ORIGINAL RESEARCH ARTICLE EXPERIMENTAL

Evaluation of anti-inflammatory potential of the multidrug herbomineral formulation in male Wistar rats against rheumatoid arthritis

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ABSTRACT

Background: Immunological and inflammatory mechanisms, which may play a role in a number of disorders like rheumatoid arthritis (RA). Ancient ayurvedic physicians had developed certain dietary and therapeutic measures to arrest or prevent these disorders. **Objective:** Rheuma off gold (RG) is a herbomineral formulation recommended by ayurvedic medical practitioners for treatment of RA. This study was carried out to lend scientific evidence to the efficacy claim for RG in the management of RA in folklore medicine. **Materials and Methods:** Arthritis was induced by complete Freund's adjuvant. Treatment with formulation 100 mg/kg and dexamethasone 2 mg/kg was given to rats intragastrically once a day from day 1 to day 21 and after which estimation of physical, biochemical, and hematological parameters were carried out. **Results:** Treatment of formulation to adjuvant induced arthritic animal showed statistically significant ($P < 0.05$) improvement in physical parameters like arthritic index, paw edema, paw thickness as well as reduction of inflammatory markers like C-reactive protein, serum rheumatoid factor, erythrocyte sedimentation rate. The treatment also produced statistically significant ($P < 0.05$) increase in hemoglobin percent and improvement in splenomegaly and thymus index. In the histopathological examination, ameliorative effect of formulation was observed in hyperplasia of synovium, pannus formation, and destruction of the joint space. **Conclusion:** The results obtained in experiments indicated that the formulation significantly inhibited the adjuvant-induced arthritis which was comparable to dexamethasone and had preferable anti-inflammatory effect without significant side effect. Thus, the formulation may be a potential preventive or therapeutic candidate for the treatment of chronic inflammation and arthritis.

Key words: Anti-inflammatory, ayurvedic, formulation, Freund's adjuvant, immunomodulatory, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is the most common systematic, inflammatory, autoimmune disease, affecting 1%-1.5% of the population worldwide.[1] In ayurveda "sandhivata" (sandhi = joint) term used to denote rheumatism. Ayurveda is a "codified" system of clinical science founded on basic principles with a logical superstructure. "Arthritis" means inflammation in joints, whereas "*sandhivata*" means vata in joints. Here, "*Vata*" conveys a definite theory of knowledge, based on which it can be inferred that *sandhivata* is a condition where pain is a characteristic clinical feature and failure of joints would possibly be its eventual dreaded result.[2]

Though several drugs are available, for example, Nonsteroidal anti-inflammatory drus (aceclofenac, diclofenac, etc.), steroids (glucocorticoid), and disease modifying antirheumatic drugs (methotrexate, cyclosporin A) for managing moderate to severe cases of arthritic pain, stiffness, and inflammation, the side effects of these drugs are often deleterious, which includes gastrointestinal irritation, cardiovascular problem, drug dependency, thymus suppression, and anemia.[3] All these drawbacks of available medications have revived the interest in our traditional system of medicine. The disease preventive and health promotive approach of Ayurveda, which takes

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into consideration the whole body, mind, and spirit while dealing with the maintenance of health, promotion of health and treating ailments is holistic and finds increasing acceptability in many regions of the world.[4] Ancient ayurvedic physicians had developed certain dietary and therapeutic measures to arrest or prevent the disease like RA. In view of drawbacks of currently available synthetic drugs, it is reasoned that the pharmacological evaluation of ayurvedic proprietary formulation may combine good efficacy, absence of side effects, and could constitute a major therapeutic improvement in treatment of immunological and inflammatory disorders.

According to the ayurvedic pharmacopoeia, rheuma off gold (RG) is a herbomineral formulation believed to have the potential for providing relief to RA patients. This formulation is prepared from parts of three different plants (*Commiphora mukul, Srychnos nux-vomica, Boswelia sereta*), maharasnadi kwath and suvarna bhasma that are used in traditional medicine for a variety of purposes [Table 1]. However, the antiarthritic properties of this formulation not been subject to any scientifically controlled investigations so far. Investigations have, therefore, been carried out using rats as experimental models, to assess the antiarthritic potential of ayurvedic proprietary formulation in animal model of Freund's adjuvant-induced arthritis.

MATERIALS AND METHODS

Materials

All the plants used in formulation were authenticated and formulation was prepared by Virgo UAP Pharma Pvt. Ltd. Sanand, (Ahmedabad, Gujarat, India) named as RG [Table 1] on the basis of an official ayurvedic formulary.[5] Complete Freund's adjuvant (CFA) was purchased from Sigma Chemicals (St. Louis, USA). C-reactive protein (CRP), serum rheumatoid factor kits were purchased from Span Diagnostics (Vadodara, India). Other chemicals used were of analytical grade.

Experiment animals

Table 1: Composition of rheuma off gold

Ingredients	Botanical name	Part used	Composition (%)
Mahayogaraj Guggulu	*Commiphora mukul*	Powder	72 mg
Maharasnadi kwath	-	Extract	112 mg
Suvarna bhasma	-	Powder	1.6 mg
Suddha kuchala	*Srychnos nux-vomica*	Seed	9.6 mg
Shallaki	*Boswelia sereta*	Exudate	4.8 mg
Colour: Sunset yellow FCF	-	-	Q.S.
Excipients	-	-	Q.S.

FCF=For coloring food, Q.S.=Quantity sufficient

Male Wistar rats weighing 250-300 g were obtained from the animal facility of Torrent Research Centre, Gandhinagar, India. They were maintained under standard environmental conditions (12 h light/dark cycle at 20° - 25°C temperature and 55% ±5% controlled humidity) and provided with feed and purified water *ad libitum*. All experiments and protocols described in present study were approved by institutions animal ethics committee and are in accordance with guidelines as per "Guide for the care and use of laboratory animal" and with permission from Committee for the Purpose of Control and Suppression of Experiments on Animals. Animals were acclimatized for one week before starting the experiment. The pharmacological work was carried out as per approved protocol (IPS/PCOL/CONS11-12/2002).

Experimental protocol

Arthritis was induced by subplantar injection of 0.1 mL of CFA in left hind paw according to a method described by Newbould *et al*.[6] Treatment with the test compound is started on the day 1 and continued for 21 days. Rats were divided into six groups of six animals each: Normal control group, control treated with RG tablet (100 mg/kg), control treated with dexamethasone (2 mg/kg), disease control group, disease treated with RG tablet (100 mg/kg), disease treated with dexamethasone (2 mg/kg). Treatment was given to rats intragastrically once a day from day 1 to day 21 days. Paw volumes and paw thickness were recorded on the day of injection as well as 5th day and 21st day, plethysmographically. Physical parameters like body weight, arthritic index, splenomegaly, and thymus weight to body weight ratio were also measured after experimental period. Blood was collected from retroorbital plexus for measurement of biochemical and hematological parameters like serum CRP, serum rheumatoid factor, hemoglobin percent, and erythrocyte sedimentation rate as an indicative of inflammation. On day 21, rats were sacrificed and ankle joints were subjected to histopathological studies.

Statistical analysis

Values are expressed as mean ± standard error of the mean. The results were analyzed using one-way factorial analysis of variance followed by Tukey's multiple comparison test using Graphpad Prism 5 software. The value of P less than 5% ($P < 0.05$) was considered as statistically significant.

RESULTS

Effect of formulation on physical parameters

Body weight

There was gradual but statistically significant reduction in body weight observed in disease control groups, while no significant difference was observed in normal control group as well as control treated with RG tablet and dexamethzone.

Treatment with RG tablet and dexamethzone produced increase in body weight as compared to disease control group but it was not statistically significant [Figure 1a].

Arthritic index

Freund's adjuvant-induced arthritic animals produced statistically significant ($P < 0.05$) increase in arthritic index in disease control group as compared to normal control group. Treatment with RG tablet and dexamethasone to diseased animal showed statistically significant ($P < 0.05$) reduction in arthritic score compared to disease control group. The formulation produced effect comparable to dexamethasone [Figure 1b].

Paw volume and paw thickness

Freund's adjuvant-induced arthritic animals produced statistically significant ($P < 0.05$) increase in paw volume and paw thickness in disease control group as compared to normal control group. Treatment with RG tablet and dexamethasone to diseased animal showed statistically significant ($P < 0.05$) reduction in paw volume and paw thickness compared to disease control group. The formulation produced effect comparable to dexamethazone. While treatment with RG tablet and dexamethasone to normal animal did not showed any significant change in both the parameters [Figures 1c and d].

Effect of formulation on secondary organ indexes

Freund's adjuvant-induced arthritic animals found to exhibit statistically significant ($P < 0.05$) decrease in spleen and thymus index in disease control group as compared to normal control group. Treatment with formulation to diseased animal showed statistically significant ($P < 0.05$) improvement in spleen and thymus index as compared to disease control group. While treatment with dexamethasone to diseased animal did not showed any protective effect on spleen and thymus index.

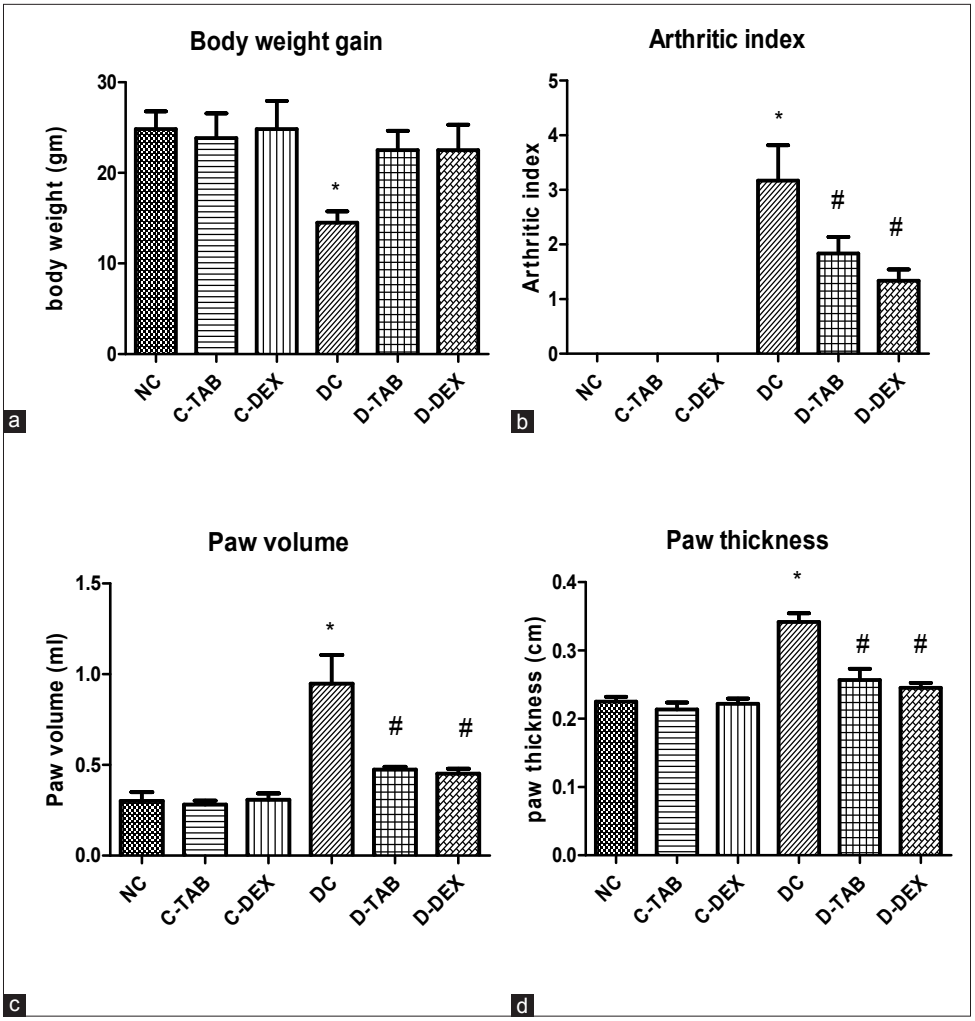

Figure 1: Effect of formulation on physical parameters: (a) Body weight gain, (b) arthritic index, (c) paw volume, and (d) paw thickness. Each bar represents mean ± standard error of the mean of six animals.*Significantly different from normal control ($P < 0.05$), #-significantly different from disease control ($P < 0.05$), NC = normal control, C-TAB = Rheuma off gold Tablet (100 mg/kg), C-DEX = control treated with dexamethazone (2 mg/kg), DC = disease control group, C-TAB = disease treated with rheuma off gold tablet (100 mg/kg), D-DEX = disease treated with dexamethazone (2 mg/kg)

Treatment with formulation did not showed any significant change in thymus index in normal animals, but treatment of dexamethasone to normal animal showed slight decrease in thymus index but was not statistically significant [Table 2].

Effect of formulation RG tablet on biochemical and hematological parameters

C-reactive protein

Rats treated with Freund's adjuvant had statistically significant ($P < 0.05$) elevated levels of CRP, erythrocyte sedimentation rate, and serum rheumatoid factor in disease control group as compared to normal control group. Treatment with RG tablet and dexamethasone to diseased animal showed statistically significant ($P < 0.05$) reduction in levels of CRP, erythrocyte sedimentation rate, and serum rheumatoid factor. While treatment with RG tablet and dexamethasone to normal animal did not showed any significant change in these levels [Table 3].

Erythrocyte sedimentation rate

Percent hemoglobin

Rats treated with Freund's adjuvant had statistically significant ($P<0.05$) decreased levels of % haemoglobin (Hb) in disease control group as compared with normal control group. Treatment with RG and dexamethasone to diseased animal showed statistically significant ($P < 0.05$) increase in levels of % Hb as compared with disease control group. The formulation produced effect comparable to dexamethazone. While treatment with RG tablet and dexamethasone to normal animal did not showed any significant change in levels of percent Hb as compared to normal control group [Table 3].

Effect of formulation RG tablet on histopathology of joints

In histopathology of ankle joints in normal control group showed normal joint structure, no cartilage destruction and no signs of inflammation or other distortion was observed. While arthritic animals showed mild to moderate hyperplasia of synovium, focal cartilage destruction, presence of pannus formation with destruction of joint space. Treatment with RG and dexamethasone group showed significant improvement in hyperplasia of synovium as compared to disease control group [Figure 2].

DISCUSSION

RA is a chronic inflammatory disease characterized by fibroblastic proliferation, infiltration of the synovial lining by inflammatory cells which leads to expression of proinflammatory cytokines and a paucity of apoptosis resulting in bone and joint destruction.[7] Despite enormous research being carried out for allergic and immune disease, it still remains a disorder which can be "controlled" and not "treated" since no satisfactory treatment is available in allopathic system. Hence, there is tremendous interest worldwide for the use of an alternative system of medicine. The research has been focused on formulations used in traditional medicine for treatment of RA.[8]

CFA is used to initiate induction of arthritis. This model is the original model of RA, has been extensively used to preclinical screening of new antiarthritis compounds and has successfully predicted activity in multiple new therapeutics. After a single injection of the adjuvant, a rapid, reliable, robust, and easily measurable polyarthritis develops. The joint pathology seen in this animal model shares the cartilage degradation, bone reportion, and cellular influx seen in human RA.[9,10]

In the present study, the change in body weight of rats was measured as one of the parameters to assess the course of the disease and the response to therapy of arthritic drugs.

Table 2: Effect of formulations rheuma off gold tablet on secondary organ indexes

Parameters	NC	C-TAB	C-DEX	DC	D-TAB	D-DEX
Splenomegaly (g)	0.096±0.007	0.094±0.007	0.096±0.006	0.61±0.050*	0.74±0.038#	0.59±0.031
Thymus weight (g)	0.119±0.013	0.109±0.018	0.108±0.013	0.073±0.004*	0.105±0.004#	0.092±0.011

Values are expressed as mean±standard error of the mean of six animals, *Significantly different from normal control (P <0.05), #significantly different from disease control (P <0.05), NC=Normal control, C-TAB=RG tablet (100 mg/kg), C-DEX=Control treated with dexamethasone (2 mg/kg), DC=Disease control group, C-TAB=Disease treated with RG Tablet (100 mg/kg), D-DEX=Disease treated with dexamethasone (2 mg/kg), [analysis of variance (ANOVA) followed by Tukey's multiple comparison test], (ANOVA followed by Tukey's multiple comparison test)

Table 3: Effect of rheuma off gold tablet on biochemical and hematological parameters

Parameters	NC	C-TAB	C-DEX	DC	D-TAB	D-DEX
CRP (mg/dL)	4.9±0.28	4.62±0.35	4.92±0.21	8.65±0.33*	6.02±0.29#	5.67±0.21#
SRF (IU/mL)	50.41±2.29	51.77±1.56	51.42±1.64	74.57±1.42*	59.58±3.23#	56.19±1.01#
ESR (mm/h)	3.54±0.19	3.85±0.17	3.82±0.2	6.32±0.15*	4.35±0.08#	4.03±0.17#
Hb (mg%)	11.83±0.95	11.72±0.82	12.63±1.03	19.76±0.4*	14.9±0.28#	14.33±0.92#

Values are expressed as mean±standard error of the mean of six animals, *Significantly different from normal control (P<0.05), #significantly different from disease control (P<0.05), NC=Normal control, C-TAB=RG tablet (100 mg/kg), C-DEX=Control treated with dexamethasone (2 mg/kg), DC=Disease control group, C-TAB=Disease treated with RG tablet (100 mg/kg), D-DEX=Disease treated with dexamethasone (2 mg/kg), [Analysis of variance (ANOVA) followed by Tukey's multiple comparison test], (ANOVA followed by Tukey's multiple comparison test)

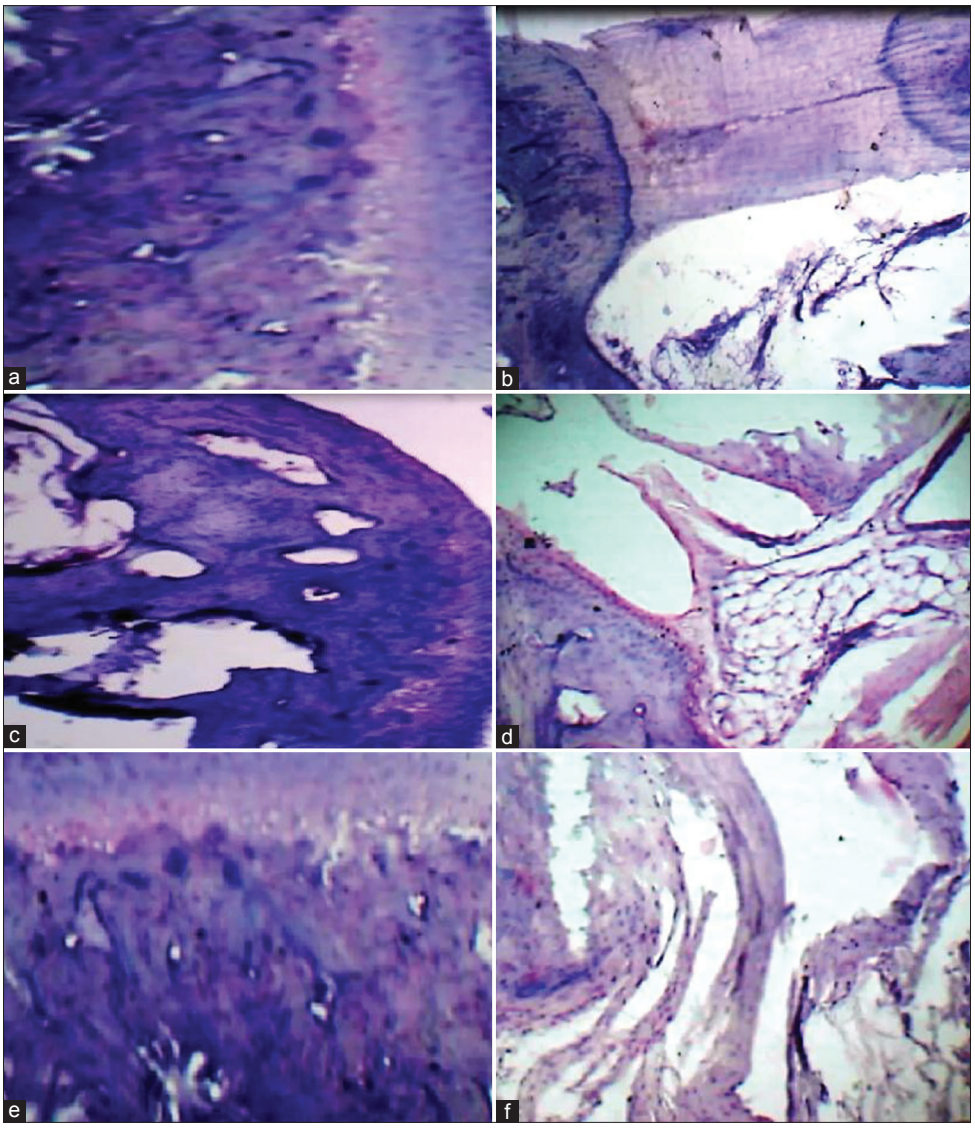

Figure 2: Effect of formulation rheuma off gold tablet on histopathology of joints: (a) Normal control group, (b) disease control group, (c) control treated with tablet, (d) disease treated with tablet, (e) control treated with dexamethazone, and (f) disease treated with dexamethazone

Finding from the studies implicated that with increase the incidence and severity of arthritis, a decrease in body weight of the rats occurred during the course of the experimental period due to alterations in the metabolic activities of diseased rats. The decrease in body weight gain in the arthritic rats compared to the normal control rats in the current study is in concordance with the fact that RA is associated with loss of lean tissues, which contain most of the body's protein. The reduction in body weight gain may also be attributed to muscle wasting in experimental arthritis, occurring due to enhanced protein breakdown by the ubiquitin-proteasome proteolytic pathway.[11,12] It has been previously reported that decrease in the body weight during inflammation is due to deficient absorption of nutrients through the intestine.[13] Chronic treatment with RG to arthritic rats improved loss of body weight. These effects may be attributed to either inhibition of loss of lean tissues containing body's protein or inhibition of muscle wasting or improvement in inflammation leads to increase in absorption of nutrients through the intestine.

Arthritic index includes the combined index of inflammation, formation of nodules, and extent of spread of the disease to other organs. This gives the full picture of the disease. In chronically affected joints, the normally delicate synovium develops many villous folds because of increased numbers and size of cells in the synovial membrane and colonization by lymphocytes and plasma cells.[14] The infiltrating cells, initially perivenular but later forming lymphoid follicles synthesize interleukin (IL)-2, cytokines, rheumatoid factor, and other immunoglobulins. Inflammation and/or nodules are observed on ears, nose, tail, fore paws, and hind paws. Arthritic index is the average of the score given to severity of the lesions in these places.

A selective reduction in the arthritis score distinguishes the immunosuppressive effects of a drug from its anti-inflammatory effects.[15] Administration of Freund's adjuvant-induced severe chronic disease of arthritic index in disease control group which was significantly different from normal control group. Formulation-treated group produced statistically significant reduction in arthritic score compared to disease control indicates possible immunosuppressant effect and these immunosuppressive effect may be attributed to presence of plant steroids like guggulsterones and boswellic acid in *Commiphora mukul* and *Boswelia sereta* in RG which have been reported to have immunosuppressive activity.[16-18]

Paw volumes and paw thickness are physical indicators of the inflammation in early as well as chronic phase of the disease. The progression of arthritis is characterized by increase of the paw footpad and tibiotarsal joint diameters after day 14, which can be attributed to the delayed immunological flare in the disease.[9] The determination of paw swelling is apparently simple, sensitive, and quick procedure for evaluating the degree of inflammation and assessing therapeutic effects of drugs. Formulation-treated group showed significant reduction in paw volume suggesting the anti-inflammatory activity of the formulation. T-cell proliferation is an important mechanism of adjuvant diseases; specifically their differentiation into Th-1 helper cells.[19] Therefore, possible mechanism for reduction in paw edema might be either suppressive effect on Th-1 helper cells.

Splenomegaly occurs as a result of profound induction of extramedullary hematopoiesis by late-phase cytokines in the red pulp in conjunction with pyogranulomatous inflammation in the red pulp and capsule resulting in disruption of spleen histology.[20,14] Administration of Freund's adjuvant decreased spleen weight to body weight ratio in disease control groups. Spleen weight to body weight ratio was found to be increased in formulation-treated group indicating effectiveness in correcting splenomegaly. Possible mechanism may involve suppression of extramedullary hematopoiesis through action on late-phase IL-6 or IL-12.

The decrease in thymus weight is related to a suppressive effect on the immune system apart from anti-inflammatory effect.[21] The observed decrease in thymus weights in the dexamethasone-treated normal rats was higher as compared to formulation-treated group indicates formulation have less thymus suppressive effect as compared to dexamethasone. Although in disease control group decrease in thymus weight was observed which may be due to alterations in the cell populations in these organs, which are related to the immune function. Treatment with formulation produced improvement in thymus weight. These observations indicate protective effect of formulation as compared to dexamethazone.

Adjuvant disease is associated with an increase in the plasma levels of CRP.[22] CRP levels are closely linked to the rate of disease progression.[23] CRP is released from liver in response to action of IL-6 in inflammatory condition and improves antigen presentation. The treatment with formulation significantly reduced the levels of CRP. The effect may be due to suppression of all stages of disease progression like inhibition of synthesis of chemical mediators because RG contains three medicinal plants (*Commiphora mukul, Srychnos nux-vomica, Boswelia sereta*) each having proved anti-inflammatory activities. These all plants have been found to contain substances like guggulsterones, boswellic acid, and brucine, which have been proved to be anti-inflammatory by the mechanisms may be associated with the inhibition of inflammatory mediator overproduction.[24-26] The formulation also contains maharasnadi kwath and suvarna bhasma which is a very famous ayurvedic medicine used in vata disorders like kampavata (Parkinson's disease), hemiplegia, paraplegia, neck pain, low back pain, RA, osteoarthritis, knee pain, and hip pain. According to the kashaya sangrahaya and ayurveda pharmacopoeia, maharasnadhi kwath is a polyherbal formulation proved to be safe and nontoxic have the potential for providing relief to RA patients.[27] This formulation is prepared from parts of 26 different plants[28] that are used in traditional medicine for a variety of purposes such as reduction of pain, reduction of inflammation, and antipyretic activity.

Rheumatoid factor is the true marker of clinical presentation of RA. Adjuvant disease shows elevated blood levels of rheumatoid factor.[29] Rheumatoid factor generation in arthritis involves B cell activation via toll-like receptors and several genetic predispositions to arthritic diseases.[30] Diseased control group showed significantly elevated levels of serum rheumatoid factor compared to normal control group. The treatment with formulation significantly reduced the levels of these biomarkers of inflammation and autoimmune stimulation in the treated rats. The results of the present study indicate that the anti-inflammatory effects of formulation may be due to inhibition of activation of B cell by medicinal plants present in formulation, because all plants of formulation contains substantial amounts of plant steroids which are reported to produce anti-inflammatory action.

ESR is the indicator of chronic inflammatory disease state.[31] Several factors which are increased in acute tissue damage, chronic inflammation, and chronic infection could have a relevant role in increased erythrocyte aggregation.[32]

RA accompanied by raised CRP levels and CRP plays important role in the induction and maintenance of increased erythrocyte aggregation in the blood of RA patients.[33] Treatment with formulation showed reduction in ESR. The outcomes may infer that formulation have protective effect on late-phase inflammatory markers. Thus, the reduction in the ESR brought about by treatment support its antiarthritic effect.

It is proposed that the reduction in the Hb count during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells. Thus, increase in the Hb count brought about by formulation further support its antiarthritic effect.

Histopathological evaluations of ankle joints in normal control group showed normal joint structure, no cartilage destruction, and no signs of inflammation or other distortion was observed. While in arthritic animals showed mild to moderate hyperplasia of synovium; focal cartilage destruction; presence of pannus formation with destruction of joint space. It also showed marked damage of articular structure indicating joint damage and inflammation. The pannus formation and bone erosion associated with inhibition of neutrophil infiltration.[34] Hyperplastic synovial tissue (pannus) may erode cartilage, subchondral bone, articular capsule, and ligaments.[35] Treatment with RG and dexamethasone group showed significant improvement in hyperplasia of synovium as compared to disease control group which showed protective effect of formulation on hyperplasia of synovium support its antiarthritic effect. The inhibition of pannus formation and bone erosion may be associated with inhibition of neutrophil infiltration.

Traditional medicine and medicinal plants are the powerful source of new drugs, they contributing about 90% of the newly discovered pharmaceuticals. Traditional medicine continues to provide health coverage for over 80% of the world population, especially in the developing world.[27] Thus, on the basis of above results, it could be concluded that formulation, a combination of three herbal plants and maharasnadi kwath and suvarna bhasma exert a significant anti-inflammatory and immunosuppressive effect. This could be due to different types of active principles and different mechanism of action. However, the overall side effects like spleenomegaly and thymus suppration observed with formulation are less then that with dexamethasone which could be due to different mechanism of action of individual constituent present in formulation. Therefore, combination of plants with minerals is beneficial because combination of two or more compounds with different mechanism of action may have synergetic effect or may be reducing side effect of the other constituent. Thus, it is suggested that the herbomineral formulation may be able to be considered as safe supplementary therapy for a long-term and effective management of arthritic patients.

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