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Original Article

Anti-arthritic and Anti-inflammatory Activity of Ayurvedic Polyherbal Formulation in Laboratory Animals

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ABSTRACT

Background: RD/M.ph.AA-01 is an Ayurvedic polyherbal formulation, developed for *amavata*, which is associated with chronic inflammatory autoimmune diseases. **Objectives:** The study was planned to evaluate the anti-arthritic and anti-inflammatory activity of RD/M.ph.AA-01 in laboratory animals. **Materials and Methods:** Anti-arthritic activity of RD/M.ph.AA-01 was evaluated at 270 and 405 mg/kg doses against Complete Freund's Adjuvant (CFA)-induced arthritis. Anti-inflammatory activity was studied using cotton pellet granuloma and formalin-induced edema in rat models. The effect of topical application of RD/M.ph.AA-01 on Croton oil ear edema in mice was also investigated. **Results:** Arthritis induction caused a significant (p<0.05) alteration in paw size, hematology, serum parameters, edematous tissue parameters, lipid peroxidation in the liver and histopathology of joints. These alterations were significantly (p<0.05) prevented by treatment with RD/M.ph.AA-01. In the cotton pellet granuloma, RD/M.ph.AA-01 supplementation caused significant (p<0.05) improvement in the weight of granuloma tissue, serum parameters, lipid peroxidation in the liver and histopathological changes in granuloma tissue. A significant (p<0.05) improvement in paw size, serum parameters and liver lipid peroxidation were observed by RD/M.ph.AA-01 treatment in the formalin-induced edema model. The RD/M.ph.AA-01 also significantly (p<0.05) suppressed Croton oil-induced ear edema. **Conclusion:** These findings showed that RD/M.ph.AA-01 possesses potent anti-arthritic and anti-inflammatory activity.

Keywords: Anti-arthritic, Anti-inflammatory, Ayurvedic polyherbal formulation.

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INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease of the joints marked by leukocyte infiltration, pannus development, and severe articular cartilage and bone loss.[1] About 1% of the world's population is affected by it.[2] Anti-inflammatory and disease-modifying drugs are the ones that are most frequently prescribed for RA. These drugs are intended to treat pain, lessen joint inflammation, stop joint deterioration, and bring back joint function in damaged joints. These medications alter the pathophysiology and improve quality of life, but they do not completely reverse the condition. Additionally, they have a number of negative side effects, including immunodeficiency, cardiovascular toxicity, gastrointestinal issues and organ damage.[3,4] This is increasing the popularity of herbal medicines as it is believed that they are safe, effective and more affordable than synthetic drugs. It is reported that about 80% of the population in developing countries depends on herbal medicines for their primary healthcare needs.[5]

The Ayurvedic polyherbal formulation, RD/M.ph.AA-01, was developed by Penta Care Pvt. Ltd., Bangalore for therapeutic use in *Amavata*, which is associated in several ways with chronic inflammatory autoimmune diseases. It is a formulation that contains aqueous extracts of eleven herbs (Table 1). Plant extracts of RD/M.ph.AA-01 has anti-cancer, anti-gout, anti-allergic, antiulcer, antioxidants, cytotoxic, anti-inflammatory, wound healing, radioprotective, antimicrobial, analgesic, immunomodulatory, antidiabetic, antidepressant, anti-pyritic, anti-neuro-inflammatory and anti-obesity activities.[6-32] An earlier investigation in our laboratory reported antinociceptive activity RD/M.ph.AA-01 with involvement of opioid, adenosine and $5HT_3$ receptors.[33] With this background, the present study was conducted to investigate the anti-arthritic and

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anti-inflammatory properties of RD/M.ph.AA-01 in laboratory animals.

MATERIALS AND METHODS

Polyherbal Formulation

The Ayurvedic polyherbal formulation (RD/M.ph.AA-01) was obtained as a gift sample from Penta Care Pvt. Ltd., Bangalore, India for research purposes.

Experimental Animals

Wistar albino rats (150-200 g) and Swiss albino mice (20-25 g) were obtained from NIMHANS, Bangalore, India. They were housed in an animal house approved by the CCSEA, New Delhi. The study was approved by the Institutional Animal Ethics Committee (Ref. No.: 997/c/6/CPCSEA).

Phytochemical Analysis

The RD/M.ph.AA-01 was studied for the presence of various phytoconstituents by standard methods.[34] It showed the presence of steroids, flavonoids and tannins in RD/M.ph.AA-01.

Dose Selection

The dose of RD/M.ph.AA-01 was calculated from daily human dose using a body surface area conversion factor.[35] The daily human dose of RD/M.ph.AA-01 is 3,000 to 4,500 mg per day and therefore doses of 270 and 405 mg/kg/day were used for the study.

Evaluation of Anti-arthritic Activity

Anti-arthritic activity of RD/M.ph.AA-01 was evaluated against Complete Freund's Adjuvant-induced arthritis in rats. Wistar albino rats were randomly divided into five groups containing six rats in each. Group I was the vehicle-treated group and received 0.5% w/v Carboxy Methyl Cellulose (CMC) solution (10 mL/kg, p.o.). All remaining groups received arthritic-inducing treatment of 0.1 mL injection of Complete Freund's Adjuvant (CFA) emulsion into the sub-planter surface of the right hind paw.[36] Group II was an arthritic control group and received 0.5% w/v CMC solution (10 mL/kg b.w, p.o.). Group III was the standard drug treatment group and received diclofenac sodium (15 mg/kg, p.o.). Group IV and V served as RD.M.ph.AA-01 treated groups and received RD.M.ph.AA-01 at doses of 270 and 405 mg/kg respectively. The RD.M.ph.AA-01 and standard drug were suspended in distilled water using CMC (0.5% w/v) and given once daily by oral route for 21 days from the day of CFA injection.

The changes in the paw size were measured on 0, 4, 7, 14 and 21st days by using a caliper. On the 21st day, blood was withdrawn from the carotid artery under ether anesthesia. The serum was separated by centrifugation at 10,000 g for 10 min. Blood and serum samples were used for estimation of Erythrocyte Sedimentation Rate (ESR), Hemoglobin (Hb), Red Blood Cells Count (RBC), total White Blood Cells Count (WBC), neutrophil, lymphocyte, Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP) and Total Protein (TP).

After blood collection, rats were sacrificed to isolate stomach, liver, bone joint and edematous tissues from the injected hind paw. The stomach was opened along with greater curvature, rinsed with normal saline and subjected to lesion counting for evaluation of the ulcerogenic potential of RD.M.ph.AA-01. The liver tissue homogenate (20% w/v in Tris-HCl buffer (0.02 mol/L, pH 7.4) was prepared and used for the estimation of lipid peroxidation activity. The edematous tissues were used to estimate hydroxyproline and hexosamine. Histopathological changes in the joint were estimated by the hematoxylin and eosin staining methods.

Evaluation of Anti-inflammatory Activity

Cotton Pellet Granuloma in Rats

Forty-two rats were used for the study. Two sterilized cotton pellets (20±1 mg) were implanted on either side of the ventral region of rats.[36] Cotton pellet implanted rats were randomly divided into four groups containing six rats in each. Group I served as granuloma-induced control and received 0.5% w/v CMC solution (10 mL/kg, p.o.). Group II was the standard drug treatment group and received diclofenac sodium (15 mg/kg, p.o.). Group III and IV were RD.M.ph.AA-01 treated groups and received RD.M.ph.AA-01 at doses of 270 and 405 mg/kg respectively. The RD.M.ph.AA-01 was suspended in distilled water using 0.5% CMC solution given once daily by oral route for eight days.

On the 9th day, cotton pellets were removed, weighed and dried at 60°C for 6 hr. The dry weight of granuloma tissue was calculated after deducting cotton pellet weight and taken as a measure of granuloma tissue formation. The cotton pellet granuloma tissue was subjected to microscopic examination. The blood was collected from the retro-orbital sinus under anesthetic conditions. The serum levels of SGOT, SGPT and ALP were estimated. After blood collection, rats were sacrificed to isolate the liver and 20% w/v liver homogenate was prepared in Tris-HCl buffer (0.02 mol/L, pH 7.4). The liver homogenate was used for the estimation of lipid peroxidation activity.

Formalin-induced Edema in Rats

Wistar albino rats were divided into four groups of six rats each. Group I served as edema control and received 0.5% w/v CMC solution (10 ml/kg b.w, p.o). Group II was the standard drug treatment group and received diclofenac sodium (15 mg/kg b.w, p.o). Group III and IV were RD.M.ph.AA-01 treated groups and received RD.M.ph.AA-01 at doses of 270 and 405 mg/kg respectively. The RD.M.ph.AA-01 was suspended in distilled water using 0.5% CMC solution given once by oral route.

After 30 min of treatment, all rats were injected with formalin (0.05 mL, 2.5% v/v) into the sub planter area of the right hind paw.[37] The paw size was measured at 0, 30, 60, 120, 180 and 240 min by using a caliper scale to determine the degree of inflammation. After the last measurement of paw size, blood was collected from the retro-orbital sinus under anesthetic conditions. The serum levels of SGOT, SGPT and ALP were estimated. Rats were sacrificed to isolate the liver and 20% w/v liver homogenate was prepared in Tris-HCl buffer (0.02 mol/L, pH 7.4). The liver homogenate was used for the estimation of lipid peroxidation activity.

Croton Oil Ear Edema in Mice

Mice were divided into five groups with six mice in each. Group I was edema control and received only the irritant solvent (Croton oil). Group II was the standard drug treatment group and received dexamethasone (0.08 mg/ear). Groups III, IV and V were RD.M.ph.AA-01 treated groups and received RD.M.ph.AA-01 at the doses of 1.5, 2.5 and 5 mg/ear respectively.

Croton oil solution (5%) was prepared using acetone. The dexamethasone and RD.M.ph.AA-01 were dissolved in this solution and 0.01 mL of RD.M.ph.AA-01 (1.5, 2.5 and 5 mg/ear) or dexamethasone (0.08 mg/ear) were applied on both sides of the right ear of mice. The left ear remains untreated (control) and receives only the Croton oil solution. Four hours after application, the animals were sacrificed and both ears were removed and weighed immediately.[38] The weight difference between the treated and untreated ears was recorded as a measure of the degree of inflammatory edema.

Statistical Analysis

Statistical analysis was performed by using a one-way Analysis of Variance (ANOVA) followed by Dunnett's test. $p<0.05$ was considered significant.

RESULTS

Administration of CFA in the paw of rats resulted in a significant ($p<0.01$) increase in paw size at the end of the experimental period (Table 2). This increase in paw size was significantly ($p<0.01$) decreased by treatment with all doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg). All doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) were found to be equipotent in this regard (Table 2). Induction of arthritis by CFA administration caused a significant alteration in blood parameters (Table 3). There was a significant ($p<0.05$) decrease in Hb, RBCs, lymphocytes and total protein content in arthritis-induced rats as compared to vehicle control rats. The ESR, WBCs and neutrophil count were significantly ($p<0.01$) increased in arthritic-control rats as compared to vehicle-control rats. These changes in blood contents of Hb, RBCs, lymphocytes, total protein, ESR, WBCs, neutrophil, SGPT, SGOT and ALP were significantly ($p<0.05$) prevented by treatment with all doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg). The higher dose of RD/M.ph.AA-01 (i.e. 405 mg/kg) and diclofenac (15 mg/kg) were found to be equipotent in preventing changes in blood contents of Hb, RBCs, lymphocytes,

Table 1: The composition of RD/M.ph.AA-01.

Common name	Botanical name	Each 500 mg contains
Triphala	*Three myrobalans*	75 mg
Guduchi	*Tinospora cordifolia*	50 mg
Rasna	*Alpinia galanga*	75 mg
Ashvagandha	*Withania somnifera*	75 mg
Shatavari	*Asperagus racemosus*	75 mg
Yashtimadhu	*Glycyrrhiza glabra*	75 mg
Pippali	*Piper longum*	50 mg
Guggulu	*Commiphora mukul*	05 mg
Haridra	*Curcuma longa*	20 mg

Table 2: Effect of RD/M.ph.AA-01 on CFA-induced arthritis in rats.

Groups	Paw size in millimeters				
	0th day	4th day	7th day	14th day	21st day
Vehicle control	0.03 ± 0.13	0.26 ± 0.09	0.05 ± 0.11	-0.03 ± 0.07	-0.06 ± 0.13
Arthritic control	2.12 ± 0.19 ^{a**}	3.59 ± 0.33^{a**}	3.33 ± 0.29^{a**}	3.21 ± 0.29^{a**}	3.23 ± 0.26^{a**}
Diclofenac sodium (15 mg/kg)	2.51 ± 0.12	2.52 ± 0.14^{b**}	2.47 ± 0.63^{b**}	1.71 ± 0.24^{b**}	1.17 ± 0.19^{b**}
RD/M.ph.AA-01 (270 mg/kg)	2.01 ± 0.13	3.23 ± 0.19^{b}	2.70 ± 0.14^{b}	2.31 ± 0.08^{b**}	2.13 ± 0.07^{b**}
RD/M.ph.AA-01 (405 mg/kg)	2.30 ± 0.48	2.69 ± 0.10^{b*}	2.33 ± 0.14^{b**}	1.94 ± 0.06^{b**}	1.36 ± 0.08^{b**}

**= $p < 0.01$ = very significant, *= $p < 0.05$ = significant, Values are expressed as mean ± SEM. [a]Comparisons are made with normal control; [b]Comparisons are made with arthritic control.

ESR, WBCs, neutrophil, SGPT, SGOT and ALP. The higher dose of RD/M.ph.AA-01 (i.e. 405 mg/kg) was found to be more potent than diclofenac (15 mg/kg) in increasing the blood content of total protein (Table 3). Arthritis induction caused a significant (p<0.01) increase in hydroxyproline and hexosamine content in edematous tissue of arthritic rats, which were significantly (p<0.05) reversed by all doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg). A higher dose of RD/M.ph.AA-01 (i.e. 405 mg/kg) and diclofenac (15 mg/kg) were found to be equipotent in this regard (Table 3). Induction of arthritis by CFA administration caused a significant (p<0.01) increase in lipid peroxidation of liver tissue. All doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) prevented this change and restored lipid peroxidation of liver tissue near to vehicle value (Table 3). Examination of the stomach for ulceration showed the presence of ulcer lesions only in diclofenac-treated rats (Table 3). Histopathology of the knee joint of arthritic-induced rats showed hyperplasia of synovial cells with trapped adjuvant oil drops, pannus formation, synovial proliferation and infiltration of inflammatory cells with neutrophils (Figure 1). Treatment with all doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) resulted in the minimum synovial proliferation and reduced inflammatory reaction in the joint capsule. The higher dose of RD/M.ph.AA-01 (i.e. 405 mg/kg) showed an almost normal synovial membrane with no pannus formation (Figure 1).

In the cotton pellet granuloma model, treatment with all doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) caused a significant (p<0.01) decrease in the weight of granuloma tissue and serum levels of SGPT, SGOT and ALP levels (Table 4). Lipid peroxidation of liver tissue is also decreased significantly (p<0.01) by treatment with all doses of RD/M.

Table 3: Effect of RD/M.ph.AA-01 on biochemical parameters in CFA induced arthritic rats.

Parameters	Vehicle control	Arthritic control	Diclofenac sodium (15 mg/kg)	RD/M.ph.AA-01 (270 mg/kg)	RD/M.ph.AA-01 (405 mg/kg)
Hb (g/dl)	16 ± 0.51	12.43 ± 0.98^{a**}	16.33 ± 0.55^{b**}	14.66 ± 0.81^{b}	16 ± 0.44^{b**}
RBCs (million/cumm)	5.21 ± 0.15	4.13 ± 0.31^{a**}	5.26 ± 0.14^{b**}	4.63 ± 0.13^{b}	5.16 ± 0.06^{b**}
Lymphocyte (%)	66.66 ± 0.76	34.33 ± 1.43^{a**}	62.66 ± 1.28^{b**}	51.55 ± 1.14^{b**}	65.66 ± 1.28^{b**}
Total Protein (g/dl)	6.58 ± 0.14	4.88 ± 0.21 ^{a*}	5.58 ± 0.15 ^{b*}	6.60 ± 0.19 ^{b**}	6.35 ± 0.14 ^{b**}
ESR (mm)(after 1 hr)	1.91 ± 0.15^{a**}	6.43 ±0.18	2.33 ± 0.21^{b**}	3.83 ± 0.30^{b**}	2.5 ± 0.22^{b**}
WBC (cells/cumm)	5383 ± 283	8466 ± 545^{a**}	4133 ± 507^{b**}	6333 ± 614^{b**}	5533 ± 190^{b**}
Neutrophil (%)	32.66 ± 1.38	63.66 ± 4.83^{a**}	38.83 ± 2.52^{b**}	48.16 ± 3.53^{b**}	34.33 ± 1.28^{b**}
SGPT (IU/L)	91.5 ± 8.78	198 ± 8.98^{a**}	107 ± 9.54 ^{b**}	128.17 ± 4.13^{b**}	96.33 ± 9.88 ^{b**}
SGOT (IU/L)	132 ± 9.21	202 ± 13.16 ^{a**}	150± 6.68 ^{b**}	162 ± 8.53 ^{b*}	134 ±12.0 ^{b**}
ALP (IU/L)	182 ± 8.45	315.6 ± 30.57 ^{a**}	209.6 ± 32.79 ^{b**}	217 ± 11.41 ^{b*}	183.66 ± 7.94 ^{b**}
Hydroxyproline (µg/g)	336.66 ± 20.03	449 ± 12.26 ^{a**}	319.77 ± 11.69 ^{b**}	393.88 ± 7.52 ^{b*}	335.88 ± 17.21 ^{b**}
Hexosamine (µg/g)	800 ± 23.52	1454.16 ± 36.45 ^{a**}	815.83 ± 12.27 ^{b**}	1020.83 ± 30.53 ^{b**}	841.66 ± 13.52 ^{b**}
Liver Lipid Peroxidation (%)	51.33 ± 2.54	100± 2.36 ^{a**}	62.0 ± 1.86 ^{b**}	51.33 ± 0.84 ^{b**}	53.50 ± 2.54 ^{b**}
Ulcer count (no.)	0.00 ± 0.00	0.00 ± 0.00	7.16 ± 0.30**	0.00 ± 0.00	0.00 ± 0.00

**= p < 0.01 = very significant, *= p < 0.05 = significant, Values are expressed as mean ± SEM. [a] Comparisons are made with normal control; [b] Comparisons are made with arthritic control.

Table 4: Effect of RD/M.ph.AA-01 on cotton pellet granuloma in rats.

Parameters	Granuloma Control	Diclofenac sodium (15 mg/kg)	RD/M.ph.AA-01 (270 mg/kg)	RD/M.ph.AA-01 (405 mg/kg)
Weight (mg) of granulama tissue	147.33 ± 7.24	91.00 ± 3.75**	122.85 ± 3.44**	98.66 ± 3.33**
SGPT (IU/L)	179.33 ± 2.61	94.83 ± 1.74**	150.50 ± 3.37**	101.00 ± 1.03**
SGOT (IU/L)	201.83 ± 4.12	126.50 ± 3.18**	159.15 ± 2.09**	133.16 ± 5.57**
ALP (IU/L)	495.16 ± 14.27	202.83 ± 5.35**	224.33 ± 7.12**	203.33 ± 13.64**
Liver Lipid Peroxidation (%)	100 ± 3.25	65.5 ± 2.93**	74.83 5 ± 3.81**	61.5 ± 1.66**

**= $p < 0.01$ = very significant, *= $p < 0.05$ = significant, Values are expressed as mean ± SEM.

Table 5: Effect of RD/M.ph.AA-01 on formalin induced paw edema in rats.

Groups	Paw size in millimeters					
	0 min	30 min	60 min	120 min	180 min	240 min
Control	2.60 ± 0.20	2.20 ± 0.13	2.22 ± 0.36	2.48 ± 0.03	2.37 ± 0.05	2.20 ± 0.15
Diclofenac sodium (15 mg/kg)	2.01 ± 0.05	1.61 ± 0.33	1.31 ± 0.03*	0.98 ± 0.14**	1.24 ± 0.13**	1.40 ± 0.13**
RD/M.ph.AA-01 (270 mg/kg)	2.24 ± 0.05	2.13 ± 0.13	2.12 ± 0.06	1.74 ± 0.28	1.49 ± 0.17**	1.64 ± 0.11*
RD/M.ph.AA-01 (405 mg/kg)	1.93 ± 0.04	1.56 ± 0.03	1.25 ± 0.09*	0.96 ± 0.24**	1.11 ± 0.01**	1.23 ± 0.11**

**= $p < 0.01$ = very significant, *= $p < 0.05$ = significant, Values are expressed as mean ± SEM.

Table 6: Effect of RD/M.ph.AA-01 on SERUM SGPT, SGOT, ALP level and lipid peroxidation in liver of formalin-induced paw edema in rats.

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Lipid Peroxidation (%)
Control	169.16 ± 2.86	196.6 ± 3.58	449.16 ± 10.66	100 ± 3.62
Diclofenac sodium (15 mg/kg)	113.50 ± 3.62**	124.66 ± 2.39**	262.50 ± 5.96**	70.16 ± 3.30**
RD/M.ph.AA-01 (270 mg/kg)	150.66 ± 2.33**	152.33 ± 3.63**	328.16 ± 14.30**	84.50 ± 1.43**
RD/M.ph.AA-01 (405 mg/kg)	117.00 ± 1.94**	121.50 ± 1.28**	254.50 ± 5.96**	66.52 ± 2.34**

**= $p < 0.01$ = very significant, *= $p < 0.05$ = significant, Values are expressed as mean ± SEM.

ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg). All doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) were found to be equipotent in decreasing serum levels of SGPT, SGOT and ALP, and lipid peroxidation of liver tissues (Table 4). Microscopy of cotton pellet granulation tissue revealed densely packed active neovascularization along with the presence of inflammatory cells (Figure 2). However, granulation tissue of rats treated with all doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) showed thick fibrous connective tissue with neovascularization and minimum inflammatory infiltration (Figure 2).

Formalin injection into the paws of rats produced paw edema during the experimental period (Table 5). The paw size was significantly ($p<0.05$) reduced by treatment with all doses of (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg). A higher dose of RD/M.ph.AA-01 (i.e. 405 mg/kg) and diclofenac (15 mg/kg) were found to be equipotent in this regard (Table 5). There was a significant ($p<0.01$) decrease in serum levels of SGPT, SGOT and ALP by treatment with all doses of (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) as compared to control rats (Table 6). Lipid peroxidation of liver tissue is also normalized significantly ($p<0.01$) by treatment with all doses of (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg). All doses of RD/M.ph.AA-01 (i.e. 270 and 405 mg/kg) and diclofenac (15 mg/kg) were found to be equipotent in decreasing serum levels of SGPT, SGOT and ALP, and lipid peroxidation of liver tissues (Table 6).

Table 7: Effect of RD/M.ph.AA-01 on croton oil ear edema in mice.

Groups	Ear Weight (mg)	% inhibition
Control	33.00 ± 4.18	-
Dexamethasone (0.08 mg/ear)	10.33 ± 2.06**	68.69
RD/M.ph.AA-01 (1.25 mg/ear)	29.16 ± 1.51	11.63
RD/M.ph.AA-01 (2.5 mg/ear)	29.21 ± 3.26	11.48
RD/M.ph.AA-01 (5.0 mg/ear)	19.50 ± 1.08**	40.90

**= $p < 0.01$ = very significant, Values are expressed as mean ± SEM.

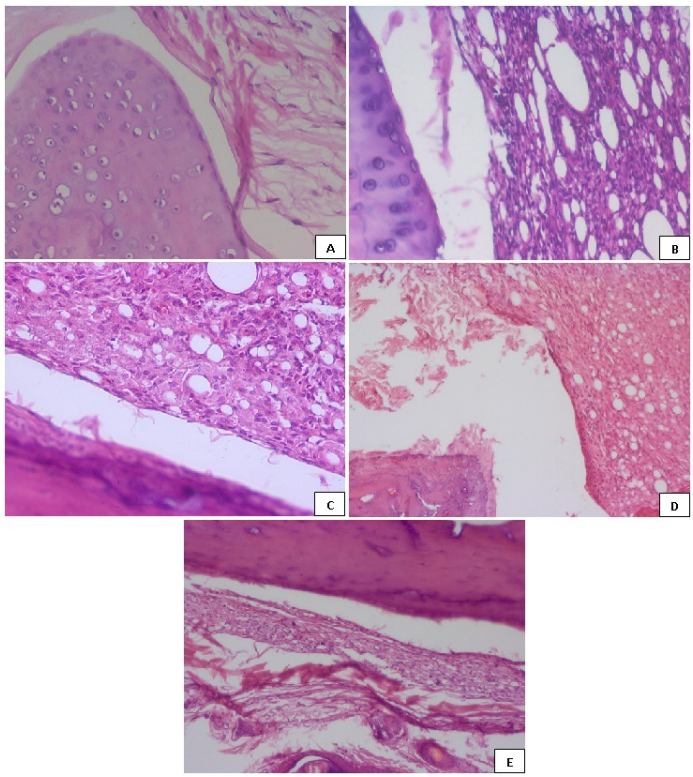

Figure 1: Pictogram of the knee joint of (A) normal control; (B) arthritic control; (C) diclofenac treated; (D and E) RD/M.ph.AA-01 treated at the dose of 270 mg/kg and 405 mg/kg body weight respectively. (H & E × 100).

In the Croton oil-induced mouse model of ear edema, topical application of a higher dose of RD/M.Ph.AA-01 (i.e. 5 mg/ear) and dexamethasone (0.08 mg/ear) were found to cause a significant (p<0.01) decrease in ear weight as compared to control rats. There was 40.90 and 68.69% inhibition of ear weight by topical application of a higher dose of RD/M.Ph.AA-01 (i.e. 5 mg/ear) and dexamethasone (0.08 mg/ear) respectively (Table 7).

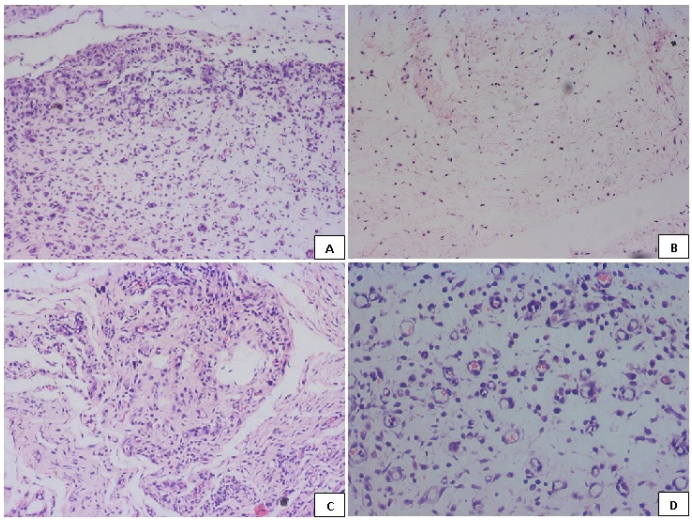

Figure 2: Pictogram of granulation tissue of (A) granuloma-induced control; (B) diclofenac treated; (C and D) RD/M.ph.AA-01 treated at the dose of 270 mg/kg and 405 mg/kg body weight respectively. (H & E × 100).

DISCUSSION

The CFA-induced RA model mimics human RA in pathological and serological changes as well as the involvement of inflammatory mediators.[36] The CFA injection caused the injected ankle of all the rats to enlarge and appear red, which is consistent with previous reports.[36] There was a significant reduction in paw edema when RD/M.ph.AA-01 treated rats were compared to arthritic control rats.

A previous report has demonstrated that CFA induction significantly lowers hemoglobin, lymphocytes and RBC counts.[36] Rats are anemic because their hemoglobin and red cell levels are low. Anemia is the most prevalent extracellular index in RA patients. Moderate hypochromic normocytic anemia is revealed by a drop in RBC count. The main cause could be a decrease in plasma iron levels, as well as iron impoundment in the reticuloendothelial system and synovial tissue, which leads to bone marrow failure. This decreased blood levels of hemoglobin, lymphocytes and RBC counts were significantly increased by treatment with RD/M.ph.AA-01.

The blood levels of WBCs, neutrophils, and ESR were significantly increased in arthritis-induced rats which is consistent with the previous report.[36] The increase in total WBC count in the arthritic group could be due to immune system activation in response to invading pathogenic microorganisms. As a result, it is obvious that inflammatory mononuclear cells have infiltrated the joints of arthritic control. ESR is an indirect indication of acute stage response of RA. During inflammatory conditions, neutrophils activate and produce mediators including platelet-activating factor and lysozyme, producing inflammatory disease. ESR can affect a variety of factors, including blood fibrinogen concentration, immunoglobulins, and hemoglobin. A higher ESR level in arthritic rats shows the chronicity and severity of the

disease.[36] The RD/M.ph.AA-01 treatment brought the changes in WBCs, neutrophil count and ESR level to the normal.

Serum levels of SGPT, SGOT and ALP were a method for determining the anti-arthritic potential of the test medication. Previous studies indicate that serum levels of aminotransferases and ALP are significantly elevated in rats with arthritic conditions, indicating that these are good indicators of liver and kidney dysfunction and are measured as characteristics of adjuvant arthritis.[36] The production of physiologically active chemical mediators such as bradykinins by serum SGOT and SGPT is critical in the inflammatory process. The liver and bone fraction isoenzymes were raised in arthritic rats, which resulted in an increase in ALP levels. The RD/M.ph.AA-01 treatment reduced the rate of increase in serum SGPT, SGOT, and ALP levels. These findings demonstrate the protective role of RD/M.ph.AA-01 in reducing bone loss and organ protection in arthritis rats.

Arthritis induction by CFA caused a reduction in serum total protein levels in rats. The treatment with RD/M.ph.AA-01 restored the elevated serum total protein level to normal. The metabolism of connective tissue is altered by significant biochemical variables during the inflammatory process. These changes result in changes in the virtual composition of connective tissue elements such as mucopolysaccharides, glycoproteins, hexosamine, and sialic acid.[39] Increased level of hydroxyproline and hexosamine in edematous tissue of CFA-induced arthritic rats was decreased by RD/M.ph.AA-01 treatment.

Gastric lesions are a common side effect of nonsteroidal anti-inflammatory medicines.[40,41] This is related to the suppression of the cyclo-oxygenase enzyme, which produces prostaglandins. In our investigation, RD/M.ph.AA-01 had no negative effects on the stomach mucosa, showing that it has anti-ulcerogenic properties. However, rats given diclofenac sodium had minimal erosions.

Microscopic sections of ankle joints of arthritis-induced rats revealed severe histopathological alterations such as inflammatory cell infiltration and synovial membrane hyperplasia. The rats treated with RD/M.ph.AA-01 showed minor histological damage when compared to the untreated arthritic rats.

Cotton pellet granuloma is a model of transudative and proliferative components of chronic inflammation. The transudate is proportional to the moist weight of the cotton pellet. The amount of granulomatous tissue is proportional to the dry weight of the pellet.[36] The RD/M.ph.AA-01 and diclofenac were found to decrease the weight of cotton pellets. This evidence supports that the RD/M.ph.AA-01 has a stronger effect on inflammatory mediators in the progression of inflammation in rats. This impact could be caused by cellular migration to wounded areas, as well as collagen and mucopolysaccharide buildup. Its effect might also be due to the stabilization of the lysosomal membrane system as the RD/M.ph.AA-01 normalized the elevated serum levels of SGPT, SGOT, and ALP in cotton pellet-induced granuloma rats. Lipid peroxidation associated with inflammatory conditions was effectively inhibited by RD/M.ph.AA-01 in both CFA-induced arthritic as well as cotton pellet modes.

Formalin-induced pedal edema in rats is one of the most suitable assays to investigate anti-proliferative, anti-arthritic and anti-inflammatory efficacy as it closely resembles human arthritis. Formalin-induced edema resulted in localized inflammation and pain. The nociceptive effect is biphasic, with a first neurogenic component followed by a tissue-mediated reaction.[42] The RD/M.ph.AA-01 caused significant inhibition of formalin-induced edema.

Croton oil ear edema is a suitable method for screening anti-inflammatory steroids. Non-steroidal anti-inflammatory drugs are less susceptible to Croton oil.[42] The RD/M.ph.AA-01 caused significant inhibition of Croton oil ear edema. This demonstrates that the anti-inflammatory action of RD/M.ph.AA-01 may be due to the presence of steroids.

Preliminary phytochemical analysis indicated the presence of steroids, flavonoids and tannins in the RD/M.ph.AA-01. Flavonoids are well known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception. Thus, the flavonoids may be responsible for the anti-arthritic and anti-inflammatory effects of RD/M.ph.AA-01.

CONCLUSION

It is concluded that the RD/M.ph.AA-01, an Ayurvedic polyherbal formulation, has significant anti-arthritic and anti-inflammatory activity. The presence of steroids, flavonoids and tannins is responsible for the effects. Further studies are needed for a better understanding of the mechanism of actions underlying the anti-arthritic and anti-inflammatory effects of RD/M.ph.AA-01.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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