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Original Article

ESTABLISHMENT OF A MECHANISM OF POLYHERBAL FORMULATION FOR ANTI-RHEUMATISM

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ABSTRACT

Objective: A marketed product Dr. Ortho has been used by many patients and is gaining a positive feedback from the patients. A study is designed to establish its mechanism of action on various anti-rheumatic models in experimental animals. No studies have been investigated on this product for its anti-rheumatic activity.

Methods: Dr. Ortho (55 mg/kg, p. o.) was administered during adjuvant-induced arthritis, phlogistic agents (Histamine, bradykinin, and serotonin) induced paw edema, Acetic acid induced writhing test in mice, and Eddy's hot plate. Histopathology (T. S. of knee joint) and various hematological parameters (WBC's, RBC's, and ESR count) were observed during the study.

Results: Dr. Ortho decreases arthritis induced inflammation by antagonizing or inhibiting the inflammatory mediators, i.e., histamine, 5-hydroxy tryptamine and bradykinin. (20% ($P<0.05$) of carrageenan, 10.01%, ($P<0.05$) of histamine, 8% ($P<0.05$) of 5-hydroxy tryptamine induced paw volume). Dr. Ortho acts as both peripheral and central analgesic. Histopathology findings revealed that there is a reduction in the neutrophil infiltration, pannus formation, and bone. Hematological parameters show that Dr. Ortho decreases the elevated level of the WBC's count.

Conclusion: The present study revealed some facts regarding the mechanism of Dr. Ortho in reducing arthritis induced inflammation. However study may be done further to illustrate its complete mechanism.

Keywords: Ortho, Adjuvant induced arthritis, Carrageenan, Histamine.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory disease affecting many tissues, but principally attacking the joints to produce a non-suppurative proliferative synovitis that frequently progresses to destroy articular cartilage and underlying bone with resulting disabling arthritis.

RA is a very common condition, with a prevalence of approximately 1%; it is three to five times more common in women than in men. The peak incidence is in the second to fourth decades of life, but no age is immune [1]. The disease affects systemically many extra-articular tissues, includes skin, blood vessels, heart, lungs and muscles [2]. The commonly used drugs such as nonsteroidal anti-inflammatory drugs (NSAIDS) and biologic (e. g., antitumor necrosis-α antibody) are effective in alleviating the symptoms of the disease. However, the prolonged use of these drugs is associated with severe adverse reactions, In addition, these drugs are expensive, and not all patients respond well to them. In view of these limitations, it is essential to continue the search for safer and less expensive alternatives to the conventionally used drugs In fact; herbal plants are being used widely as medicine around decades for the treatment of arthritic disease. Herbal medicine constitutes important resources for the treatment of various ailments [3]. Natural plant products represent a promising group of therapeutic agents for arthritis. However, one of the major concerns in seriously considering these products for therapeutic purposes is that the mechanisms of action of many of them are poorly defined, if at all [4,5].

Alternate medicines like Ayurveda, Unani, Chinese, etc. are more and more used for the treatment of diseases, illness prevention and maintenance of health. An ayurvedic polyherbal formulation named-Dr. Orthocapsules, SBS biotech, Ambala city, Haryana (India) containing extracts of different herbal medicinal plants-Boswellia Serrata (Shallaki), Commiphora Mukul (guggal), Pluchea Lanceolata (Rasna), Trigonella foenumgraecum (Methi), Zingiber Officinale (ginger), Withania Somnifera (Ashwagandha powder), Purified Asphaltam (shilajeet) and Strychnos Nux-vomica. The constituents of this capsule are used in folk medicine for the treatment of inflammation and pain associated with arthritis. Therefore, the present study was done to determine the mechanism of Dr. Ortho capsule.

MATERIALS AND METHODS

Animals

Adult wistar albino rats (180-230 g) of either sex or Swiss albino mice (25-30 g) were used for pharmacological activities. For both experiments, the animals were kept in polypropylene cages (3 per cage) at 25±2 °C with relative humidity 45-55% under 12h light and dark cycles. All the animals were acclimatized to laboratory condition for a week before use. They were fed with standard animal feed and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocols, Reg no. 837/AC/04/CPCSEA.

Drugs and chemicals

The Dr. Ortho capsule was obtained from SBS Biotech, Himachal Pradesh (manufacturing unit). Indomethacin from Sigma-Aldirich. Complete Freund's Reagent, histamine, 5-hyrdoxy tryptamine, bradykinin, carrageenan from Sigma Aldirich. AST, ALT and TP kits for estimation were obtained from Transasia bio-medicals limited, Mumbai (India).

Anti-arthritic activity–adjuvant induced arthritis in rats [6]

Albino rats weighing 150-200 g were divided into four groups of six animals. Controls animals were administered the vehicle. Group 2 (Arthritic control) animals were injected with CFA 0.1 ml to subplantar region of right hind paw, For Group 3 and Group 4 animals, respectively, Dr. Orthocapsule (55 mg/kg, p. o.) and indomethacin (10 mg/kg, p. o.) were administered twice a day, from

the day of injection of CFA (0.1 mL in the subplantar region) and continued up to 14th post CFA challenge day. Paw volume was measured using a mercury plethysmograph on 0, 4, 7, 14, 21 d from the day of CFA injection [7]. At the end of the 21st day, blood was withdrawn from retro-orbital plexus, WBC count was found, and serum was separated by centrifugation (Remi) at 3000 rpm for 15 min. [8]. Estimation of ALT, AST and total protein [9] were done using standard Erba-chem kits, Germany (marketed by Transasia medico labs, Mumbai, India) and auto analyzer. Histopathological assessment of the knee joint was done on the 21st day after euthanasia. The groups defined to be:

Group 1: Control

Group 2: CFA-induced (0.1 ml)

Group 3: Dr. Ortho (55 mg/kg, p. o.)

Group 4: Indomethacin (10 mg/kg, p. o.)

Anti-inflammatory activity–phlogistic agents induced paw edema in rats [10, 11]

Male Albino Wistar rats weighing between 150-200 g were divided into twelve groups of six rats each. Animals of Group 1 receive vehicle (distilled water 10 ml/kg p. o.), animals of groups were subjected to inflammation by injecting phlogistic agents prior to administration of the vehicle. Groups of animals were administered with poly herbal formulation (55 mg/kg p. o.) followed by 5-HT [0.1 ml (1 mg/ml)], bradykinin [0.1 ml (20 µg/ml)] 60 min thereafter and carrageenan [0.1 ml (1%)], histamine [0.1 ml (1 mg/ml)] 30 min thereafter animals of the group were also administered with indomethacin (10 mg/kg, i. p.) followed by respective phlogistic agents. The following was the groups prepared-

Group 1: Histamine [0.1 ml (1 mg/ml)]

Group 2: Dr. Ortho (55 mg/kg, p. o.)+histamine

Group 3: Indomethacin (10 mg/kg, p. o.)+histamine

Group 4: 5-hydroxy tryptamine [0.1 ml (1 mg/ml)]

Group 5: Dr. Ortho (55 mg/kg, p. o.)+5-hydroxy tryptamine

Group 6: Indomethacin (10 mg/kg, p. o.)+5-hydroxy tryptamine

Group 7: Carrageenan (0.1 ml (1%)]

Group 8: Dr. Ortho (55 mg/kg, p. o.)+Carrageenan

Group 9: Indomethacin (10 mg/kg, p. o.)+Carrageenan

Group 10: Bradykinin [0.1 ml (20 µg/ml)]

Group 11: Dr. Ortho (55 mg/kg, p. o.)+Bradykinin

Group 12: Indomethacin (10 mg/kg, p. o.)+Bradykinin

The paw volumes were measured immediately (0 h) and then at predetermined intervals; for Carrageenan at (30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h), for Bradykinin at (10 min, 20 min, 30 min, 60 min, and 120 min), for Histamine and 5-HT at (30 min, 60 min, 90 min, 120 min, 150 min, and 180 min) using plethysmometer. Percent change in paw volume was calculated using the following formula.

%Inhibition = 100-(Control mean/Treated mean x 100)

Analgesic activity

Acetic acid induced writhing test in mice [12]

Mice of either sex with a weight between 20 and 25 g are used. Three groups were made, one group treated as a control and received 0.6% v/v acetic acid i. p., second one received diclofenac sodium (20 mg/kg, p. o.) as a standard drug and the last group was given Dr. Ortho (55 mg/kg, p. o.).

Group 1: Control (0.6% v/v acetic acid, i. p.)

Group 2: Diclofenac sodium (20 mg/kg, p. o.) as standard

Group 3: Dr. Ortho (55 mg/kg, p. o.)

Test animals are administered the drug or the standard at various pretreatment times prior to acetic acid administration. The mice are placed individually into glass beakers and five min are allowed to elapse [13]. The mice are then observed for a period of 10 min and the number of writhes is recorded for each animal. The formula for computing percent inhibition is-

$$\frac{\text{Control mean- treated mean}}{\text{Control mean}} \times 100$$

Eddy's hot plate

Three groups of six mice each of either sex with an initial weight of 18 to 22 g are used for each dose. The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is controlled for 55 ° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded with a stopwatch. The latency is recorded before and after 20, 60 and 90 min following oral or subcutaneous administration of the standard or the test compound [14]. The three groups are as follows-

Group 1: Control

Group 2: Tramadol (25 mg kg_1, p. o.), as standard

Group 3: Dr. Ortho (55 mg/kg, p. o.)

Histopathology

After euthanasia on day 21, the hind paws amputated above the knee joint were fixed in 7.4% formalin solution. The sections of articulation of the tarsal joints were stained with haematoxylin and eosin and were examined microscopically for mononuclear infiltration, pannus formation, and bone destruction [15].

Statistical analysis

All the results were expressed as mean±SEM and analysis were carried out by one-way ANOVA, Post-hock analysis was done by Tukey's multiple comparison tests to estimate the significance of the difference between various individual groups. $P<0.05$ was considered as significant.

Evaluation of parameters

Body weight changes were measured by an electronic weighing balance. Haematological parameters like RBC, Hb (hemoglobin), ESR, and WBC was measured by Autoanalyzer [16].

RESULTS

Anti-arthritic activity–adjuvant induced arthritis in rats

Paw volume

Dr. Ortho decreased the paw volume on day 4, 7, 14, and 21 respectively as shown in table 1.

Lysosomal enzymes

Dr. Ortho decreased (but not significant) the lysosomal enzyme ALT, AST and total protein compared with arthritic rats, as shown in the fig. 2.

Histopathological study

Section of the synovial joint of a normal control rat shows intact articular hyaline cartilage (fig. 3(A), subchondrial bone layer and adjacent synovial layer. The synovial layer shows synovial lining cells within a normal range. The synovial subepithelium shows fibro-collagenous stroma with vascular spaces (fig. 3 (A)). Section of the synovial joint of CFA treated rat shows partially distorted articular hyaline cartilage with intact subchondrial bone layer and partially effaced synovial layer. The distorted articular cartilage, i.e., bone erosion is replaced by mixed inflammatory cells comprising predominantly neutrophil and some lymphocytes. The synovial sub epithelium shows fibro collagenous stroma with vascular spaces (fig. 3 (B)). Section of the synovial joint of Dr. Ortho treated shows intact articular hyaline cartilage (fig. 3 (C)), subchondrial bone layer and adjacent synovial layer. The synovial layer shows synovial lining cells within normal range. The synovial sub epithelium shows fibrocollagenous stroma with vascular spaces (fig. 3 (C)).

Table 1: Effect of Dr. Ortho on paw edema

Treatment	Paw volume (ml) (mean±sem)				
	0 d	4th day	7th day	14th day	21st day
Control	1.23±0.01	1.37±0.034	1.17±0.086	1.26±0.0140	2.10±0.034
CFA	1.1±0.037	2.77±0.054**	3.2±0.041***	2.9±0.037**	2.5±0.044*
Dr. Ortho	1.2±0.031	2.5±0.043	2.5±0.023	2.10±0.037	1.70±0.033
Indomethacin	1.21±0.022	2.50±0.013	2.53±0.021	2.10±0.019	1.70±0.080

Values are expressed as mean±SEM n= 6; one way ANOVA followed by Turkey's test, ***p<0.001 v/s control, **p<0.01 and *p<0.05 v/s control.

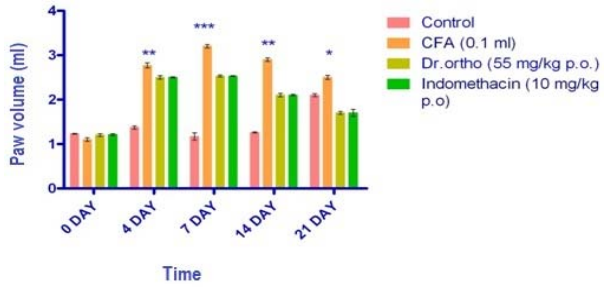

Fig. 1: Effect of Dr. ortho on paw edema
Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY's multiple comparison test. ***P<0.001 vs control, **P<0.01 vs arthritic control, *P<0.05 vs arthritic control

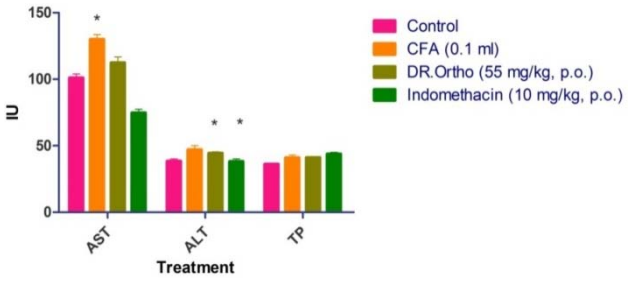

Fig. 2: Effect of Dr. ortho on AST and ALT
Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY's multiple comparison test, *P<0.05 vs arthritic control

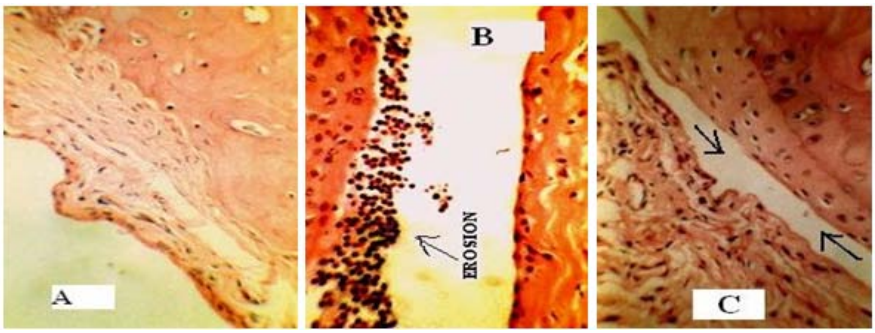

Fig. 3: (A)-TS of the knee joint of normal control rat, showing no mononuclear infiltration and pannus formation [H&E, 100×]. (B)-TS of the knee joint of with bone erosion [H&E, 400×]. (C)-TS of knee joint of with reduced bone erosion [H&E, 100×]

Anti-inflammatory activity–phlogistic agents induced paw edema in rats

Dr. Ortho significantly reduced the Carrageenan (fig. 4), Histamine (fig. 5), 5-HT (fig. 6), Bradykinin (Fig.7) induced paw edema respectively.

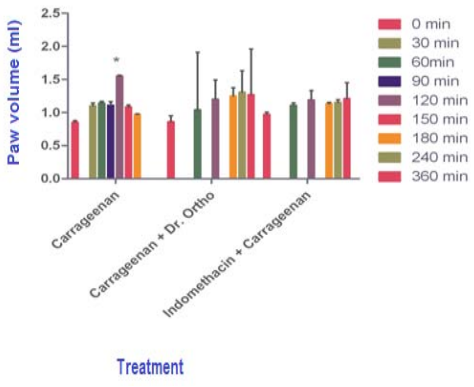

Fig. 4: Effect of Dr. Ortho on carrageenan-induced paw edema in rat
Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY's multiple comparison test. *P<0.05 vs arthritic control

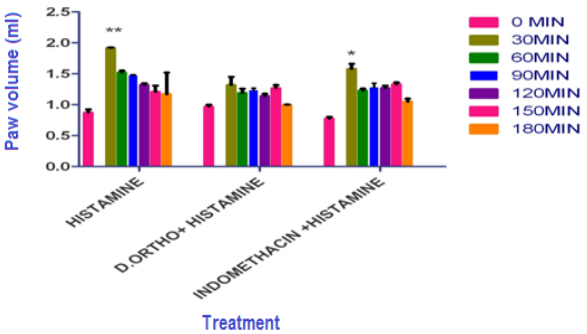

Fig. 5: Effect of Dr. Ortho on histamine-induced paw edema in rat
Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY's multiple comparison test. **P<0.01 vs arthritic control, *P<0.05 vs arthritic control.

Analgesic activity

Acetic acid induced writhing test in mice

Dr. Ortho when compared to control animals, significantly (P<0.01) decreased the number of acetic acid induced writhing episodes and

the percentage protection was 41.2%, while the reference drug, Diclofenac sodium showed 66.4% protection.

Eddy's hot plate

Fig. 8 shows the time interval of the anti nociception produced by Dr. Ortho. The effect reached a peak significantly at 120 min after administration and then gradually decreased.

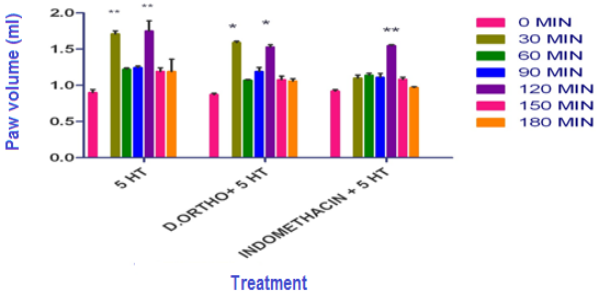

Fig. 6: Effect of Dr. Ortho on 5-HT induced paw edema in rat Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY's multiple comparison test. **P<0.01 vs arthritic control, *P<0.05 vs arthritic control

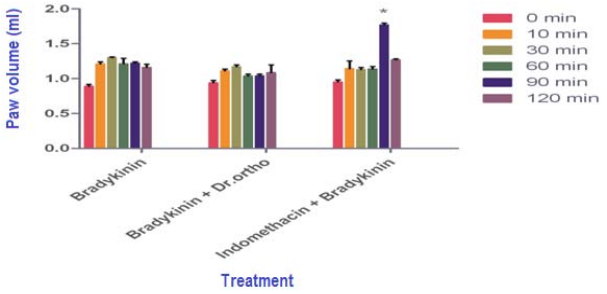

Fig. 7: Effect of Dr. ortho on bradykinin-induced paw edema in rat Values are expressed as mean±SEM. n=6; One-way ANOVA followed by TURKEY's multiple comparison test. *P<0.05 vs arthritic control

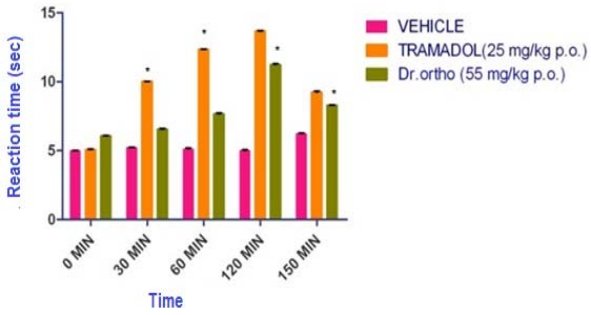

Fig. 8: Analgesic activity of Dr. Ortho using Eddy's hot plate Values are expressed as mean±SEM n=6; One-way ANOVA followed by TURKEY's multiple comparison test. *P<0.05 vs arthritic control

Body weight changes

Dr. Ortho (55 mg/kg) inhibits the loss of body weight observed during arthritic condition in a disease control group. The results are shown in table 2.

Hematological changes

Dr. Ortho and indomethacin-treated groups significantly decreased the total WBC count while ESR count regains its normal value. The results are shown in table 3.

DISCUSSION

In the present study an attempt is made to illustrate mechanism of the action of Dr. Ortho. Inflammation, pain and pannus formation are the integral parts of arthritis. Hence, we tried to elucidate the mechanism of action of Dr. Ortho by studying its effect on these three components. Inflammation, an integral part of arthritis, causes various abnormalities like swelling, redness, loss of function through numerous mediators like Cytokines, Bradykinin, Histamine, Eicosanoids, and Platelet activating factor. So in our study, we selected some of the known phlogistic agents like Carrageenan, Histamine, 5-HT and Bradykinin to understand the anti-inflammatory component of Dr. Ortho. Adjuvant-induced arthritis in rats is the most widely used models of experimental arthritis in screening programs for anti-inflammatory drugs [17].

Table 2: Changes in body weight in adjuvant arthritis in rats

S. No.	Groups	Before treatment(g)	After treatment on 21st day(g)	Mean change in body weight
1.	Control	172.1	173.9	1.8±0.150
2.	CFA induced	167.3	160.1	-7.2±0.25#
3.	Dr. Ortho	169.5	171.0	1.5±0.135
4.	Indomethacin	174.7	176.4	1.7±0.605

Table 3: Effect on hematological parameters in adjuvant induced arthritis in rats

S. No.	Treatment	Total WBC count cells/µl	RBC count (million/µl)	Hb (g%)	ESR (mm/h)
1	Control	8102.6±0.6	6.45±0.191	10.23±0.11	12.75±0.01
2	CFA induced	14002.6±214	4.14±0.046**	10.82±0.15	17.26±0.22
3	Dr. Ortho	7597±8.57	5.09±0.17	10.20±0.002	6.27±0.28
4	Indomethacin	6057.4±8	7.19±0.11	12.11±0.11	6.02±0.15

All values are in mean±SEM **P≤0.03 is considered significant, *p<0.0001 considered more significant when compared to the control (n=6)

We found that Dr. Ortho inhibited 20% (P<0.05) of Carrageenan-induced paw volume at 30 min., 10.01%, (P<0.05) of Histamine induced paw volume at 30 min respectively. 8%, 7% (P<0.05) of 5-HT induced paw volume at 30 min and 120 min. 3% (P<0.01) of Bradykinin-induced paw volume at 20 min. respectively. These results show that, though not very potent, Dr. Ortho decreased the inflammation by antagonizing or inhibiting the inflammatory mediators i.e., Histamine, 5-HT and Bradykinin. Analgesic activity of Dr. Ortho was illustrated through, acetic acid induced; prostaglandins mediated pain, where percentage protection was found to be 45.1% (P<0.001). Analgesic activity of Dr. Ortho using Eddy's hot plate was found to be maximum of 120 min. This indicates that Dr. Ortho acts as both peripheral and central analgesic.

Pannus formation is one of the major events which lead to cartilage destruction and bone erosion in rheumatoid arthritis. The pannus formed due to CFA was reduced by Dr. Ortho. It is supported by

histological studies of knee joints [18]. fig. 3 (A) is TS of knee joint of control rat. Severe neutrophil infiltration, pannus formation and bone erosion are seen in the knee joint of arthritic control rat as shown in fig. 3 (B). On treatment with Dr. Ortho, there is a reduction in the neutrophil infiltration, pannus formation and bone as shown in fig. 3 (C), respectively. Loss of body weight occurs in arthritis due to deficient absorption of nutrients through the intestine and distress caused by the severity of arthritis [19]. Dr. Ortho decreased the elevated level of WBC, thus leading to a reduction in mononuclear infiltration and hence reduced pannus formation.

CONCLUSION

The ayurvedic product is purely based on ancient Indian data for the treatment of the pain coupled with arthritis. The study was taken up with an aim to explicate the mechanism of the anti-arthritic action of the mentioned polyherbal product.

In summary, the anti-rheumatic mechanism of Dr. Ortho is due to-

I. Inhibition of inflammation by inhibiting inflammatory mediators, i.e., Histamine, 5-HT, and Bradykinin.

II. Protection against prostaglandins mediated pain in mice, by inhibiting cyclo-oxygenase synthesis.

III. Reduction in pannus formation by inhibiting mononuclear infiltration.

Therefore, the data clearly indicate the antiarthritic action of a polyherbal formulation.

CONFLICT OF INTERESTS

All the authors declare no conflict of interest

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