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Antiarthritic activity of Majoon Suranjan (a polyherbal Unani formulation) in rat

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***Background & objectives*: Majoon Suranjan (MS) is a polyherbal formulation used in Unani system of medicine for the treatment of rheumatoid arthritis (RA). The present study evaluates the antiarthritic efficacy of this formulation in three different experimental models.**

***Methods*: The anti-inflammatory activity of MS (in doses of 450, 900 and 1800 mg/kg body wt) was evaluated using the turpentine oil induced paw oedema model and the antiarthritic efficacy was evaluated using the formaldehyde and complete Freund's adjuvant (CFA) induced arthritis models. Aspirin (100 mg/kg body wt) was used as the standard drug in all the models. In order to assess the safety of the test drug, oral acute and 28 day toxicity studies were also carried out.**

***Results*: MS produced a dose dependent protective effect in all the experimental models. Its antiarthritic efficacy was comparable to aspirin in formaldehyde induced arthritis and was superior to aspirin in turpentine oil induced paw oedema and CFA induced arthritis. MS also inhibited the delayed increase in joint diameter as seen in control and aspirin treated animals in CFA induced arthritis. Oral LD_{50} of MS was found to be >5000 mg/kg in rats. Chronic administration did not produce any significant physiological changes in the tested animals.**

***Interpretation & conclusions*: Results of the present study suggest that the antiarthritic activity of MS was due to the interplay between its anti-inflammatory and disease modifying activities, thus supporting its use in traditional medicine for the treatment of RA.**

Key words Adjuvant arthritis - analgesia - formaldehyde - Majoon Suranjan - paw edema - turpentine oil

Rheumatoid arthritis (RA) is a progressive, disabling, chronic multisystem disease of unknown cause characterized by pain, swelling and stiffness of synovial joints. An inflammatory reaction, increased cellularity of synovial tissue and joint damage are the pathological hallmarks of RA[1]. Though conventional treatment options for this condition have improved in terms of effectiveness, the use of non-steroidal anti-inflammatory drugs (NSAIDs) like etoricoxib, disease modifying anti-rheumatic drugs (DMARDs) like methotrexate, sulphasalazine, leflunomide, hydroxychloroquine, and corticosteroids like prednisolone, methylprednisolone have all been associated with adverse effects. Because of this reason, patients suffering from chronic musculoskeletal disorders are likely to seek alternative methods for

symptomatic relief and are amongst the highest users of complementary and alternative medicine[2]. This revival of herbal and other complementary therapies in the management of chronic diseases (RA and other inflammatory disorders) is well documented[3]. However, despite an increase in use, evidence for effectiveness and safety of these complementary therapies is limited.

Unani system identifies and attributes diseases like RA to a weak immune and digestive system. It suggests a number of polyherbal formulations as being effective in the treatment of this condition. Majoon Suranjan (MS) is one such polyherbal formulation composed of *Lawsonia inermis, Foeniculum vulgare, Capparis spinosa, Terminalia chebula, Ipomoea turpethum, Apium graveolens, Zingiber officinalis, Convulvulus scammony, Colchicum luteum, Cassia angustifolia, Piper nigrum, Coriandrum sativum, Rosa damascus, Origanum vulgare, Pyrethrum indicum, Plumbago zelanicum, Verbascum thapus, Ricinus communis* oil[4]. Even though this formulation has been used in the Unani system of medicine for hundreds of years, its efficacy in rheumatoid arthritis has not been validated using modern scientific parameters. Therefore, the present study was carried out to evaluate the antiarthritic potential of the polyherbal formulation Majoon Suranjan using experimental models of arthritis.

Material & Methods

Animals: The study was carried out in the Department of Pharmacology after approval of the protocol by the Institutional Animal Ethics Committee, All India Institute of Medical Sciences (AIIMS), New Delhi. Inbred adult male Wistar rats (150-200 g) from the Central Animal Facility, AIIMS, were used in the study. Animals were housed under standard laboratory conditions at 25 ± 2^0C in groups of three with access to food and water *ad libitum*. They were acclimatized to the laboratory conditions for a period of 5 days before the study. After completion of the study, all the animals were euthanized by an overdose of anaesthetic ether and the carcasses were disposed in accordance with institute regulations.

Drugs and chemicals: The polyherbal formulation Majoon Suranjan was procured from Majeedia Hospital Pharmacy, Jamia Hamdard (Hamdard University), New Delhi. Aspirin (acetylsalicylic acid) was used as commercially available powder (Sigma-Aldrich, USA). Both drugs were suspended in 1 per cent gum acacia (vehicle) and administered by oral gavage. Aspirin was administered in a dose of 100 mg/kg body wt and MS was administered in a dose of 450, 900 or 1800 mg/kg body wt. The doses that were used in the study were calculated from clinically used anti-arthritic doses of MS in man[4]. Formaldehyde and turpentine oil were purchased from Sigma-Aldrich, USA and complete Freund's adjuvant (CFA) was purchased from Difco Laboratories, USA.

Turpentine oil induced paw oedema: Five groups of male Wistar rats (n=6) were used in this study. Animals were fasted overnight with free access to water before the experiment. On the day of the experiment, baseline paw volume was recorded by using a plethysmometer (Ugo Basile 7140, Italy). Thereafter group I received the vehicle (2 ml/kg body wt), group II received aspirin (100 mg/kg body wt) and groups III, IV and V received MS in doses of 450, 900 and 1800 mg/kg body wt, respectively. Thirty minutes after administration of the vehicle/drug, oedema was induced by administration of 0.05 ml of turpentine oil into the subplantar surface of the left hind paw of the animal[5,6]. Increase in volume of the injected was measured at 1, 3 and 6 h post turpentine oil administration.

Formaldehyde induced arthritis: Five groups of male Wistar albino rats (n=6) were used in this study. Baseline recording of the joint diameter was made by using a micrometer screw gauge. Grouping of animals and drug treatments was same as for turpentine oil induced paw oedema. Drugs/vehicle was administered for a duration of 10 days. Thirty minutes after administration of vehicle/drugs, arthritis was induced by subplantar administration of 0.1 ml formaldehyde (2% v/v) into the left hind paw of all the animals on days 1 and 3[7,8]. Increase in joint diameter of the injected paw was measured on days 8, 9 and 10, 30 min after administration of the respective vehicle/drug treatment.

Adjuvant induced arthritis: Five groups of male Wistar albino rats (n=6) were used in this study. Baseline recording of the joint diameter was made by using a micrometer screw gauge. Grouping of animals and drug treatments was same as for turpentine oil induced paw oedema. Thirty minutes after administration of the vehicle/drug, arthritis was induced by subplantar administration of 0.1 ml of CFA (0.05% w/v *Mycobacterium butyricum* in mineral oil) into the left hind paw of all the rats[9,10]. This was designated as day 0. After immunization with CFA, all the groups were maintained on vehicle/drug treatment for 20 more days. Joint diameter of the injected paw was again

measured on days 7, 14 and 21, 30 min after vehicle/drug administration.

Toxicity studies of the plant extract: Evaluation of oral acute toxicity of MS was carried out according to the Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals (425)[11]. A limit test (5000 mg/kg body weight) was performed using five male Wistar rats (150-200 g) from our breeding stock. All the animals were observed for behavioural changes and mortality till 14 days after administration of the dose.

Evaluation of oral 28 day toxicity of MS was carried out according to the OECD guidelines for testing of chemicals (407)[12]. Twelve male Wistar rats (150-180 g) from our breeding stock were divided into two groups (n=6). Group I received the vehicle (2 ml/kg body weight, 1% gum acacia) and served as normal control and group II received MS in a dose of 1800 mg/kg body weight (maximum dose tested in antiarthritic studies). Drug/vehicle was administered daily for a duration of 28 days.

Statistical method: Difference between groups was compared by using One-way ANOVA followed by Dunnett's Multiple Comparison. $P<0.05$ was considered significant.

Results

Effect of MS on turpentine oil induced paw joint oedema in rats: Subplantar administration of turpentine oil produced paw oedema that was maintained throughout the entire observation period (Fig. 1). Even though aspirin and MS treatment reduced the paw oedema as compared to control animals, the difference was significant only at 6 h after turpentine oil administrations. MS produced a dose dependent reduction in paw oedema throughout the observation period. Even though maximum inhibition of paw oedema was produced by MS at a dose of 1800 mg/kg, there was only a marginal difference in the efficacies of the higher two doses (900 and 1800 mg/kg).

Effect of MS on formaldehyde induced arthritis in rats: Administration of 2 per cent formaldehyde on days 1 and 3 produced ankle joint swelling in the injected limb all the animals. This joint swelling was sustained throughout the observation period of 10 days (Fig. 2). The increase in joint diameter was less in the aspirin and MS treated groups as compared to the control, and this difference was significant ($P<0.01$) on all observational days. Even though MS produced a dose dependent inhibition of joint swelling, maximum inhibition was produced by aspirin. Only a marginal difference was observed in the efficacies of the higher two doses of MS (900 and 1800 mg/kg).

Effect of MS on CFA induced arthritis in rats: Immunization with CFA produced an increase in the ankle joint diameter of the injected limb in all the animals (Fig. 3). The standard drug aspirin produced a significant decrease in the joint diameter as compared to control on all observation days. Even though MS produced a dose dependent reduction in joint swelling as compared to control, the difference was significant only on day 21 in the higher two dose treated groups. Maximum joint swelling was observed in all the groups on day 7. However, in the control and aspirin treated groups, after the initial decrease in joint diameter, from day 7 to 14 there was a slight increase in the joint diameter up to day 21. This trend was not seen in any of the MS treated groups. Maximum reduction of joint

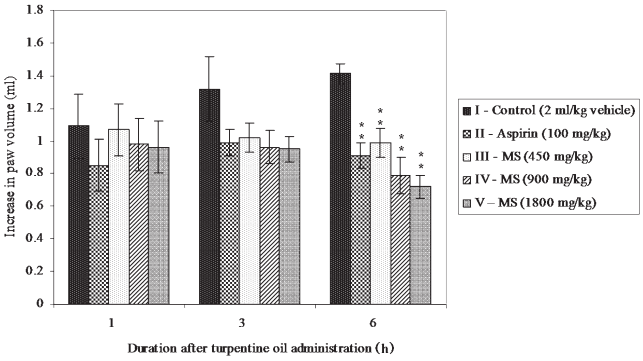

Fig. 1. Effect of MS on turpentine oil induced paw oedema in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. $^{**}P<0.01$ compared to control.

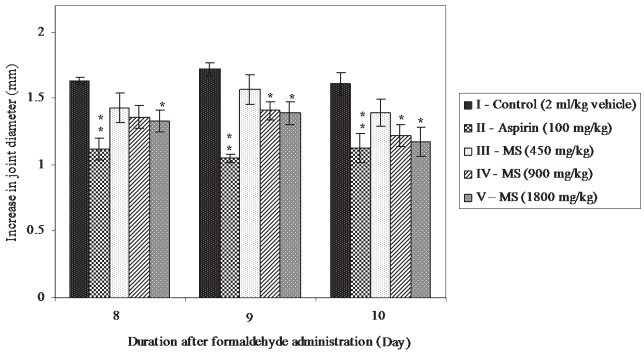

Fig. 2. Effect of MS on formaldehyde induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. $^{*}P<0.05$, $^{**}P<0.01$ compared to control.

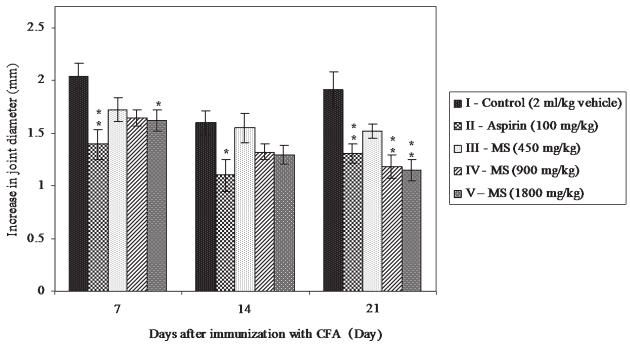

Fig. 3. Effect of MS on Complete Freund's Adjuvant (CFA) induced arthritis in rats. All values are mean ± SE from 6 animals in each group. Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison. $^{*}P<0.05$, $^{**}P<0.01$ compared to control.

swelling was produced by MS on day 21 in the highest dose treated group (1800 mg/kg).

Toxicity profile of the test plant: Administration of MS in a dose of 5000 mg/kg body weight did not produce any behavioural abnormalities in the animals. As all tested animals survived, the oral LD_{50} of MS in rats was found to be >5000 mg/kg body weight.

Chronic administration of MS in a dose of 1800 mg/kg body weight for 28 days did not produce any significant physiological changes in the tested animals as compared to normal control (data not shown). There was a marginal increase in body weight, bleeding time, RBC count and SGOT (serum glutamate oxaloacetate transaminase) levels as compared to normal control, but this increase was not significant. WBC count, %Hb and percentage organ weight of liver showed a marginal decrease from normal control, but this difference was also not statistically significant. All other parameters remained unaltered.

Discussion

Majoon Suranjan (MS) is a polyherbal formulation that is used in the Unani system of medicine for treatment of RA and other joint disorders[4]. It is composed of the extracts of 18 individual medicinal plants which are formulated in a sugar base. Some of the individual constituents of this polyherbal formulation have been evaluated for their anti-inflammatory activity. *Lawsonia inermis* has been shown to be efficacious in cotton pellet granuloma, granuloma pouch and formalin induced paw oedema models of inflammation in rats[13]. Chebulagic acid from the immature fruit of *Terminalia chebula* has been shown to suppress the onset and progression of collagen induced arthritis in mice[14]. *Colchicum luteum* has been shown to afford symptomatic relief in patients with rheumatoid arthritis in a 90 day trial[15,16], *Coriandrum sativum* has been shown to be efficacious in reducing carrageenan induced paw oedema[17], *Pyrethrum indicum* has been shown to induce synoviocytes apoptosis and suppress proliferation of synoviocytes in adjuvant-induced arthritis rats[18], *Zingiber officinalis* has been shown to decrease pain and swelling in arthritis patients[19] and *Foeniculum vulgare* has been found to be effective in reducing carrageenan induced paw oedema[20]. In present study turpentine oil induced paw oedema was used to evaluate the anti-inflammatory activity and the formaldehyde and CFA induced joint arthritis models were used to evaluate the antiarthritic efficacy of the formulation.

Turpentine oil induced paw oedema is characterized by a triphasic release of inflammatory mediators. The initial phase is mediated by histamine and serotonin, intermediate phase by kinin like substance and the late phase by cycloxygenase and lipoxygenase products[5,6]. In the present study, inhibition of turpentine oil induced paw oedema was observed in the test drug treated groups throughout the observation period. This suggests that MS influenced all the phases of turpentine oil induced inflammation in the rat paw. However, maximum inhibition of paw oedema was seen during the late phase of inflammation, thus suggesting a prominent cycloxygenase/lipoxygenase inhibitory activity.

In formaldehyde induced inflammatory arthritis MS was able to significantly reduce joint swelling in the treated group. Even though the reduction in joint swelling in the MS treated groups was lower than that observed in the aspirin treated group, on day 10 at the higher doses tested (900 and 1800 mg/kg) efficacy of MS was comparable to aspirin.

Complete Freund's adjuvant induced arthritis is one of the most widely used models as it has been shown to share a number of clinical and immunological features with human arthritis[9]. Therefore, this model is used with a relatively high degree of validity for evaluating agents with potential antiarthritic activity. In the vehicle treated animals (control), there was an increase in the joint diameter after day 14, which can be attributed to the delayed immunological flare in the disease[21]. This increase in joint diameter was also seen in the aspirin treated group, demonstrating the absence of disease modifying activity in the standard drug. However, this trend of delayed increase in joint diameter was not observed in the MS treated groups, suggesting the involvement of mechanisms other than inhibition of inflammatory autocoids in the antiarthritic

activity of the test drug. The most probable mechanism might be the inhibition of proinflammatory cells by *Colchicum luteum*[22] and *Terminalia chebula*[14], which could have led to an alteration in the immunological milieu during the delayed phase of the response.

Results of the present study contribute towards validating the traditional use of this polyherbal formulation in the treatment of rheumatoid arthritis. However, no animal model completely depicts the pathophysiology and disease progression in this debilitating disease. Therefore, further investigational studies are required to elucidate the exact mechanism of antiarthritic activity of this polyherbal formulation.

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Conflict of interest: None to declare.

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