Evaluation of Anti-Arthritic Activity of Polyherbal Formulation in Collagen Induced Arthritic Model

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Abstract

Aim: The study was conducted to evaluate anti-arthritic activity of polyherbal formulation (PHF) in collagen induced animal model. The PHF was formulated using the herbs which have known antiarthritic effects at particular ratio to enhance the pharmacological activity of individual herb and reduce the dose of single plant extract. Ethanolic extract of Sida Cordifolia, Root of vitex negundo, Ricinus Communis, Aerva lanata, Trachyspermum ammi, Curcuma longa and Trigonellum foneum graceum were used in PHF.

Methods: In the study animals were divided into five group with 6 animal each and they were induced disease by administration of chicken sternal collagen type 2 on zeros and seventh day and followed the treatment with higher and lower dose of polyherbal formulation from twentieth day to fortieth day. RESULT: The evaluation of body weight, arthritis index, paw volume, hematological parameters were performed and the histopathological analysis of ankle joint also done.

Conclusion: Polyherbal formulation can nullify the most ill effect produced by the CIA in female wistar rat

Keywords: Polyherbal formulation, anti arthritisis, in vivo study

INTRODUCTION

Rheumatoid arthritis is a systemic inflammatory disease, which manifests itself in multiple joints of the body. The inflammatory process primarily affects the lining of the joints (synovial membrane), but can also affect other organs. The inflamed synovia leads to erosions of the cartilage and bone and sometimes joint deformity. Pain, swelling, and redness are common joint manifestations. The prevalence of RA in Indian subcontinent is 0.4–0.6% of population. The epidemiological ratio of arthritis in female and male is 3:1 and the prevalence is 1% of the world population.(1)

The pathogenic mechanisms of synovial inflammation are likely to result from a complex interplay of genetic, environmental, and immunologic factors that produces dysregulation of the immune system and a breakdown in self-tolerance. It caused by number of pro-inflammatory molecules released by macrophages including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like COX and LOX are the potential target for chronic inflammatory conditions.

Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal agents and immunosuppressant are usually used as RA treatment. However, their side effects and toxicity call for alternative, safer and more effective natural product based drugs. Now it is a growing concern allover for the development of new safe, potent, less toxic ant arthritic drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded.(4)

Objective of the present study is to formulate a polyherbal formulation (PHF) and evaluate its anti arthritic potential in animals. The PHF was formulated using the herbs which have known anti arthritic effects at particular ratio to enhance the pharmacological activity of individual herb and reduce the dose of single plant extract. In Ayurveda two principles are used for drug formulation viz., single herb or more Ethanolic extract of Sida Cordifolia, Root of vitex negundo, Ricinus Communis, Aerva lanata, Trachyspermum ammi, Curcuma longa and Trigonellum foneum graceum were used in PHF. In traditional system of Indian medicine combined extract of individual plants rather than individual ones to achieve maximum theuraputic efficacy.

Collagen-induced arthritis (CIA) is an experimental autoimmune disease that can be induced with type II collagen in the appropriate rodent strains and non-human primates [6]. When compared with other experimental arthritis models, the characteristics of the CIA model resemble human RA more closely in its clinical, pathological, immunological and histological aspects. In addition, CIA facilitates the understanding of RA pathogenesis in human and help to generate new therapeutic regimen for RA. Therefore, this research was designed to study the effects of ethanolic extract of Polyherbal formulation on joint inflammation in female wistar rats with CIA.

MATERIALS AND METHODS

Collection

The formulation runaport was collected from sps Biosciences Ernakulam district from Kerala, India.

Chemicals

Chicken sternal collagen type II (Sigma Aldrich, USA), Incomplete Freunds Adjuvant (Sigma Aldrich, USA), Prednisolone (Symed Pharmaceuticals Pvt. Ltd., Hyderabad). All other chemicals and reagents used for the study were of analytical grade procured from approved organization. 2.5. Animals Eighteen healthy female wistar rats weighing between 150–170 g were obtained from Small Animal Breeding House, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala and housed at Animal house of University College of Pharmacy, Cheruvandoor campus,
Ettumanoor, Kottayam, Kerala. The experiments were carried out after obtaining the permission of Institutional Animal Ethics Committee, University College of Pharmacy, Cheruvandoor campus, Ettumanoor, Kottayam, Kerala, India under IAEC.

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

• group I: vehicle treated collagen induced arthritic rats;
• group II: indomethacin (10 mg/kg) treated collagen induced arthritic rats;
• group III: rumaport (100 mg/kg) treated collagen induced arthritic rats.

Arthritic score
The severity of inflammation in each limb was monitored by set visual criteria for the degree of inflammation, the extent of erythema and edema of the periarticular tissues, and the enlargement, distortion, or ankylosis of the joints. Findings were scored on a scale of 0–4, where 0 = no inflammation, 1 = inflammation of 1 joint, 2 = unequivocal inflammation of at least two joints of the limb or moderate inflammation of one joint, 3 = severe inflammation of one joint and 4 = maximum inflammation of ≥ one joint in the limb. The arthritis score was the sum of the scores for all four limbs (maximum score = 16).

Body weight
Alteration of the body weight is a key factor of RA and is measured using digital weighing balance from one to 40 days from the day of CIA injection [13].

Paw volume
Both hind paws volumes of all animals were measured just before collagen + IFA injection on day 0 and thereafter at different time intervals till day 41 using a plethysmometer. The change in paw volume was measured as the difference between final and initial paw volumes.

Hematological parameters
Hematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), rheumatoid factor, were evaluated using routine laboratory methods. Blood withdrawal by retro orbital after applying lidocaine ointment

Histopathological parameter
On day 41, animals were sacrificed by cervical dislocation and the ankle joints of the hind limbs were excised and fixed in Bouin’s fluid; subsequently the specimens were decalcified with 10% EDTA for seven days, dehydrated and embedded in paraffin blocks. Sections of ankle joints (5 m thick) were cut mounted on slides and stained using haematoxylin and eosin. Grading of cellular infiltration, synovial hyperplasia, pannus formation, joint space narrowing, and cartilage and bone erosion of the ankle joints was blindly investigated by two independent examiners using a semi quantitative scale from; 0 = normal, 1 = mild changes, 2 = moderate changes, 3 = severe changes and 4 = very severe changes. Histopathological scores were combined and expressed as the sum of both ankle joints, with a maximum histological score of eight for each histological parameter per rat [16].

Statistical analysis
All results were expressed as mean ± standard error of mean (SEM). Data were statistically evaluated by one-way ANOVA followed by Tukey’s Multiple Comparison test using Graph Pad Prism 8. The differences among groups were considered to be significant at P < 0.05.

RESULT
Collagen induced arthritis. The developments of arthritis in most of the rats were produced on 12th day after the primary immunization. CIA animals showed an increase in paw volume and a decrease in body weight on day 12. The disease incidence reached 100% by day 18. Treatment was started on day 20, CIA rats were administered with 0.05% CMC (group I or control), CIA rats received 10 mg/kg of prednisolone (group II or standard) orally and CIA rats received 500 mg/kg of RUMAPORT (group III or test) orally till the 40th day.

3.2.1. Effect of Rumaport on arthritic score
In this study, rats with no signs of inflammation were taken, from day 3 all induced rats began to show an increase in arthritis scores. The arthritic control animals (group I) showed a highest arthritic score (12.74 ± 0.010) till 36th day, but index became slightly reduced and stabilized to a mean arthritic index of 11.41±0.348 till day 40. In group II (CIA animals treated with indomethacin), showed an increase in arthritic index till day 20.
During the treatment, animals showed a marked decrease in arthritic index. From day 24, they had an arthritic index of \(1.30 \pm 0.026\). Group 3 animals (CIA animals treated with rumaport) showed an increase in arthritic index before the treatment. From 21st day, the arthritic index of animals showed a constant decrease till day 40. The animals showed a lowest arthritic index of on 40th day \(1.82 \pm 0.179\). CIA rats treated with indomethacin and rumaport (group II and III) showed significantly lower (\(P < 0.001\)) arthritis scores compared with CIA rats (group I) (Fig. 1).

### Effect of Rumaport on body weight

Rats of weight between 150–170 g were taken for the study. CIA rats had shown a steady increase in body weight till day 4. all rats showed a decrease in weight due to the induction of arthritis. The body weight of CIA rats (group I) decreased very relentlessly till day 40(\(131 \pm 3.331\)), due to severe arthritis. In group II rats (CIA rats treated with indomethacin) showed a gradual decrease in body weight till day 20 and after that weight get increased to \(166.6 \pm 0.714\) on 40th day. Meanwhile in group III rats (CIA rats treated with rumaport(high dose and low dose) showed a steady increase in body weight from day 28today 40 day 40 (\(164.8 \pm 3.123\) and \(170.3 \pm 1.342\))

### Hematological Parameters

**RBC**

The result shows that, there is a significant rise in RBC count in arthritic control group while in all treated group there is a significant increase (\(p<0.001\) in group2; \(p<0.01\) & \(p<0.001\) in Rumaport, low dose and high dose respectively.

**WBC**

The result shows that, there is a significant rise in WBC count in arthritic control group while in all treated group there is a significant increase (\(p<0.001\) in DFC group; \(p<0.01\) & \(p<0.001\) in Rumaport low dose and high dose respectively.

**ESR**

In inflammatory condition infection will be more and therefore ESR level will be also high. In arthritic condition ESR level was increased and it will decrease after treatment with anti-inflammatory drugs. Values shown indicate that ESR rate is increased in case of arthritic group. As compared to this group test group show a significant reduction of \(p<0.001\) in ESR level. \(p<0.001\) in indomethacin group and Rumaport treated groups.
Values were expressed as MEAN ± SEM where n=6; Statistical analyses were done using One-way ANOVA followed by Tukey’s multiple comparison tests. a P<0.05,  b P < 0.01, c P < 0.001 Vs control. 
& e P< 0.05, f P < 0.01,  g P < 0.001 Vs positive control.

RF: Rheumatoid factor is an antibody present in blood plasma. The amount of RF was very high in arthritic conditions. After proper treatment it will began to lowers. The result showed that, there is an increase in RF value in arthritic control group. As compared to arthritic group, Rumaport and indomethacin treated groups shows a significant reduction of p<0.001 in RF value.

Histopathology
Histopathological evaluation of the tibio-tarsal joint of CIA rats showed massive influx of inflammatory cells, synovial hyperplasia, pannus formation, cartilage destruction, bone erosion and joint space narrowing. Treatment with indomethacin (10 mg/kg) showed moderate signs of hyperplasia, cartilage destruction and pannus formation. Tibio tarsal joints of rats treated with rumaport (200 mg/kg) showed mild signs of cellular infiltration. It did not show any signs of joint destruction or damage to cartilage (Fig. 6).

**POSITIVE CONTROL:** Due to arthritis synovial membrane was found to be damaged, and joint space was absent. High amount of lymphocyte was present due to inflammation.

**INDOMETHACIN (Std):** After the treatment with anti-inflammatory drug, synovial membrane was began to normal and joint space was appeared. Absence of lymphocyte.

**RUMAPORT (LD):** After treatment low dose of Rumacure (100mg/kg) synovial membrane & joint space was appeared normal and lymphocyte was absent.

**RUMAPORT (HD):** Above results showed that joint was found to be normal; synovial membrane was normal and joint space was appear normal, inflammatory cells was absent.

**DISCUSSION**

Arthritis score is a clinical assessment of joint swelling [17]. The alteration in plasma protein induces the synthesis of proinflammatory cytokines, prostaglandins, leukotriene and matrix metalloproteinase that caused fluid accumulation in the synovium. This results in an increase in arthritis scores due to damage in joints and bones of the rat’s paw [18].Indomethacin is a quick acting drug that reduces the symptoms of CIA by inhibiting the production of pro-inflammatory cytokines, which will in turn inhibit the inflammatory response and reduce the symptoms of arthritis.

The reduction and inhibition of TNF- and IL-production cause disturbance to the cyclooxygenase-2 activity expression and reduction in the prostaglandins secretion. Thus, these changes will reduce arthritis scores. Therefore, this suggests that Rumaport has the capability in delaying the inflammatory response and reducing the occurrence of the joints inflammation symptoms. Arthritic disease resulted in reduction in body weight; this is mainly due to the cytokine driven hyper-metabolism as well as reduction in rearing movements in the induced animals. Indomethacin and low dose and high dose of rumaport completely prevented arthritic disease on day 40 (P < 0.001) as compared to arthritic control animals. Indomethacin are known to possess a general anti-anabolic and catabolic effect on bone and muscle growth. This might be due to inhibition of cytokine driven hyper-metabolism by inhibiting cytokines in arthritic animals, which further enhanced rearing movements in animals due to the reduction in inflammation of joints.

TNF- was shown to be a prominent inducer of COX-2 expression and eicosanoid production and this resulted in an increased PGE2 level. PGE2 played an important role in inflammation and it triggered an acute inflammatory response characterized by edema, pain and infiltration of leucocytes. Moreover, the elevated paw volume CIA rats (group I) was complemented by the infiltration of inflammatory cells was evidenced from the histopathological analysis. Hence, the administration of rumaport significantly reduced PGE2 levels with concomitant inhibition of edema and reduction in inflammatory infiltration. Monoclonal antibodies to tumor necrosis factor- were shown to be efficacious in animal models of inflammatory diseases and demonstrated to be effective drugs in RA treatment. Therefore, by inhibiting the production of TNF- represents a good strategy for therapeutic intervention of immune and inflammatory diseases [22]. From our results, local tumor necrosis factor- gene transcription existed and may contribute to pathophysiology of inflammatory processes in hind limb in the chronicphase.

The inhibitory effect of rumaport on tumor necrosis factor-mRNA correlated with its effect on joint lesion reduction. The scoring was done according to the procedure, scores as the sum of two hind limbs. CIA rat showed severe destruction of the joint and CIA rat treated with prednisolone showed moderate destruction of joint. CIA rat treated with rumaport showed only a mild destruction of joint. From this, it is evident that the Rumaport have a potent ant arthritic activity as indomethacin as it minimizes most of the ill effects of CIA. The mechanism behind the activity may be by decreasing the expression of pro-inflammatory cytokines such as TNF- and also the suppression of COX enzymes, which results in the reduction of joint inflammation and destruction.

To conclude, in the present study showed promising anti-inflammatory as well as anti arthritic activity. The
results shows there is need to further explore the activities in molecular level to understand the mechanism behind action.

**REFERENCE**


