Original Research

Evaluation of therapeutic efficacy of Majoon Suranjan, a Unani formulation, in the treatment of rheumatoid arthritis: an experimental study

Mythily Subramaneyaan[1], Shagufta Yasmeen[1], Rafat S Ahmed[1], Vinod K Arora[2], Asok K Tripathi[1] and Basu D Banerjee[1]

[1]Department of Biochemistry, University College of Medical Sciences and GTB Hospital (University of Delhi), Dilshad Garden, Delhi 110095, India; [2]Department of Pathology, University College of Medical Sciences and GTB Hospital (University of Delhi), Dilshad Garden, Delhi 110095, India

Corresponding author: Rafat S Ahmed. Email: rafatnizam@rediffmail.com

Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder. Allopathic treatments for RA have various side-effects and limitations. Majoon Suranjan (MS) is a polyherbal Unani formulation used to treat RA. Although it is widely used, evidence-based toxicity and efficacy data are not available. The present study was designed to assess the safety and therapeutic efficacy of MS in experimental animals. Acute (14 days) and long-term (90 days) toxicity studies were carried out at three doses of MS, i.e. 440, 880 and 1760 mg/kg body weight in male and female Wistar rats. Arthritis was induced in male rats by immunization with bovine collagen type II and they were treated with vehicle, methotrexate (0.25 mg/kg body weight, intraperitoneal once weekly) and MS (880 mg/kg body weight, orally, daily) for 20 days. Serum rheumatoid factor, anticyclic citrullinated peptide antibody, antinuclear antibody and C-reactive protein (CRP) were estimated. None of the rats exhibited overt toxicity or mortality and MS was found to be safe at the tested doses. No abnormal findings were observed in haematological and biochemical parameters, necropsy and histopathology at therapeutic effective dose. MS significantly inhibited the footpad swelling in arthritic rats while serum autoantibodies and CRP levels were significantly decreased. The present study demonstrates that at therapeutic doses, the Unani medicine, MS is relatively safe. Furthermore, MS was found to be effective in decreasing the biomarkers of RA, thus providing scientific evidence in support of its traditional use in the treatment of RA.

Keywords: autoimmune disease, rheumatoid arthritis, collagen-induced arthritis, traditional medicine, Majoon Suranjan, Unani system of medicine, methotrexate

Experimental Biology and Medicine **2013; 238: 1379–1387. DOI: 10.1177/1535370213498983**

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune and systemic inflammatory disease that targets synovial joints. Its prevalence is estimated at 2.5–3% in adults above 50 years of age.[1] Although the exact aetiology and pathogenesis of RA is not yet fully understood, it is likely that an autoimmune-mediated attack on the joints plays a crucial role in the pathogenesis of RA.[2] The present therapeutic measures aim only at relief of symptoms and improvement of general health of the patients. A number of allopathic drugs currently in use, such as disease-modifying antirheumatic drugs, non-steroidal anti-inflammatory drugs, slow-acting antirheumatic drugs, provide symptomatic relief; however, their adverse effects compel the patients to discontinue their use. Since long-term ingestion of these drugs results in various deleterious side-effects, the use of alternative medicines is another therapeutic approach for the treatment of such chronic diseases.[3]

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health, namely, Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy.[4] The Unani system of Medicine originated in Greece and was introduced in India by the Arabs. In classical Unani literature, the illness of joints pain is mentioned under the caption '*Waja-ul-Mafasil*'. The term '*Waja*' means pain and '*Mafasil*' means joints; hence, '*Waja-ul-Mafasil*' means pain in joints.[5] The general term of *Waja-ul-Mafasil* denotes the condition of

joint pain, in which the symptoms closely resemble the symptoms of RA as mentioned in the allopathy system.

The basic pathology of *Waja-ul-Mafasil* lies in the accumulation of abnormal or vitiated phlegm, which is formed due to the defective metabolism in liver. The drugs generally used for the treatment of RA are Majoon Suranjan (MS), Habb-e-Asgand, Majoon filasfa, Majoon jograj guggal, etc.[6] Although medicinal herbs and herbal formulas are generally considered to be safer than conventional drugs, there is no evidence for the quality, safety and efficacy of most commonly used herbal formulations including MS.

MS is a polyherbal Unani formulation, commonly used in the treatment of RA. Most of the ingredients of this formulation are of plant origin and are used as spices/condiments in India. Its name is due to the ingredient Suranjan Shirin (*Colchicum luteum*). Although this formulation has been used in the Unani system of Medicine for years and is claimed to alleviate the symptoms of RA, not much scientific studies are available regarding its safety and efficacy. Till date we have come across only one study reporting the antiarthritic activity of MS in an adjuvant-induced rat arthritic model.[7] Hence, this study was designed to assess the safety and efficacy of MS in experimentally induced RA.

Materials and methods

Study drug and dose calculation

The polyherbal Unani formulation, MS was procured from Majeedia hospital pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India. Its constituents are listed in Table 1.[8] MS was prepared by powdering all the ingredients and mixed with base (*Qiwam*) made of purified honey. Human effective dose (HED) of MS is 10 g/day. The therapeutic effective dose (TED) for rats has been calculated as per the following formula

$$\text{HED(mg/kg body weight)} = \text{Animal dose (mg/kg)} \times \text{Animal } k_m/\text{Human } k_m$$

where k_m represents the basal surface area, which is 6 for rats and 37 for adult humans.[9] Hence, the TED of MS for rats as calculated is 880 mg/kg body weight.

Experimental animals and housing conditions

Pathogen-free Wistar albino rats of both genders (100–150 g of body weight, 6–8 weeks of age) were obtained from central animal house facility of the institute. The animals were housed in polypropylene cages and allowed to acclimatize for one week before the initiation of the experiment. The rats received human care in compliance with CPCSEA and were fed pellet food (Hindustan Lever Ltd, Mumbai, India) and tap water *ad libitum* and maintained under the following standard conditions: light:dark cycle, 14:10 h; ambient temperature, $22 \pm 2^{\circ}$C and humidity, 40–45%. The study design was approved by the institutional animal ethics committee (approval no: UCMS/IAEC/18/2009).

Table 1 Ingredients of Majoon Suranjan

Name of the ingredient	Botanical name	Each 10 g contains
Berg Hina	*Lawsonia inermis Linn.*	0.05 g
Asaroon	*Asatum weropaeum Linn.*	0.06 g
Post Haelela Zard	*Terminalia chebula*	0.25 g
Tukhm Karafts	*Apium graveolens Linn.*	0.05 g
Zanjabeel	*Zingiber officinale*	0.10 g
Samundar Jhag	cuttle bone/sea foam	0.05 g
Sana Makki	*Cassia angustifolia*	0.40 g
Filfil Safaid	*Piper nigrum Linn.*	0.05 g
Gul Surkh	*Rosa centifolia*	0.10 g
Namak Sambhar	Salt from Sambhar lake	0.05 g
Sugar	*Saccharum officinarum*	7.90 g
Badyan	*Foeniculum vulgare Mill*	0.05 g
Baikh Kiber		0.06 g
Turbud Safaid	*Operculina turpethum*	0.54 g
Chita Lakri	*Plumbago zeylanica*	0.06 g
Saqmooniya	*Convolvulus scammonia*	0.10 g
Suranjan Shirin	*Colchicum luteum*	0.20 g
Satar Farsi	*Satureja hortensis Linn.*	0.05 g
Kishneez Khushk	*Coriandrum sativum*	0.10 g
Mahi Zehraj		0.06 g
Roghan Arandi	*Riccinus communis*	0.40 g

Safety evaluation studies

Grouping and treatment

Acute oral toxicity study. Adult male and female rats were randomly divided into eight groups (four groups of each sex and five rats/group). Group 1 animals of both sexes received 440 mg/kg body weight of MS dissolved in distilled water (0.5 times the TED), group 2 animals of both sexes received 880 mg/kg body weight of MS (equivalent to TED) and group 3 animals of both sexes received 1760 mg/kg body weight of MS (twice the TED) orally for 14 days. The control group of each sex received an equivalent amount of saline.

Long-term oral toxicity study. For long-term toxicity study, adult male and female rats were randomly divided into eight groups, four of each sex and 10 animals per group. The animals of each sex and different groups were treated in doses similar to that of the acute toxicity study. However, instead of 14 days, the animals received the treatment for 90 days.

Acute oral toxicity in rats (14 days). Mortality and clinical signs were continuously observed for 0, 1, 2, 3, 4, 5 and 6 h after dosing on day 1 and once per day from day 2–14. On day 15, all animals were euthanized and gross histological examination of various internal organs was carried out.

Long-term oral toxicity in rats (90 days). The rats were carefully observed daily for any overt or apparent signs

or symptoms of toxicity. The body weight change of individual rats was noted initially and thereafter weekly during the study period. The water and food intake, activity of the animals, mortality and clinical signs were observed daily.

Animals were fasted overnight before necropsy and blood sampling. Animals were anaesthetized for blood sampling from retro-orbital plexus vein. Blood samples were processed for serum biochemical and haematological parameters. Haematological parameters such as haemoglobin (Hb) content and total and differential leukocyte count were estimated as per standard protocol. Biochemical parameters like serum urea, creatinine, alkaline phosphatase, aspartate transaminase (AST), alanine transaminase (ALT), total protein, albumin and bilirubin were estimated in an autoanalyser (Olympus AU-400) using standard kits.

The rats were euthanized by CO_2 inhalation and organs such as liver, spleen and kidney were excised and preserved in 10% neutral formalin buffer for histopathological examination. Paraffin sections of the tissues of 5 μm thickness were prepared by routine microtomy and fixed in neutral formalin buffer. The sections were stained with haematoxylin–eosin using conventional methods and were observed under a light microscope for histological changes.

Therapeutic efficacy study

Grouping and treatment. For therapeutic efficacy study, male rats were randomly divided into four groups as follows: Group I: control (normal saline); Group II: induced (treated with collagen type II [CII] for induction RA + normal saline); Group III: induced + treated with methotrexate (MTX) (0.25 mg/kg body weight, intraperitoneal once weekly after the induction of arthritis [25th day]); Group IV: induced + treated with MS (880 mg/kg body weight, orally daily for 20 days after the induction of arthritis [25th day]).

Induction of arthritis. Arthritis in rats was induced by CII (Sigma Aldrich, USA) as described by Campo *et al*.[10] Briefly, CII was dissolved in 0.1 M acetic acid (2 mg/mL) at 4°C overnight and emulsified with an equal volume of complete Freund's adjuvant. Each rat was immunized with a dose of 200 μg of CII subcutaneously at the base of the tail. Rats were boosted with the same antigen preparation on day 14.

Assessment of arthritic index and footpad thickness in collagen-induced arthritic rats. A blinded independent observer with no knowledge of the treatment protocol performed evaluation of joint inflammation. Arthritis development was monitored with a macroscopic scoring system of the four limbs ranging from 0 to 15 (1 point for each swollen or red toe, 1 point for mid-foot digit or knuckle and 5 points for a swollen ankle). The scores of the four paws were added. A total score of 60 for each rat was considered as RA induction.[11] The severity of the clinical arthritis in each affected paw was graded at the end of treatment protocol by the same macroscopic scoring system. Clinical severity was also assessed by measuring the footpad thickness. The degree of footpad swelling was measured using a dial-gauge calliper. The increased thickness of footpad in percentage was compared with control rats.

Evaluation of antiarthritic activity of the test drug. The biomarkers of RA and inflammation, rheumatoid factor (RF), anticyclic citrullinated peptide antibody (a-CCP), antinuclear antibody (ANA) and C-reactive protein (CRP), were measured in rat serum using standard ELISA kits (Omega Diagnostics Ltd., UK), as per manufacturers' instructions.

Statistical analysis

All values are expressed as mean ± SD. Data were analysed by one-way ANOVA using SPSS version 16 (SPSS, Chicago, USA) statistical program and the individual comparisons of the treatment were obtained by Tukey's test for multiple comparisons. All P values were two-sided. A difference of $P < 0.05$ was considered to be statistically significant.

Results

Acute oral toxicity

MS did not cause any behavioural changes and did not produce any signs or symptoms of toxicity or mortality up to a dose of 1760 mg/kg body weight, clearly indicating that the formulation is unlikely to induce any drastic effect. Gross histological examination of liver, spleen and kidney did not show any gross lesion or pathological changes in all the groups.

Long-term oral toxicity

Clinical observations. No apparent sign of toxicity was found clinically during the experimental period. Food and water consumption remained unaffected in all the groups. A significant reduction in body weight gain was observed in MS-treated rats (data not shown).

Haematological profile. The effect of MS on haematological profile is presented in Table 2. Hb levels were decreased significantly in both male and female rats only at the highest dose, i.e. 1760 mg/kg body weight. Total and differential leukocyte count decreased significantly in rats of either sex treated at TED and TED × 2 of MS.

Biochemical parameters. The effect of MS on various biochemical parameters is presented in Table 2. In male rats treated with MS, serum urea levels were increased significantly at all the doses. At the highest dose, creatinine, AST and ALT were significantly increased while bilirubin decreased significantly as compared with control. A significant increase in total protein and albumin was observed in the TED-treated group as compared with control. In female rats, urea, creatinine, AST, ALT and total bilirubin levels were increased at the highest dose while total protein and albumin was increased significantly at both TED and TED × 2, as compared with control.

Table 2 Haematological and biochemical parameters in rats treated with Majoon Suranjan.*

	Majoon Suranjan							
	Male rats ($n = 10$ per group)				Female rats ($n = 10$ per group)			
Parameters	Control (saline)	Group 1 (440 mg/kg body weight)	Group 2 (880 mg/kg body weight)	Group 3 (1760 mg/kg body weight)	Control-a (saline)	Group 1a (440 mg/kg body weight)	Group 2a (880 mg/kg body weight)	Group 3a (1760 mg/kg body weight)
Haemoglobin (g/dL)	14.22 ± 0.08	14.04 ± 0.18	14.08 ± 0.23	13.78 ± 0.22†	14.52 ± 0.08	14.54 ± 0.19	14.32 ± 0.18	14.14 ± 0.22†
TLC (/mm^3)	6777.6 ± 59.9	6702.4 ± 18.9†	6601.6 ± 65.3†	6434.4 ± 73.28†	7148.4 ± 89.9	7052 ± 97	6963.6 ± 58.08†	6765.2 ± 138.93†
Neutrophil (% of TLC)	8.65 ± 0.04	8.57 ± 0.05†	8.38 ± 0.03†	8.03 ± 0.09†	8.75 ± 0.06	8.67 ± 0.06	8.46 ± 0.03†	8.11 ± 0.08†
Lymphocyte (% of TLC)	85.96 ± 0.32	84.9 ± 0.32†	82.4 ± 0.53†	80.6 ± 0.78†	87.8 ± 0.42	85.06 ± 0.49†	82.84 ± 0.39†	79.66 ± 0.97†
Urea (mg/dL)	36.8 ± 4.39	40.8 ± 2.25†	41.6 ± 2.17†	43.8 ± 3.12†	39.4 ± 4.74	37.2 ± 0.79	40.2 ± 1.81	43.6 ± 2.17†
Creatinine (mg/dL)	0.5 ± 0.07	0.54 ± 0.05	0.56 ± 0.11	0.6 ± 0.08†	0.52 ± 0.08	0.54 ± 0.05	0.56 ± 0.05	.64 ± 0.11†
AST (IU/L)	132.8 ± 12.19	132.4 ± 6.02	132.6 ± 4.04	138.4 ± 4.09	130.8 ± 12.51	130.4 ± 2.17	131.4 ± 4.45	134 ± 2.58
ALT (IU/L)	52 ± 5.85	51.80 ± 1.81	53 ± 2.49	68.4 ± 3.92†	50 ± 4.06	50.2 ± 2.04	51 ± 3.46	58.4 ± 1.58†
ALP (IU/L)	181.6 ± 33.06	181.4 ± 3.63	183.6 ± 4.97	191 ± 2.49	184.8 ± 4.83	184.8 ± 3.55	185 ± 4.81	189.4 ± 1.96
Total protein (g%)	8.12 ± 0.27	8.88 ± 0.16†	8.6 ± 0.15†	8.26 ± 0.22	7.92 ± 0.29	7.98 ± 0.12	8.9 ± 0.24†	8.74 ± 0.11†
Albumin (g/dL)	4.32 ± 0.17	4.4 ± 0.24	4.56 ± 0.11†	4.76 ± 0.11†	4.32 ± 0.17	4.38 ± 0.12	4.68 ± 0.12†	5.04 ± 0.05†
Bilirubin-direct (mg/dL)	0.28 ± 0.08	0.26 ± 0.08	0.24 ± 0.05	0.2 ± 0.07†	0.28 ± 0.08	0.24 ± 0.05	0.28 ± 0.04	0.34 ± 0.11
Bilirubin-total (mg/dL)	0.5 ± 0.09	0.5 ± 0.09	0.48 ± 0.08	0.4 ± 0.06†	0.4 ± 0.09	0.42 ± 0.08	0.44 ± 0.05	0.52 ± 0.04†

TLC: total leukocyte count; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase.
*Values are mean ± SD ($n = 10$ per group).
†Significantly different from respective control, $P < 0.05$.

Histological examination. Gross examination showed that liver, spleen and kidney were normal in size and appearance. Histopathological examination of liver, spleen and kidney showed mild to moderate cellular changes. Control group showed normal histological structure of the liver, the hepatic lobules were normal and consisted of normal hepatocytes, blood vessels and bile ducts. Hydropic degeneration, hepatocyte ballooning, arbitration of sinusoids and prominence of Kupffer cells were observed in rats treated with TED. Rats treated with the highest dose revealed granulomatous inflammation and dilatation of sinusoids (Figure 1). Splenic tissue of controls rats showed normal red and white pulp. However, rats treated with TED and TED × 2 of MS showed extra-medullary haematopoiesis and increase in white pulp proportion (Figure 2). Renal tissues of control rats and rats treated with TED showed normal histological structures of the glomeruli and renal tubules in the cortical and medullary portions. At TED × 2, dilatation of tubules, vessels, interstitial inflammation and focal chronic inflammation were observed (Figure 3).

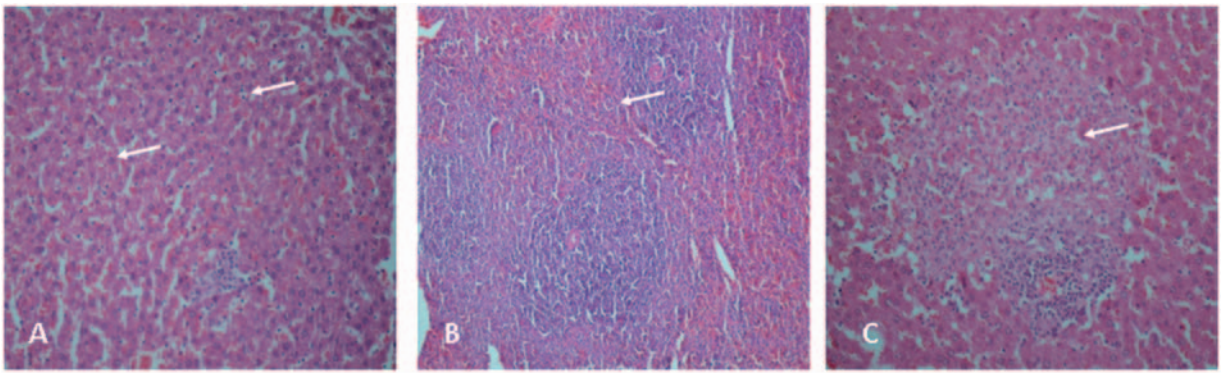

Figure 1 Effect of MS on liver: (A) hepatocytes show fatty changes (H&E stain × 200); (B) sinusoids show dilatation (H&E stain × 100) and (C) granulomatus inflammation (H&E stain × 400) at highest dose of MS. MS: Majoon Suranjan; H&E: haematoxylin–eosin. (A color version of this figure is available in the online journal)

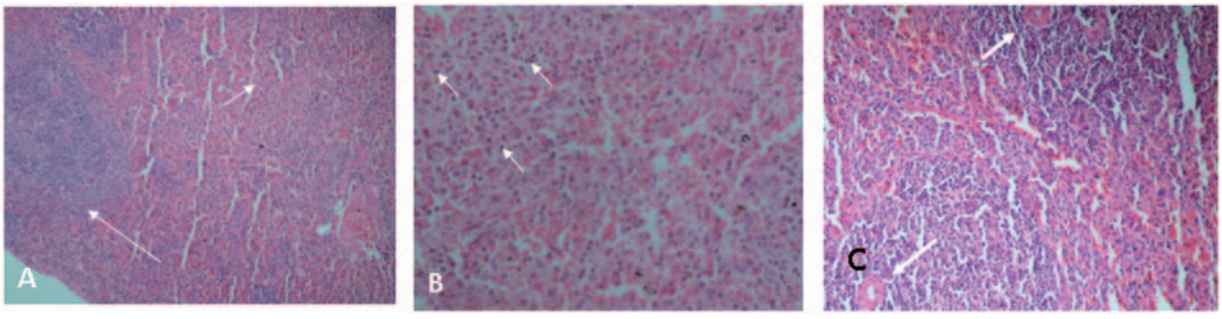

Figure 2 Effect of MS on spleen: (A) control rats showed normal architecture of spleen – white pulp (long arrow) and red pulp (short arrow) (H&E × 200); (B) rats treated with MS (880 mg/kg body weight) showed deposition of hemosiderin pigments and (C) rats treated with MS (1760 mg/kg body weight) showed extramedullary haematopoiesis (H&E stain × 200) in spleen. MS: Majoon Suranjan; H&E: haematoxylin–eosin. (A color version of this figure is available in the online journal)

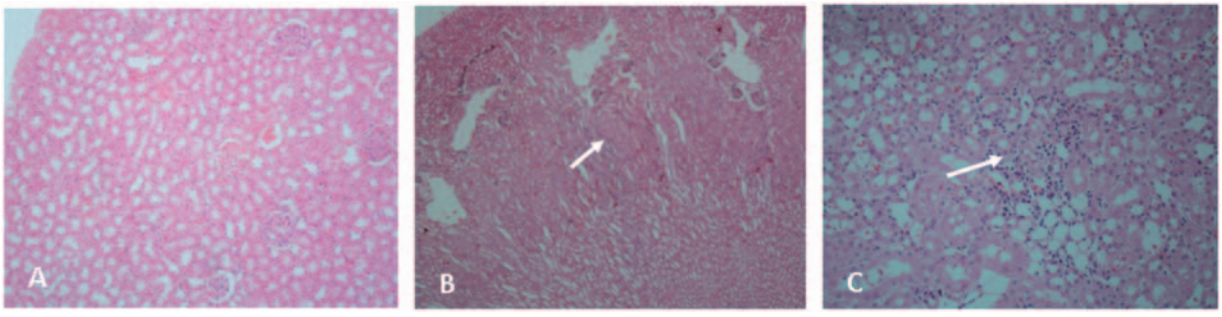

Figure 3 Effect of MS on kidney: (A) control rats showed normal glomeruli and tubules (H&E × 200); (B) rats treated with 880 mg/kg body weight of MS showed tubular dilatation (H&E stain × 100) and (C) at highest dose of MS, rats showed chronic inflammation (H&E stain × 400). MS: Majoon Suranjan; H&E: haematoxylin–eosin. (A color version of this figure is available in the online journal)

Antirheumatic activity

Clinical signs of collagen-induced arthritis (CIA) appeared four days after second immunization with CII. CIA rats showed periarticular erythema and oedema in paws and ankles. The extent of erythema and oedema was reduced significantly in MS-treated rats as compared with the CIA rats (Figure 4).

The CIA rats showed an increased arthritis score. A significant decrease in the arthritis score was observed in the MTX- and MS-treated rats (Figure 5(a)). The arthritic animals showed a maximum increase in footpad thickness. Treatment with MTX and MS showed significant inhibition of the footpad swelling (Figure 5(b)).

The autoantibodies, RF, a-CCP and ANA, were significantly increased in CIA rats. Treatment with MS showed a significant decrease in RF, a-CCP, ANA and CRP, which was comparable with that of MTX-treated rats (Table 3).

Discussion

RA is a chronic, systemic, immune-mediated inflammatory disease associated with decreased life expectancy and poor quality of life. The therapy of RA is mainly based on MTX and biological agents that directly target molecules involved in the pathogenesis of RA, e.g. the human soluble tumour necrosis factor (TNF) receptor etanercept (Enbrel).

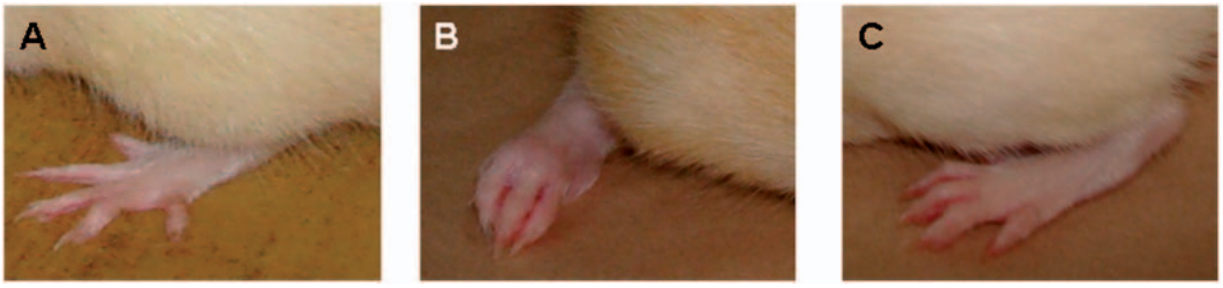

Figure 4 Representative picture of the hind paw of (A) untreated rat (0 day), showing inflammation and joint swelling in: (B) induced rat (25th day) and (C) MS-treated (45th day) rat. (A color version of this figure is available in the online journal)

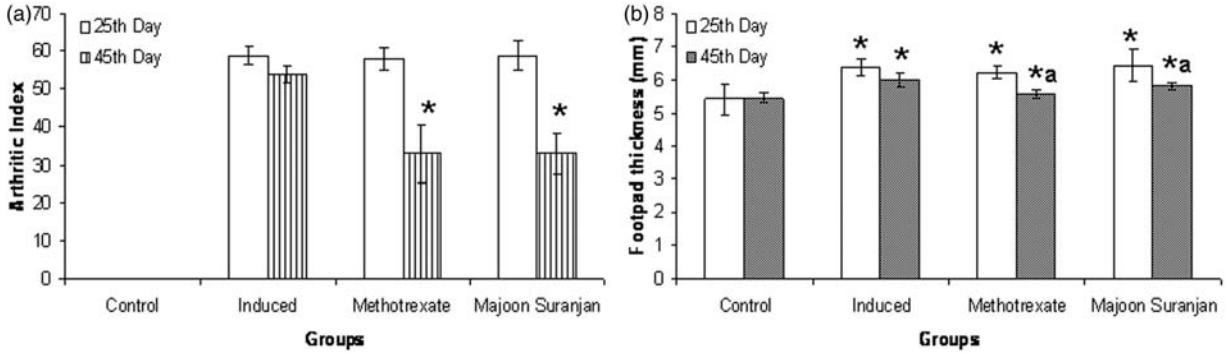

Figure 5 Effect of methotrexate and MS on: (a) arthritic index and (b) footpad swelling in collagen-induced arthritic rats. MS: Majoon Suranjan. *Significantly different from control, $P < 0.05$. †Significantly different from induced group, $P < 0.05$

Table 3 Effect of treatment with Majoon Suranjan on biomarkers of rheumatoid arthritis in arthritis-induced male rats.*

Groups ($n = 6$)	RF (IU/mL)	a-CCP (U/mL)	ANA (IU/mL)	CRP (ng/mL)
Control	17.21 ± 0.19	35.21 ± 0.13	1.05 ± 0.029	2.024 ± 0.004
Induced (CII, 200 μg)	37.51 ± 0.27†	135.22 ± 0.13†	2.59 ± 0.004†	3.05 ± 0.005†
Methotrexate (0.25 mg/kg body weight)	22.26 ± 0.16†‡	81.11 ± 0.08†‡	1.66 ± 0.005†‡	2.39 ± 0.003†‡
Majoon Suranjan (880 mg/kg body weight)	20.93 ± 0.07†‡§	68.04 ± 0.07†‡§	1.57 ± 0.004†‡§	1.70 ± 0.003†‡§

RF: rheumatoid factor; a-CCP: anticyclic citrullinated peptide antibody; ANA: antinuclear antibody, CRP: C-reactive protein; CII: Collagen type II.
*Values are mean ± SD ($n = 6$ per group).
†Significantly different from control group, $P < 0.05$.
‡Significantly different from induced group, $P < 0.05$.
§Significantly different from methotrexate-treated group, $P < 0.05$.

However, cytokines targeted with such biological agents also play a general role in the immune defence and the risk of infections increases with the use of these agents.[12] As a result, many patients look for complementary and alternative medicine (CAM) options in coping with this debilitating disease. Research has indicated that people suffering from chronic pain, as in RA, and those dissatisfied with current treatment are very likely to seek alternative treatments and an estimated 60–90% of the persons with arthritis use CAM, mostly chiropractic and herbal therapies.[13]

Millions of Indians use herbal drugs regularly, as spices, home-remedies, health food as well as over-the-counter as self-medication or also as drugs prescribed in the non-allopathic systems.[14] Although there are many traditional herbal medicine interventions available and some have been verified by clinical trials, their efficacy and safety are still questioned by consumers.[15] Hence, there is a need for a scientific evaluation of known ancient Indian Ayurvedic and Unani formulations for their safety and efficacy. The present study is one such effort in this direction.

Acute toxicity studies on the drug MS did not show any significant effects on the behavioural profile or on any other parameters in experimental animals. From this it can be inferred that MS is not toxic at all the doses, including the TED. During long-term toxicity study, no treatment-related overt toxicity and/or mortality was observed. Alterations observed in haematological and biochemical parameters at TED were within the normal range (Table 2).

The decrease in the Hb content at the highest dose might be due to the effect of the drug on erythropoiesis or the haematopoietic system. Total and differential leukocyte count decrease might be due to the immunosuppressive action of the drug. These results are in accordance with the histopathological findings of splenic and renal tissues. The observed pathological changes of these tissues were more pronounced at TED × 2. Liver is the organ most sensitive to biochemical changes.[16] Fatty degeneration and accumulation of lipid droplets in the hepatocytes, which were observed in this study, might be due to the metabolism of this drug in the liver in an attempt to collect all biotransformation compounds invading the cell in these vacuoles prior to excretion (Figure 1).

At present, the current modalities for treating RA are mostly symptomatic. The present study is focused to assess the effect of MS on the biomarkers of RA in experimental animals. Animal models of arthritis are used to study pathogenesis of disease and to evaluate potential antiarthritic drugs for clinical use. The CIA model is a highly dynamic model with time-dependent disease progression and is a frequently used animal model of human RA. In this model, immunization with CII, the collagen found in joint cartilage, induces T-cell activation, anti-CII autoantibody production and inflammation and joint destruction similar to that observed in human RA.[17]

Although the exact pathogenesis of RA is not fully understood, the immune and inflammatory systems are intimately linked. The arthritic index represents the grade of arthritis and was used to assess the efficacy of the treatment. Treatment with MS resulted in a decrease in the arthritic score, which was comparable with that of MTX treatment (Figure 5(a)). The swelling of footpad in CIA rats represents the severity of arthritis and is the most important index for evaluation of arthritis. Treatment with MTX as well as MS resulted in a decrease in footpad thickness, as compared with CIA rats (Figure 5(b)). Our findings are consistent with the results obtained by Singh *et al.*,[7] wherein they have reported that MS was able to inhibit turpentine oil-induced paw oedema The reduction in paw oedema might be due to some ingredients of MS like *Coriandrum sativum* and *Foeniculum vulgare; C. sativum* and *F. vulgare* have been found to be effective in reducing carrageenan-induced paw oedema.[18,19]

CRP is an acute phase plasma protein produced as part of the inflammatory process by the liver and adipocytes. The reduction in CRP is relevant for inhibition of arthritic progression. High levels of CRP induced in the CIA group were significantly decreased by treatment with both MS and MTX. These results indicate that MS has the ability to reduce systemic inflammation, which may be attributed to some individual constituents such as *Riccinus communis*[20] and *Apium graveolens,*[21] which are known to possess anti-inflammatory activity.

Biomarkers for RA are much needed if we are to understand and measure disease progression and to facilitate the development of novel treatments of RA. A number of autoantibodies like antiperinuclear factor, antikeratin antibodies, RF, a-CCP and ANA are known to be associated with RA[22–24] and the progression of the disease. RF is an autoantibody produced against the Fc portion of IgG and is most relevant in RA. a-CCP are autoantibodies produced against filaggrin. a-CCP is more specific than RF and is used to distinguish various causes of arthritis.[25] MS was found to be effective in significantly reducing the levels of RF as well as a-CCP antibodies and the results are comparable with that of MTX (Table 3). ANAs are unusual antibodies, detectable in the blood, that have the capability of binding to certain structures within the nucleus of the cell. ANAs are found in patients whose immune system may be predisposed to cause inflammation against their own body tissues. Treatment with MS resulted in a significant decrease in these antibodies, suggesting a decreased inflammatory response.

From the results of the present study, it can be said that MS suppressed the autoimmune response and has the ability to reduce the progression of the disease by decreasing the circulation/production of autoantibodies and reducing the inflammatory response. The efficacy of MS in the suppression of autoimmunity might be due to the synergistic antiarthritic activity of the individual constituents. *Colchicum luteum*, the main ingredient of MS, has been shown to afford symptomatic relief in patients with RA in a 90-day trial.[26] *Terminalia chebula* has been shown to suppress the onset and progression of CIA in mice.[27] Piperine, the phenolic component of *Piper nigrum,* has anti-rheumatic effects in animal models and anti-inflammatory effects on interleukin (IL)-1β-stimulated fibroblast-like synoviocytes.[28]

To the best of our knowledge, this is the first systematic study that reports the efficacy of MS in treatment of RA, as

indicated by reduction in the biomarkers of RA. Furthermore, this formulation has also been found to be non-toxic at the TED. However at twice the TED, fatty changes and necrosis of liver cells was observed. Hence, care should be taken to avoid the overdose of this formulation. In an earlier study, we have reported that *Habb-e-Asgand*, a poly-herbal Unani formulation is non-toxic, free of any side-effects and cost-effective and also showed efficacy in treating RA in animal model.[29] Although there is vast experience-based evidence for the use of such drugs, there is lack of scientific evaluation. Results of the present study contribute towards validating the traditional use of this polyherbal formulation in the treatment of RA. Drugs that block pro-inflammatory cytokines such as TNF-α and IL-1β can improve outcomes for RA and Kineret and Enbrel have been developed to block IL-1β receptor and neutralize the TNF-α;[30,31] however, these protein drugs are costly in the treatment of RA.[32] Herbal drugs and Indian medicinal plants are also rich sources of beneficial compounds including antioxidants. Newer approaches using modern technology in combination with established traditional health principles will yield rich dividends in improving health, especially among people who do not have access to the use of costlier western systems of medicine.

Author contributions: MS and SY carried out the experimental work, data collection and analysis; VKA conducted the histopathological studies; BDB and AKT carried out the toxicological and immunological studies and RSA did the concept designing, funding, manuscript preparation and editing.

ACKNOWLEDGEMENT

The authors are thankful to Central Council for Research in Unani Medicine (CCRUM), Department of AYUSH, Ministry of Health and Family Welfare (Government of India), for providing financial support for this study (F. No – 3-22/2008-CCRUM/EMR).

REFERENCES

1. David M, Weinblatt M. Rheumatoid arthritis. *Lancet* 2001;**358**:903–11
2. Jawaheer D, Thomson W, MacGregor AJ, Carthy D, Davidson J, Dyer PA, Silman AJ, Ollier WE. "Homozygosity" for the HLA-DR shared epitope contributes the highest risk for rheumatoid arthritis concordance in identical twins. *Arthritis Rheum* 1994;**37**:681–6
3. Rathore B, Mahdi AA, Paul BN, Saxena PN, Das SK. Indian herbal medicines: possible potent therapeutic agents for rheumatoid arthritis. *J Clin Biochem Nutr* 2007;**41**:12–7
4. Vaidya ADB, Devasagayam TPA. Current status of herbal drugs in India: an overview. *J Clin Biochem Nutr* 2007;**41**:1–11
5. Razi Z. *Kitabul Hawi Fill Tibb*. Vol 11. Urdu translation by CCRUM, Government of India. New Delhi, India: CCRMU, 2002:75–100
6. Ahmad B, Akhtar J. Unani system of medicine. *Phcog Rev* 2007;**1**:210–14
7. Singh S, Nair V, Gupta YK. Anti-arthritic activity of Majoon suranjan (a polyherbal Unani formulation) in rat. *Indian J Med Res* 2011;**134**:384–8
8. Said HM. *Hamdard Pharmacopoeia of Eastern Medicine. Indian Medical Science Series*. New Delhi: Sri Satguru Publications, 1997
9. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* 2007;**22**:659–61
10. Campo GM, Avenoso A, Campo S, Ferlazzo AM, Altavilla D, Calatroni A. Efficacy of treatment with glycosaminoglycans on experimental collagen-induced arthritis in rats. *Arthritis Res Ther* 2003;**5**:R122–31
11. Holmdahl R, Carlsen S, Mikulowska A, Vestberg M, Brunsberg U, Hansson A, Sunduall M, Jansson L, Pettersson U. Genetic analysis of murine models for rheumatoid arthritis. In: Adolpho KW (ed.). *Human Genome Methods*. New York: CRC Press, 1998:215
12. Shanahan JC, Moreland LW, Carter RH. Upcoming biologic agents for the treatment of rheumatic diseases. *Curr Opin Rheumatol* 2003;**15**:226–36
13. Rao J, Mihaliak K, Kroenke K, Bradley J, Tierney W, Weinberger M. Use of complementary therapies for arthritis among patients of rheumatologists. *Ann Inter Med* 1999;**131**:409–16
14. Gautam V, Raman RMV, Ashish K. *Exporting Indian Healthcare (Export Potential of Ayurvedha and Siddha Products and Services). Road Beyond Boundaries (The Case of Selected Indian Healthcare Systems)*. Mumbai, India: Export-Import Bank of India; 2003:14–54
15. Cheng CW, Bian ZX, Wu TX. Systematic review of Chinese herbal medicine for functional constipation. *World J Gastroenterol* 2009;**15**:4886–95
16. Hasan HA. Hemato-biochemical changes induced by fed intake of edible tiger nut (Cyerus esculentus) to senile rats. *J Egypt Ger Soc Zool* 2005;**48**:217–36
17. Bevaart L, Vervoordeldonk MJ, Tak PP. Evaluation of therapeutic targets in animal models of arthritis: how does it relate to rheumatoid arthritis? *Arthritis Rheum* 2010;**62**:2192–205
18. Ammar NM, Al-Okbi SY, Mohamed DA. Study of the anti-inflammatory activity of some medicinal edible plants growing in Egypt. *J Islamic Acad Sci* 1997;**10**:113–22
19. Ozbek H. The anti-inflammatory activity of the Foeniculum vulgare L. essential oil and investigation of its median lethal dose in rats and mice. *Int J Pharmacol* 2005;**1**:329–31
20. Saini AK, Goyal R, Gauttam VK, Kalia AN. Evaluation of anti-inflammatory potential of *Ricinus communis* Linn leaves extracts and its flavonoids content in Wistar rats. *J Chem Pharm Res* 2010;**2**:690–95
21. Fazal SS, Singla RK. Review on the pharmacognostical & pharmacological characterization of *Apium graveolens* Linn. *Indo Global J Pharmaceut Sci* 2012;**2**:36–42
22. Vincent C, de Keyser F, Masson-Bessiere C, Sebbag M, Veys EM, Serre G. Anti-perinuclear factor compared with the so called 'antikeratin' antibodies and antibodies to human epidermis filaggrin, in the diagnosis of arthritides. *Ann Rheum Dis* 1999;**58**:42–8
23. Goldbach-Mansky R, Lee J, McCoy A, Hoxworth J, Yarboro C, Smolen JS, Steiner G, Rosen A, Zhang C, Ménard HA, Zhou ZJ, Palosuo T, Van Venrooij WJ, Wilder RL, Klippel JH, Schumacher HR Jr, El-Gabalawy HS. Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. *Arthritis Res* 2000;**2**:236–43
24. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000;**43**:155–63
25. De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, Lebeer K, Wyns B, Vincent C, Mielants H, Boullart L, Serre G, Veys EM, De Keyser F. Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Ann Rheum Dis* 2004;**63**:1587–93
26. Javed M, Khan JA, Siddiqui MM. Effect of Colchicum luteum baker in the management of rheumatoid arthritis. *Indian J Tradit Know* 2005;**4**:421–3
27. Lee SI, Hyun PM, Kim SH, Kim KS, Lee SK, Kim BS, Maeng PJ, Lim JS. Suppression of the onset and progression of collagen-induced arthritis by chebulagic acid screened from a natural product library. *Arthritis Rheum* 2005;**52**:345–53
28. Bang JS, Oh da H, Choi HM, Sur BJ, Lim SJ, Kim JY, Yang HI, Yoo MC, Hahm DH, Kim KS. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1beta-stimulated fibroblast-like synoviocytes and in rat arthritis models. *Arthritis Res Ther* 2009;**11**:R49–R57

29. Subramaneyaan M, Yasmeen S, Arora VK, Tripathi AK, Banerjee BD, Ahmed RS. Safety evaluation and therapeutic efficacy of *Habb-e-Asgand,* a commonly used anti-rheumatic poly-herbal Unani formulation. *Altern Ther Health Med* 2012 (in press)
30. Calabrese LH. Molecular differences in anticytokines therapies. *Clin Exp Rheumatol* 2003;**21**:241–8
31. Louie SG, Park B, Yoon H. Biological response modifiers in the management of rheumatoid arthritis. *Am J Health Syst Pharm* 2003;**60**:346–55
32. Lubeck DP. A review of the direct costs of rheumatoid arthritis managed care versus fee-for-service settings. *Pharmacoeconomics* 2001;**19**:811–8

(Received February 15, 2013, Accepted May 28, 2013)