IJPSR (2024), Volume 15, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 23 February 2024; received in revised form, 26 March 2024; accepted, 24 April 2024; published 01 August 2024

IN-VIVO ANTI-ARTHRITIC EFFECT OF COMPARATIVE STUDY OF POLYHERBAL FORMULATIONS (MUNIPAYN, RUMALAYA FORTE) IN FCA INDUCED ARTHRITIC RATS

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Keywords:

Rumalaya forte, Munipayn, Freund's complete adjuvant

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ABSTRACT: Rheumatoid arthritis (RA) is one of the most prevalent chronic inflammatory joint diseases which affect approximately 1% adult population in the worldwide. Polyherbal ayurvedic formulations (Munipayn and Rumalaya forte) reported to be effective as anti-inflammatory and anti arthritic activity. *In-vitro* anti-inflammatory of individual formulations (Munipayn and Rumalaya forte) were evaluated by albumin denaturation and Red blood cell membrane stabilization assay methods. *In-vivo* anti-arthritic activity of Munipayn (25 mg/kg and Rumalaya forte 40 mg/kg, bw, po) were evaluated against Frund's complete adjuvant induced arthritis in rats. After the injection of FCA, the polyherbal formulations, standard was administered for 28 days. Both the formulations and standard showed a significant ($P<0.001$) reduction in paw volume, paw thickness, increase in WBC when compared to FCA treated rats. Histological reports also suggested that histological changes reversed in both formulations treated rats. Present study resulted that Munipayn and Rumalaya forte has significant anti-inflammatory, anti-arthritic activity against FCA-induced arthritis rats.

INTRODUCTION: Rheumatoid arthritis (RA) is a systemic autoimmune chronic inflammatory disease and characterized by hyperplasia of synovial membrane, infiltration of inflammatory cells, neo-vascularization, cartilage erosion and joint destruction [1, 2]. It mainly affects joints and multiple joints at the same time [3, 4]. RA may proceed to severe impairment, with direct negative effects on lifestyle and a rise in mortality rate [5, 6]. The prevalence of RA is about 0.5–1.0% of the general population [7].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(8).2346-52

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(8).2346-52

Persons of all ages are susceptible to developing RA, although the prevalence has increased significantly in people over the age of 40 years, particularly women, who are two to three times more susceptible than men [3, 6].

The imbalance between pro-inflammatory and endogenous anti-inflammatory mediators causes synovial membrane inflammation and joint destruction in RA. Interleukins (IL-1, IL-1, IL-6, IL-18, and IL-20), tumor necrosis factor (TNF)-, C reactive protein (CRP), monocyte chemo attractant protein-1 (MCP-1), receptor activator of nuclear factor-kB ligand (RANKL) fractalkine, matrix metalloproteinase-9 (MMP-9) and adhesion molecules all contribute to the development of RA, joint and cartilage destruction [3, 4]. Inflammation in joints is by complex interplay between different dendritic cell (DC) subtypes, T cells, B cells,

macrophages, neutrophils, fibroblasts and osteoclasts [3]. Presently rheumatoid arthritis is treated with a wide variety of medicines such as disease modifying anti-rheumatoid drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids help in reducing inflammation of the joint(s) and decreasing pain but they are accompanied by serious adverse effects [7, 8]. So, there is a dire need to find safe drug for RA with minimal adverse effects that can be used for long term treatment.

The medicinal plant-based drugs are favored over conventional medicines by patients because of decreased mobility, fear of surgery, ever-growing medicinal cost and adverse effects of novel drugs. Herbal remedies diminish the manifestations of illness and increase the quality of life. Therefore, there is a tremendous increase in the use of plant-based health products in developing and developed countries.

Ayurveda treasure house has a number of remedies, which can be beneficial in conditions of arthritis. We have selected two polyherbal formulations Munipayn and Rumalaya forte. Munipayntablet; is the solution for pain, inflammation anywhere in the body it is a proprietary Ayurveda medicine manufactured in muniyal ayurveda pharmaceuticals [9]. Rumalaya Forte tablet; is a fully natural and herbal ingestible tablet that helps the body in a variety of ways. This revolutionary all-natural tablet has both anti-inflammatory properties as well as can double down as a painkiller. The Rumalaya has an immunomodulator ingredient that helps to modulate the humoral and cell-mediated response of the natural immune system of your body to pain. It also helps in the treatment of cartilage damage [10]. Hence the present study was taken to evaluate the in-vitro anti-inflammatory and *in-vivo* Antiarthritic efficacy of polyherbal formulations (Munipayn and Rumalaya forte). Therefore, the present study is undertaken to compare the anti-arthritic efficacy of formulations (Munipayn, Rumalaya Forte) in FCA induced arthritic rats.

MATERIAL AND METHODS:

Drugs and Chemicals: Munipayn and Rumalaya Forte were purchased from Pharmacy in Bangalore. Freund's complete adjuvant was purchased from Sigma Aldrich. Diagnostic assay kits such as alkaline phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and C-reactive protein (CRP) were purchased from Unitron Bio Medicals Bangalore. The other chemicals used in the study protocol were of analytical grade.

Experimental Animals: Male Wistar albino rats (150–200g) were procured from Sri Raghavendra enterprises, (841/PO/Bt/S/04/CPCSEA) were used in the study. The study has been approved by Institutional Animal Ethical Committee, Nargund College of Pharmacy (IEAC/NCP/116/2021). Animals were housed in polypropylene cages with paddy husk as a bedding material, which was changed thrice a week. They were maintained under standard environmental conditions (temperature 25±2°C; relative humidity 60±5% and 12 h light/dark cycles;) and were fed with standard pellet diet and distilled water *ad libitum*. The procedures performed in the study were in accordance to Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines. The biomedical disposal was sent to Maridi Bio industries Pvt. Ltd, Bangalore.

Experimental Design: Based on the calculation of Human dose animal dose calculation, the dose for the study was selected for the present study was Munipayn (40 mg/kg, bw, po) and Rumalaya Forte (25 mg/kg, bw, po).

30 rats were divided into five groups and each group contains six animals. Indomethacin (3 mg/kg, bw, po) was used as standard [11]. Both the standard and formulation suspensions were prepared with suspending agent (1% Sodium carboxy methyl cellulose) and used immediately.

Experimental Groupings:

Group I: Normal control (Rats received standard feed and water *ad libitum).*

Group II: Arthritic control (0.1 ml of FCA injected to intraplantar region of left paw of rats).

Group III: Standard control (Rats received Indomethacin (3 mg/kg, bw, po) for 28 days from the day of induction of arthritis.

Group IV: Rumalaya Forte (received 25 mg/kg, bw, po) for 28 days from the day of induction of arthritis.

Group V: Munipayn (revived40 mg/kg, bw, po) for 28 days from the day of induction of arthritis.

As mentioned above, the treatments were given for 28 days. The body weight, paw volume and paw thickness of experimental rats were measured on 0, 7, 14, 21 and 28thday. The mean change in paw volume and pw thickness with respect to initial paw volume and paw thickness was calculated on respective days and the percentage inhibition of paw edema was calculated by formula-

% Inhibition = Control (Vc) – Test (Vt)" / Control (Vc) × 100

At the end of the experiment, the blood was collected through retro-orbital plexus from all the experimental rats by anaesthetizing. Later, they were euthanized with overdose of pentobarbitone sodium (150 mg/kg, ip) and the proximal interphalangeal joints from all the experimental animals were isolated, fixed in 10% formalin solution for histopathological studies [12].

Hematological Parameters: The hematological parameters such as WBC counts, and erythrocyte sedimentation were estimated manually using fresh rat blood.

Biochemical Parameters: The serum was isolated by centrifugation of blood at 3000 rpm for 20 min [12]. Liver markers such as alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT) and C-reactive protein (CRP) were measured using standard kits by Robonik Pritest Bio autoanalyzer.

Statistical Analysis: The values were expressed as mean ± standard error of the mean. Statistical difference between normal to control and control to drug treatments were analyzed by One-way analysis of variance and Student's t-test was used to assess differences between multiple group**s** by using Graph pad prism. The value of P < 0.05, 0.01, 0.001 and 0.0001 was considered statistically significant.

RESULTS:

Body Weight: Polyherbal formulations treated groups showed significant increase in body weight when compared to arthritic control. Standard and arthritic control rats did not show significant change in body weight.

TABLE 1: EFFECT OF FORMULATIONS (RUMALAYA FORTE AND MUNIPAYN) ON BODY WEIGHT OF FCA INDUCED ARTHRITIC RATS

Animal group	Body weight in Mean ±SEM				
	0 Day	7th Day	14th Day	21st Day	28th Day
Group I	160.4±9.14	182±6.37	203.5±3.97	218.2±3.72	226±5.06
Group II	167.2±3.06^{ns}	179.2±4.55^{ns}	210.3±5.10 ^{ns}	218.7±6.11 ^{ns}	228.5±7.60 ^{ns}
Group III	161.2±2.66 ^{ns}	182.7±2.39 ^{ns}	208.8±2.50 ^{ns}	216.2±3.62 ^{ns}	225.8±4.66 ^{ns}
Group IV	178.8±4.779	194.2±4.84***	224.3±4.32^{ns}	236±5.40^{ns}	252.3±6.04*
Group V	183±4.63	197.3±6.51***	231.5±6.970*	238±5.70*	259.5±6.33*

Group I: Normal control; Group II: Arthritic control; Group III: Standard treatment; Group IV: Rumalaya Forte treatment; Group V: Munipayn treatment. Values were expressed as Mean±SEM (n=6). *represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001); ns is non-significant.

Paw Volume: Polyherbal formulations Rumalaya Forte and Munipayn treatment showed significant (p<0.001& p<0.01) reduction in paw volume and 37% & 30% inhibition in paw volume respectively when compared to arthritic control. Standard treatment showed significant (p <0.001) reduction in paw volume and 43.3 % inhibition in paw volume when compared to arthritic control. The results were shown **Table 2.**

TABLE 2: EFFECT OF FORMULATIONS (RUMALAYA FORTE AND MUNIPAYN) ON PAW VOLUME OF FCA INDUCED ARTHRITIC RATS

Animal group	Paw volume in Mean ±SEM				
	0 Day	7th Day	14th Day	21st Day	28th Day
Group I	0.65±0.03	0.69±0.07	0.71±0.07	0.74±0.06	0.77±0.05
Group II	0.78±0.06^{ns}	1.11±0.03^{***a}	1.22±0.05^{***a}	1.39±0.02^{****a}	1.71±0.12^{****a}

Group III	0.71±0.0^{ns}	0.92±0.04^{*b} (17.3%)	0.97±0.06^{**b} (20.4%)	0.94±0.05^{****b} (32.3%)	0.9±0.04^{****b} (47.3%)
Group IV	0.68±0.03 (ns)	1.03±0.01** 7%	1.08±0.03* 12%	1.07±0.04*** 23%	1.07±0.03*** 37%
Group V	0.74±0.05 (Ns)	1.05±0.02* 5%	1.11±0.01* 10%	1.05±0.06*** 24%	1.18±0.05*** 30%

Group I: Normal control; Group II: Arthritic control; Group III: Standard treatment; Group IV Rumalaya Forte treatment; Group V: Munipayn treatment. Values are expressed as Mean±SEM (n=6). * represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001). a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

Paw Thickness: Polyherbal formulations Rumalaya Forte and Munipayn showed significant (p<0.001 & p<0.001) reduction in paw thickness and 24% & 25% inhibition in paw thickness respectively when compared to arthritic control. Standard treated group showed significant (p <0.001) reduction in paw thickness and 32% inhibition in paw thickness when compared to the arthritic control. The results were shown in **Table 3.**

TABLE 3: EFFECT OF FORMULATIONS (RUMALAYA FORTE AND MUNIPAYN) ON PAW THICKNESS OF FCA INDUCED ARTHRITIC RATS

Animal group	Paw thickness in Mean ±SEM				
	0 Day	7th Day	14th Day	21st Day	28th Day
Group I	2.88±0.08	3.09±0.19	4.12±0.17	4.13±0.15	4.20±0.14
Group II	3.38±0.33^{ns}	4.20±0.38a^{*a}	5.42±0.17^{***a}	5.59±0.14^{****a}	5.75±0.09^{****a}
Group III	2.8±0.14^{ns}	3.95±0.16^{ns} (5.95%)	5.05±0.18^{ns} (6.78%)	4.57±0.18^{**b} (18.24%)	4.08±0.16^{****b} (29.04%)
Group IV	2.9±0.28^{ns}	3.99±0.16^{ns} (10%)	5.08±0.03* (6%)	4.66±0.10^{***b} (17%)	4.35±0.01^{***b} (24%)
Group V	2.9±0.28^{ns}	3.99±0.16^{ns} (10%)	5.08±0.03* (6%)	4.66±0.10^{***b} (17%)	4.35±0.01^{***b} (24%)

Group I: Normal control; Group II: Arthritic control; Group III: Standard treatment; Group IV: Rumalaya Forte treatment; Group V: Munipayn treatment. Values are expressed as Mean±SEM (n=6). *represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001). a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

Hematological Parameters: Polyherbal formulations Rumalaya Forte and munipayntreated groups showed significant (p<0.01) increase in WBC count when compared to arthritic control. Standard (indomethacin) treated group showed significant (p<0.01) increase in WBC count when compared to arthritic control group. The results were shown in **Table 4**.

TABLE 4: EFFECT OF FORMULATIONS (RUMALAYA FORTE AND MUNIPAYN) ON HEMATOLOGICAL PARAMETERS OF FCA INDUCED ARTHRITIC RATS

Animal groups	WBC in (thousand cells/mm$^{3)}$	ESR (mm/hr)
Group I	7.57±0.32	0.33±0.21
Group II	11.41±0.28^{***a}	7.1±0.6^{***a}
Group III	8.7±0.34^{***b}	0.50±0.22^{***b}
Group IV	9.095±0.301^{***b}	0.67±0.21^{***b}
Group V	9.340±0.199^{***b}	0.75±0.41^{***b}

WBC: White blood cell; ESR: Erythrocyte sedimentation rate: Group I: Normal control; Group II: Arthritic control; Group III: Standard treatment; Group IV:Rumalaya Forte treatment; Group V: Munipayn treatment ;Values are expressed as Mean±SEM (n=6). * represent statistical significance (*p<0.05, **P<0.01, ***P<0.001,****p<0.0001).a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

Biochemical Parameters: Polyherbal formulations -Rumalaya Forte and Munipayntreated groups showed significant (P<0.001andp<0.05) reversal of ALP, AST, ALT and CRP levels respectively. Standard treatment showed significant(P<0.001) reduction in elevated serum ALP, ALT, AST and CRP levels as compared to arthritic control rats. The results were shown in **Table 5.**

TABLE 5: EFFECT OF FORMULATIONS (RUMALAYA FORTE AND MUNIPAYN) ON BIOCHEMICAL PARAMETERSOF FCA INDUCED ARTHRITIC RATS

Animal groups	ALP (U/L)	AST (U/L)	ALT (U/L)	CRP (mg/dl)
Group I	121.7±9.53	127.4±5.72	54.89±4.03	0.059±0.01
Group II	280.9±5.70^{****a}	221.8±8.73^{***a}	74.32±4.76^{****a}	0.308±0.02^{****a}
Group III	143.6±7.94^{****b}	140.1±7.59^{***b}	60.88±9.33^{****b}	0.159±0.01^{**b}
Group IV	214±10.65^{***b}	133.6±9.98***	57.30±4.3^{*b}	0.169±0.03^{*b}
Group V	210.±11.9^{***b}	177.7±5.45**	61.05±3.67^{nsb}	0.174±0.02^{**b}

ALP: Alkaline phosphatase; AST: Aspartate amino transferase; AST: Aspartate amino transferase; CRP: C-reactive protein. Group I: Normal control; Group II: Arthritic control; Group III: Standard treatment; Group IV: Rumalaya Forte treatment ; Group V Munipayn treatment; Values are expressed as Mean±SEM (n=6). *represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001). a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

Histopathological Study: The histopathological results of interphallangeal joints were carried out for all the experimental rats. Normal control specimen showed intact joint space with intact synovial tissue and synovial tissue is lined by intact synovial lining with underlying fibro connective tissue. Arthritic control group showed significantly eroded synovial surface, cartilage and bone. Joint space is significantly narrowed. Synovial showed formation of pannus with dense fibro collage nous tissue deposit and severe infiltration of chronic inflammatory cells composed of lymphocytes, histiocytes and plasma cells along with numerous tiny blood vessels lined by endothelial cells. Standard treatment, polyherbal formulation Rumalaya Forte showed reduction in synovial cartilage and bone erosion, proliferation of collage nous tissue and increase in joint space. It also showed reduced infiltration of chronic inflammatory cells composed lymphocytes, histiocytes and plasma cells. When compared to arthritic control group, Rumalaya Forte showed significant reversal of all the above-mentioned pathological conditions Munipayn shows moderate effect observed.

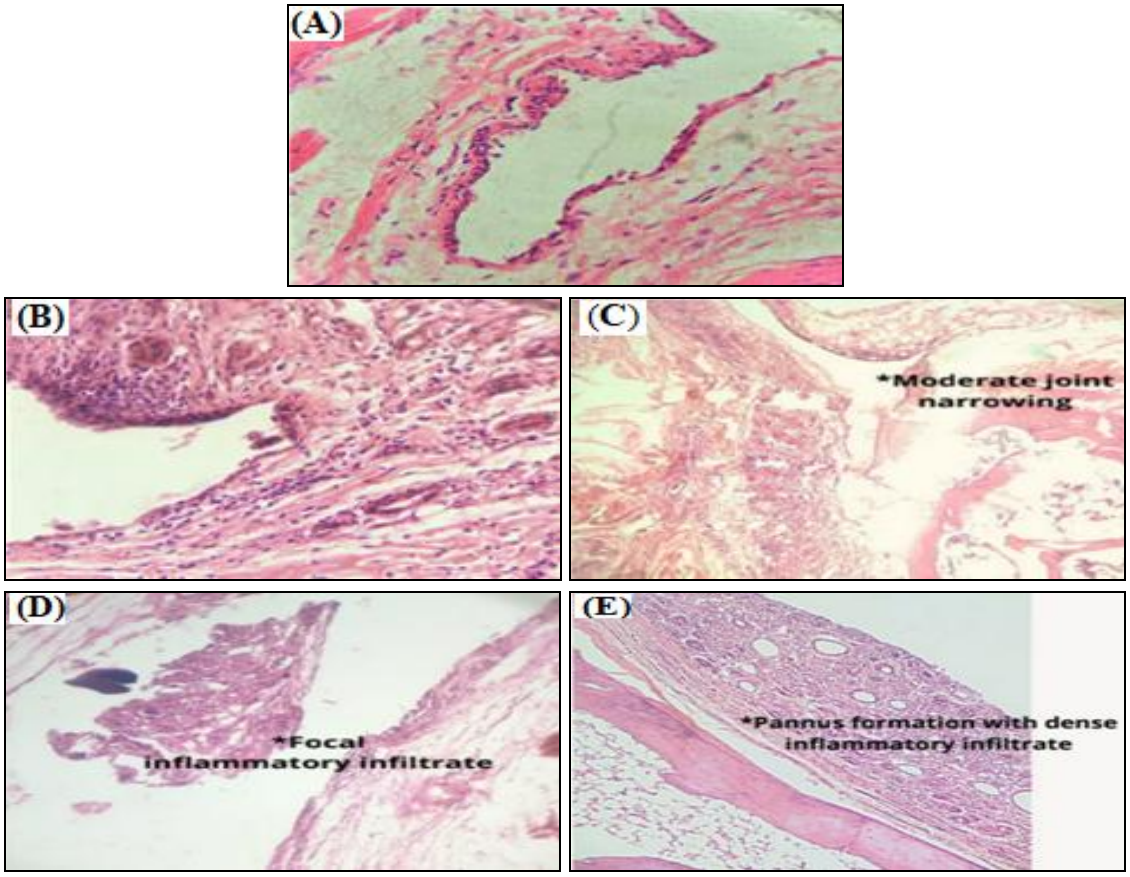

FIG. 1: HISTOPATHOLOGICAL RESULTS OF INTERPHALANGEAL JOINTS ON ADJUVANT-INDUCED ARTHRITIC RATS. a) Group-I (normal control); b) Group-II (Arthritic control); c) Group-III (standard); d)Group-IV(Rumalaya Forte); e) Group-V (Munipayn)

DISCUSSION: FCA-induced arthritis is one of the standard animal model of chronic polyarthritis, exhibiting characteristics similar to human RA and it involves cell mediated autoimmunity [13]. Therefore, Inhibition of FCA model is frequently used to evaluate anti-arthritic drugs. The bacterial peptidoglycan and muramyl dipeptide found in the FCA are responsible for the induction of adjuvant arthritis [12].

The anti-inflammatory effects on RA have been evaluated using changes in the rheumatoid indices, such as paw volume and paw thickness [14, 15]. From the results obtained, it was observed that indomethacin (3mg/kg bwp.o) and Rumalaya Forte, Munipayn (at dose 25mg/kg, and 40mg/kg respectively) treated group showed significant decrease in the paw volume and paw thickness when compared to arthritic control group. The antiarthritic effect of the Rumalaya Forte showed better effect when compared to the Munipayn.

Change in the body weight is also a parameter to assess the course of the disease and the response to therapy. Standard Indomethacin did not show significant change in body weight. But polyherbal formulation containing Rumalaya Forte and Munipayn treated groups show little significant change in body weight as compared to arthritic control. White blood cells are an important part of the body's immune system. Infections and inflammatory diseases are indications for a WBC count [12]. In the present study, arthritic control rats' WBC counts were found to be higher, which may be due to the stimulation of the immune system against the invading [1, 15], whereas the group treated Rumalaya Forte, Munipayn and Indomethacin showed significant decrease in WBC count when compared to arthritic control group.

A frequent diagnostic sign of individuals with chronic arthritis is an increase in erythrocyte sedimentation rate, which was seen in the present investigation in the arthritic control group rats. The ESR is correlated with the number and size of RBCs as well as with the relative plasma protein concentration, particularly with fibrinogen and β globulins. An increase in the ESR is asign of active but unidentified disease processes. The acute phase proteins in ESR cause inflammation comparable to that caused by an injection, injury, and surgery or tissue necrosis [12]. There was a decrease in ESR in groups treated with Rumalaya Forte, Munipayn when compared to arthritic control group which showed significant recovery from the arthritic progress. The assessment of the serum levels of ALP, ALT, AST and CRP is a tool to measure the antiarthritic activity of a particular drug.

AST and ALT play animportant role in the production of biologically active chemical mediators such as bradykinins in the inflammatory process. The elevated levels of ALP in the adjuvant-induced arthritic rats may be due to the increase in the liver and bone fraction. As a result, there is a localized loss of bone in the form of periarticular osteopenia and bone erosion. This is because the enzyme is released into circulation during the formation and resorption of bone [16]. CRP is a prototypic inflammatory biomarker of systemic inflammation that belongs to the class of acute phase proteins.

The level of CRP raises during the inflammatory process that occurs in the body and the increase in the level of CRP is due to the rise in the plasma concentration of IL-6, which is generated by the macrophages and the adipocytes [1]. In the present study, the arthritic control rats in the control group showed elevated levels of ALP, ALT, AST and CRP when compared to normal control group. There was a significant decrease in ALP level, AST level, ALT level and CRP levelin the group treated with Rumalaya Forte, Munipayn when compared to arthritic control group. In arthritis, Rumalaya Forte treated group showed moderate effect on ALP, AST, ALT and CRP level as compared to Munipayn. Histopathological study shows the differences in the normal ankle joint and adjuvant-induced arthritic rat joint [1].

In the present study arthritic control group showed significantly eroded synovial surface, cartilage and bone. Joint space is significantly narrowed. Synovial showed formation of pannus with dense fibro collagenous tissue deposit and severe infiltration of chronic inflammatory cells composed of lymphocytes, histiocytes and plasma cells along with numerous tiny blood vessels lined by endothelial cells. The group treated with indomethacin and Rumalaya Forte showed marked reduction in all pathological above-mentioned

conditions but in developed phase moderate effect is observed. Thus, this study suggested that the antiarthritic effect of formulation containing Rumalaya Forte, Munipaynon joints, bone, and cartilage in FCA -induced arthritic rats was probably mediated by anti-inflammatory action.

CONCLUSION: The current study has demonstrated that polyherbal formulations - Rumalaya Forte, Munipayn (at doses of 40 mg/kg,and 25mg/kg bw, po) has anti-inflammatory potential and exerts an antiarthritic activity by significantly reversing the pathogenesis during FCA-induced arthritis in male albino wistar rats. The antiarthritic potential of polyherbal formulations -Rumalaya Forte, Munipaynwas comparable with that of indomethacin, additionally formulation of Rumalaya Forte is more effective than Munipayn.

ACKNOWLEDGEMENT: I am very grateful to the Principal, Prof. Dr. Shachindra L Nargund and Dr. L V G Nargund, Founder of Nargund College Pharmacy, Bangalore, for providing me the their support and necessary infrastructure, atmosphere to carry out my research work. I thank the VGST (GRD-178) for supporting the present work.

CONFLICTS OF INTEREST: Nil

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How to cite this article:
Bhargav A, Shrikanta S, Nargund RR, Nargund SL, Nisarga HJ and Keshava H: *In-vivo* anti-arthritic effect of comparative study of polyherbal formulations (Munipayn, Rumalaya forte) in FCA induced arthritic rats. Int J Pharm Sci & Res 2024; 15(8): 2346-52. doi: 10.13040/IJPSR.0975-8232.15(8).2346-52.