACT

Acalypha indica is a plant possessing various medicinal properties. The aim of present study was to investigate the Anti-Arthritic activity of the hydroalcoholic extract (HEAI) obtained from the whole plant at the dose of 200 mg kg^{-1} and 400 mg kg^{-1} on Type II Collagen induced Arthritic rats. Administration of extract improved the body weight significantly when compared to Arthritic rats. On day 21, treatment was assessed by measuring Haematological parameters like white blood cells (WBC) count and erythrocyte sedimentation rate (ESR). The investigated results showed that extract inhibited the Type II Collagen induced Arthritis in a dose dependent manner and this effect was more significant with 400 mg kg^{-1} dose. ESR levels in Arthritic induced rats were improved to near values as that of standard group (IV) in dose dependent manner. In addition, HEAI also significantly decreased WBC count in Type II Collagen induced Arthritic rats. The serum from each animal is used for estimation of RF, IL-6, CRP levels. Results suggested that there is no significant change in IL-6, CRP and RF levels in Group II animals (200mg/kg of HEAI) when compared to negative control (I). Group III (400mg kg^{-1} of HEAI) showed significant decrease in values of IL-6, CRP and RF when compared to Negative control (I). Histopathological analysis further confirmed the potent Anti-Arthritic effect of HEAI. The standard drug Methotrexate (0.6mg/kg) also produced significant Anti-Arthritic effect in rats. The results suggested that the hydroalcoholic extract of *Acalypha indica* at a dose of 400 mg kg^{-1} exhibits significant Anti-Arthritic effect.

Keywords: Anti-Arthritic, *Acalypha indica*, Type II Collagen, Rats, HEAI, Histopathology

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[1]. 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[2]. 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[3]. 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[4]. Ayurvedic medicines are largely based upon herbals and herbomineral 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 filtered and concentrated in vacuum and dried in desiccator. The percentage yield was found to be 5.6%w/w. The duration of the experiment was 21 days.

Preliminary Phytochemical Screening

The hydroalcoholic extract of *Acalypha indica* was subjected to preliminary phytochemical screening for their presence or absence of active phytochemical constituents[5].

Animals

The experiment was carried out on Wistar rats weighing 150 – 200grms. The animals were procured from King's institute, Guindy, Tami 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 ± 2ºC 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

mL of cold emulsion into base of the tail. A booster injection was also given on day 7 for induction of arthritis.

Grouping of Animals

The animals were divided into different groups on day 14 after the development of Arthritis. Drugs were administered on day 14 with the onset of arthritis.

Group I : Arthritic control (negative control)

Group II : Treated with HEAI 200 mg kg^{-1} p.o., from day 14 to 21

Group III : Treated with HEAI 400 mg kg^{-1} p.o., from day 14 to 21

Group IV : Treated with standard drug Methotrexate 0.6 mg kg^{-1} i.p., from day 14 to 21

Pharmacological Evaluation

Haematological parameters estimation

At the end of day 21 blood was collected (under light ether anaesthesia) from retro-orbital veins from each animal in heparinised tubes for the estimation of total white blood cells (WBC) according to the method of Chesbrough and McArthur in an improved Neubauer chamber and Erythrocyte Sedimentation Rate (ESR) by the method of Westergren.

Measurement of Inflammatory Mediators in Serum

At the end of Day 21, blood was collected (under light ether anaesthesia) from the retro-orbital veins of each animal and was placed in a non-heparinized tube for serum separation and it was then kept at -20° C for the quantitative determination of the levels of Rheumatoid Factor (RF), cytokine IL-6 and C-reactive protein (CRP) by ELISA (following manufacturer instructions).

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 were then 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 to 90% alcohol overnight. Joint samples were then transferred into 100% alcohol for 3 hr, then into xylene until they become transparent. At this point, the samples were fixed in paraffin to performed 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 Heamotoxylin 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

Phytochemical screening

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

Haematological parameters estimation

The total WBC counts were remarkably increased in Negative control. 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 1: Table shows the effect of HEAI on Haematological parameters using blood obtained on day 21 of the treatment regimens

Group	WBC($\times 10^3$ /mm^3)	ESR (mm/h)
Negative control	15.26±0.67	12.12±0.20
HEAI 200mg/kg	14.50±0.64	11.86±0.11
HEAI 400mg/kg	12.02±0.4***	7.4±0.13***
Standard 0.6mg/kg	10.98±0.25***	3.46±0.17***

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) during the arthritic condition; however, these Day 21 levels were significantly ($p<0.05$) reduced to near normal by HEAI 400mg/ kg and standard treatments.

The data reflects the levels of IL-6 in negative control rats were increased significantly. However, a significant protective effect against this increase was observed by treatment of the rats with HEAI 400mg/ kg and standard treatments.

The C-reactive protein (CRP) levels were found to be increased in negative control rats significantly. However, the treatments with HEAI 400mg/ kg and standard drug resulted in significant decrease ($p<0.05$) of C-reactive protein levels to near normal values. HEAI 200 mg kg^{-1} does not show any significant effect on RF, IL-6, CRP levels when compared to negative control. The results were tabulated in Table 2.

Table 2: Table shows the effect of HEAI on Inflammatory mediators in serum using blood obtained on day 21 of treatment regimens

Group RF (IU/ mL)	IL-6 (pg/ mL)	CRP (ng/ mL)
Negative control 17.36±0.21	74.46±1.47	411±6.23
HEAI 200mg/kg 16.54±0.18	72.46±1.47	397±5.38
HEAI 400mg/kg 12.48±0.23***	66.36±1.10	282±8.92***
Standard 0.6mg/kg 6.08±0.09***	51.02±2.62***	119±3.80***

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 control rats. Severe neutrophil infiltration, pannus formation and bone erosion is seen in knee joints of Arthritic 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)[6]. 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[7].

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

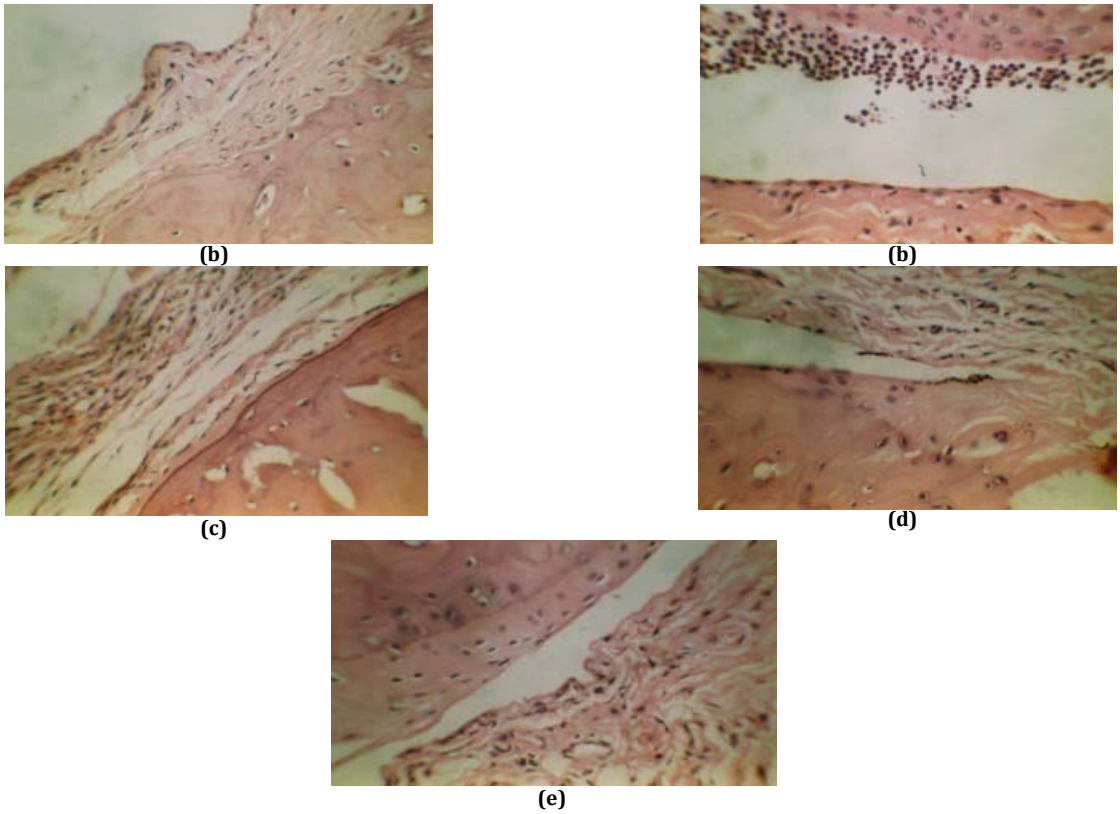

Fig. 1: It shows Histopathological changes observed in knee joints. (a) Control group (normal saline) (b) Negative control group (inducing agent) (c) Group II (200mg kg^{-1} HEAI) (d) Group III (400mg kg^{-1} HEAI) (e) Group IV (Standard)

Rheumatoid Factor is useful as a measure for assessing the severity of Rheumatoid Arthritis. Intrasynovial B lymphocytes produce Rheumatoid Factors. RF is an antibody against the Fc portion of IgG, which is itself an antibody[8]. It has been reported that RF can be synthesized by B-cells and Plasma cells that have infiltrated into the synovium of RA patients [9]. 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. C5a, which is a cleavage product of complement component 5, is potently chemotactic for neutrophils and macrophages. The bound CII antibodies activate through C5a 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[10].

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[11]. IL-6 levels are significantly elevated in patients with RA[12] 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-α 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[13]. 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, PGE_2[14].

Osteoblasts are among the cells that express the transmembrane IL-6 receptor. However, although the level of expression increases during osteoblast differentiation[15], 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[16].

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 osteoblasts resulting in bone resorption[17]. 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[18]. Synovial fibroblasts also produce RANK-L after stimulation by IL-6[19]. A highly significant correlation between IL-6 level and the severity of chronic arthritis in rats has been showed.

IL-6 is the main stimulator of C-Reactive protein that rises in the blood which indicates inflammation[20]. 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, Flavanoids**,** Phenols, Carbohydrates, Proteins and amino acids, Terpenes, Gums and mucilage in the hydroalcoholic extract. Presence of wide range of constituents indicates such as Alkaloids, Flavanoids, 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[21].

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 investigate on to isolate and identify the active principles and to elucidate the exact mechanism of action. As a number of Disease Modifying Anti Rheumatic Drugs in monotherapy often have unexpected side effects, combined treatment at lower doses may be necessary in order to expand the margin between efficacy and toxicity[22]. It is therefore necessary to develop new agents from natural sources, which when used in combination with other Anti Rheumatic drugs will be less toxic and at the same time, be affordable and effective for preventing Rheumatoid Arthritis[23].

In conclusion, the present study demonstrates that HEAI exerts inhibitory effect on Type II Collagen induced Arthritic rat model. The whole plant extract of *Acalypha indica* has shown significant decrease in various parameters such as WBC count, ESR levels and serum levels of IL-6, RF and CRP with reference to standard drug Methotrexate. Higher dose of extract (400mg/kg) has shown better significant effect on various parameters than the lower dose (200mg/kg). From the results observed in the current investigation, it may be concluded that hydroalcoholic extract of *Acalypha indica* possess potentially useful Anti -Arthritic activity. This study warrants the investigation on to isolate and identify the active principles and to elucidate the exact mechanism of action.

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