Original Research

Anti-inflammatory activity of Zandu Rhumayog Forte and Rhumasyl Gel in acute and chronic inflammatory models

Amitabha Dey, Satyajyoti Kanjilal, Anjan Adhikari, Bibhuti Nath Bhatt, Tulika Chakraborty, Pallabi Chakraborty, Deepa Gandhi, Avinash Narwaria, Chandra Kant Katiyar

Research and Development Centre, Healthcare Division, Emami Limited, Kolkata, West Bengal 700056.

Corresponding Author's Email: ksatyajyoti@gmail.com

Date of Submission: 20 Sept 2019, Date of Acceptance : 23 Nov 2019

Abstract

Aim: The purpose of the present study was to evaluate the anti-inflammatory activity of two Ayurvedic preparations (Rhumayog Forte: RF and Rhumasyl Gel: RG) commercially available for the treatment of musculoskeletal inflammation.

Methods: The anti-inflammatory activity of the test samples (RF & RG) were assessed in carrageenan-induced acute paw inflammation and complete Freund's adjuvant-induced chronic inflammation in rats. Three oral doses (100, 200 and 300 mg/kg) of RF was used and the gel was applied topically to swollen hind paw of test animals. Diclofenac sodium was used as standard. *In vitro* heat induced protein denaturation assay was also performed.

Results: RF significantly and dose dependently inhibited carrageenan-induced paw inflammation as compared to control. The combined effect of RF & RG was better in both acute and chronic condition and the effect at dose 300 mg/kg was comparable to that of the standard diclofenac (10 mg/kg) treated animals. RF at concentration 159.4 µg/ml exhibited 50% inhibition in heat induced protein denaturation.

Conclusion: These observations established the anti-inflammatory effect of both RF & RG and reaffirmed traditional Ayurvedic claims for their use in musculoskeletal inflammation.

Key Words: Inflammation, Carrageenan, Complete Freund's Adjuvant, Ayurvedic Taila, Ayurvedic Bhasmas

Annals Ayurvedic Med 2019 :8 (3-4) 104-113

Introduction

Inflammation is the body's immune response to any injury or infection and it has been concerned in the prognosis of chronic inflammatory diseases including arthritis and cancer.[1] Pain and inflammation are one of the common symptoms that are being experienced by the patients in their life time suffering from chronic diseases. The primary signs of inflammation are pain, swelling, heat, redness and loss of movement. The symptoms of inflammation are due to the production and release of pro-inflammatory mediators, which triggers series of events such as alteration in vascular permeability, stimulating adherence of circulating leukocytes to vascular endothelium, and finally, chemotactically attracting and activating inflammatory cells to accumulate at the site of the injury.[2,3] Over the period of time and if untreated, it leads to the accumulation of inflammatory cells at local tissue sites and is involved in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis or cancer.[2]

Cyclooxygenase (COX) is an enzyme that catalyses the production of inflammatory mediators (prostaglandins) from arachidonic acid. Two isoforms of cyclooxygenase (COX-1 and COX-2) are identified, in this COX-1 is responsible for the production of prostaglandins that are required for normal physiological functions and is called as 'housekeeping' enzyme. Whereas, COX-2 is expressed in immune cells which is a key player in starting the inflammatory response by the production of prostaglandin E_2 (PGE2), and triggering the production of other pro-

inflammatory chemokines and cytokines.[1,3] Eventually understanding of the mechanisms have eased the identification of inflammatory pathways for the development of effective therapeutics.

At present, the therapeutic strategies such as use of non-steroidal anti-inflammatory drugs (NSAIDS) or disease-modifying anti-inflammatory drugs (methotrexate or leflunomide) and Biological response modifiers (BRMs: anakinra, etanercept, monoclonal antibodys etc.) are involved for the treatment of chronic inflammatory diseases including arthritis. Prolonged use of these medications caused severe adverse effects and toxicity. Thus, alternative therapeutic approaches based on Ayurvedic natural products or Polyherbal formulation, complementary and alternative medicine (CAM) are becoming increasingly popular in Asian, American and European countries.[1,3]

Ayurvedic medicines have a long history of usage with minimum or no side effects reported. For treatment of inflammatory diseases there are descriptions available of a large number of preparations in Ayurvedic texts. With this background and ideology, two Ayurvedic preparations coded as RG and RF in different dosage forms were formulated with an intended use in musculoskeletal inflammatory diseases.

RG was prepared by amalgamating four medicinally important Ayurvedic oils such as *Mahamash Taila*, *Vishgarbha Taila*, *Narayana Taila* and *Gandhapuro Taila* (oil of *Gaultheria procumbens*) in 25% each equal quantities.[4-7] Each of these four oils were prepared as per the standard guidelines under the supervision of the subject experts.[8] These Ayurvedic oils contains permutation and combination of multiple herbs and are traditionally used in treatment of various pains associated with rheumatological and neurological conditions, such as *Amavata* (Rheumatism),[9] *Sandhigatavata* (Osteoarthritis) *Gridhasi* (Sciatica), *Vatarakta* (Gout), *Avabahuka* (Brachialgia), *Visvachi* (Brachial neuralgia) and also in *Bahu sosa* (Muscular wasting of forearm), *Manya Stambha* (Neck rigidity/Torticollis), *Sarvanga grahana* (Stiffness and Tightness in all limbs)[10]

Each RF tablet contains *Maharasnadi quath* (470 mg); *Yograja guggulu* (60 mg), *Lauha Bhasma* (20 mg), *Banga Bhasma* (10 mg), *Mandura Bhasma* (10 mg), *Makshika Bhasma* (10 mg), and *Abhraka Bhasma* (10 mg).[11,12] Ayurvedic *Bhasmas* are incinerated calcined herbo-mineral/metallic preparations that are biologically produced nanoparticles. They are being used in many Ayurvedic formulations commercially available for the treatment of various inflammatory diseases including rheumatoid arthritis.[13]

Based on the Ayurvedic texts, the ingredients of both the formulations are being recommended for rheumatism, osteoarthritis, gout, lower backache and for injuries.[8] However, no scientific studies are available on the anti-inflammatory / anti-arthritic activity of these ingredients as a formulation. Hence, the present study was undertaken with an objective to investigate the anti-inflammatory and anti-arthritic activity of RG and RF tablet in albino rats.

Materials and Methods

Sample formulations

The Herbo-mineral oral tablet formulation (coded as RF) and the Gel formulation with Ayurvedic oils (coded as RG) are being manufactured and marketed by Emami Ltd, Kolkata under the brand name of Zandu Rhumayog Forte and Zandu Rhumasyl respectively. The preparation of oil ingredients and *bhasmas* are followed as per the standard Pharmacopoeial norms. RF and RG samples were obtained from Emami Limited, Kolkata of the commercial batch (RF: Batch No. FH0001, Mfg. Date – April, 2014; RG: Batch No. DH0001, Mfg. Date – April, 2014).

Animals

Wistar albino rats of both sexes (150 ± 10 g body weight) were procured from Central Animal House, Kolkata. They were randomly selected and group-housed (six animals per cage; either sex) in polypropylene cages provided with husk bed at an ambient temperature (25±1°C) and relative humidity (50±10%) with a 12:12 h light/dark cycle. All the animals were acclimatized to laboratory conditions for 15 days before the start of the experiments. They were fed with standard rodent diet and water *ad libitum*. The waste in the cages was removed daily to ensure hygienic condition

and maximum comfort for animals. Ethical clearance for animal experimental work was obtained from the Institutional Animal Ethical Committee, R G Kar Medical College and Hospital, Kolkata prior to the commencement of experiments (RKC/IAEC/13/41; Dated: 20.03.2014).

Animal Grouping and Treatments

Experimentally naive rats of either sex were randomly assigned into eight groups of six animals each for a single set of experiment. Group I animals were served as control and were treated with normal saline (5 ml/kg/day, orally). Group II animals were treated with standard diclofenac sodium (10 mg/kg/day, orally), Group III animals were orally treated with standard diclofenac sodium (10 mg/kg/day) and topically with standard Diclofenac Sodium Gel (Sun Pharmaceutical Industries Ltd.) to the plantar region of rat hind paw. The three test groups (Group IV – VI) were treated with graded oral doses of RF tablets (100, 200 and 300 mg/kg/day). Group VII animals were treated with RG to the plantar region of rat hind paw and Group VIII animals were orally treated with RF tablets (300 mg/kg/day) and topically with RG to the plantar region of rat hind paw. During the course of an experiment, all animals were closely observed for apparent behavioural abnormalities.

Carrageenan induced acute paw inflammation

Acute paw inflammation induced by carrageenan is a validated animal model for assessing inflammatory responses to antigenic challenges. The experimental procedure was described elsewhere in details [14] and was followed with little laboratory modification. The animals of all the groups were deprived of food for 14 h before induction of inflammation, but were allowed to free access of water.

Paw inflammation was induced by subcutaneous injection of 0.1ml carrageenan sodium salt solution (Sigma-Aldrich, 1% w/v in normal saline) into the sub-plantar region of the rat right hind paw. RF was administered orally at different concentration levels (100, 200 and 300 mg/kg/day) for three consecutive days prior to the carrageenan injection. On day 3, standard diclofenac was administered 30 min before the carrageenan injection. However, for topical application, RG was applied immediately after injection to the respective group of the animals. The readings of normal paw volume at 0 min before carrageenan injection and inflamed paw volume after injecting carrageenan was measured at 1, 2 and 3 hrs with the aid of plethysmometer.[1]

The acute anti-inflammatory activity was expressed as the average percent inhibition of oedema in each group, which was calculated as:

$$\% \text{ inhibition} = 100 — (100 \times Vt / Vc)$$

Where, Vt and Vc represent the increase in paw volumes of rats treated with drug and control, respectively.

Complete Freund's adjuvant induced chronic inflammation in rats

The experimental procedure was described elsewhere in details [15] and was followed with little laboratory modification. Chronic inflammation or poly-arthritis in rats was induced by injecting 0.05 ml of Complete Freund's adjuvant (FCA) (1 mg/ml of *Mycobacterium tuberculosis*, heat killed and suspended in mineral oil; Sigma) into the sub-plantar surface of the hind paw. RG and the standard drugs were administered to the respective group of the animals on each day starting from day 0 and continued for 21 days. The paw volume of each rat was measured plethysmometrically on 0, 15 and 21 day of the experiment. The anti-arthritic activity was expressed as the average percent inhibition of swelling in each group, which was calculated as:

$$\% \text{ inhibition} = 100 — (100 \times Vt / Vc)$$

Where, Vt and Vc represent the increase in paw volumes of rats treated with drug and control, respectively.

The severity of secondary lesions on the fore & hind paws, ears and tail were also assessed. The severity of secondary lesion was scored on day 21 as follows:

- Fore Paws = absence of inflammation (0), inflammation at least one joint (1);
- Hind Paws = absence of inflammation (0), slight inflammation (1), moderate inflammation (2), marked inflammation (3);

- Ear = absence of nodules (0), presence of nodules (1);
- Nose = no swelling of connective tissue (0), swelling of connective tissue (1);
- Tail = absence of nodules (0), presence of nodules (1).

After autopsy, the ulcerative index was assessed using standard visual score.[16] Briefly, the stomachs were cleaned and lesions (if any) were carefully recorded and the ulcer index was calculated using the following scores:

Score 1: Presence of edema, hyperemia & single submucosal punctiform hemorrhages, Score 2: Presence of submucosal punctiform hemorrhagic lesions with small erosions, Score 3: Presence of deep ulcers with erosions and invasive lesions.

***In-vitro* anti-inflammatory activity**

Inhibition of heat induced protein denaturation

The experimental procedure followed was described elsewhere in details.[17] The reaction mixture was prepared with equal volume of test samples at different concentration (25, 50, 100 and 200 µg/ml) and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 0.1 N HCl at 37°C. Diclofenac sodium was used as a standard drug. The reaction mixtures were incubated at 37°C for 20 min and then heated to 50 ± 2°C for 20 min. The tubes were further allowed to stand at room temperature for cooling followed by measuring the turbidity spectrophotometrically (at 660 nm). The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows,

% Inhibition of protein denaturation = $[(OD_{CONTROL}$ " $OD_{TEST}) / OD_{CONTROL}]/ \times 100$

Data and statistical analysis

Data were expressed as mean and standard deviation. Statistical analysis was performed by two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test and Student's *t*-test as appropriate. GraphPad Prism-5 (GraphPad Software Inc., California, USA) software was used for statistical analysis. A *p* value less than 0.05 was considered as statistically significant.

Results

Carrageenan-induced Acute Paw Inflammation

The results summarized in Table 1 have revealed the anti-inflammatory activity of both RF and RG. In untreated (control) rats, paw volume gradually increased after carrageenan injection from 0.54 ml to 1.75 ml within 3 hrs. A significant reduction in paw inflammation was observed in all the treated groups after their respective oral or topical treatments as compared to control animals. The animals treated with three graded oral doses of RF (100, 200 and 300 mg/kg/day) has shown a dose dependent reduction in paw inflammation. Around 64% reduction in paw inflammation was observed with standard diclofenac sodium (10 mg/kg, p.o. + topical gel) after 3 hours of carrageenan challenge. RG has also shown 40% protection against carrageenan induced inflammation 3 hours after its topical application (Figure 1). Both RF and RG together have shown maximum protection against carrageenan-induced inflammation (47.43% at 3 hr) as compared to their single effects.

Freund's complete adjuvant induced chronic inflammation in rats

The results are summarized in Table 2. In control animals, the severity in paw inflammation was observed after the FCA injection. A gradual increase in initial paw volume was observed after 15 days; however, the severity of pain and inflammation in rat hind paw was persisted even after 21 days. A significant reduction in paw inflammation was observed in the treated animals after their respective oral or topical treatments as compared to control animals. RF has shown a dose dependent reduction in paw inflammation [17.91% at 100 mg/kg, 23.99% at 200 mg/kg and 30.74% at 300 mg/kg] observed after its repeated daily use for 21 days. Both RF and RG combined have shown maximum protection against paw inflammation after their concomitant use (35.47%) which was better than their single use effects (Figure 2). The standard combination diclofenac sodium (10 mg/kg, p.o. + topical gel) have

shown 44.59 % reduction in paw inflammation after 21 days of FCA challenge**.**

Inhibition of heat induced protein denaturation

The results are summarized in Table 3. RF has shown inhibition of thermally induced protein (Albumin) denaturation in a dose dependent manner.

Discussion

In Ayurvedic system of medicine there are many oils, minerals, calcined metal combination preparations made up of herbs, minerals and metals. These preparations are claimed to have analgesic and anti-inflammatory benefits. Currently many of these are commercialized in India and being exported to other countries with an intent to be used in management of various inflammatory disorders majority been arthritis. However, systematic protocol based studies are not so apparent which scientifically validates the therapeutic claims of these preparations. There are few reports available mentioning their chemical properties and biological activities.[10,18-22] In view of this, the claimed therapeutic reputation was verified in a scientific manner for the formulation which are based on Ayurvedic wisdom.

In the present study, anti-inflammatory potential of two different dosage form of Ayurvedic preparations (RG & RF) were evaluated in the scientifically validated rodent models. Observations revealed that both RG and RF have potent anti-inflammatory activity in both acute and chronic inflammatory conditions.

Carrageenan injection in living animal tissue causes release of pro-inflammatory mediators (histamine, prostaglandins, leukotrienes, bradykinin, TNF-á, etc.) and is considered as standardized rat paw inflammation model.[14] In a biphasic inflammatory response carrageenan triggers the release of histamine, serotonin, and kinins during the first hour of its injection and a sustained release of prostaglandins occurs after 2-3 hours. The second phase inflammation is sensitive to commonly used steroidal and non-steroidal anti-inflammatory drugs.[1,23] The significant acute anti-inflammatory activity of the test preparations (RG and RF) and standard diclofenac (10 mg/kg) observed in the present study may be due to the inhibition of the inflammatory mediators such as prostaglandins, histamine and cytokines. The concomitant use of both the test formulations together has shown better anti-inflammatory activity than their individualised usage. This observation suggests that concomitant use of both oral and topical preparations together is an effective remedy for managing inflammatory conditions. However, their clinical efficacy would also depend upon on the allostatic load of the patient.

In the chronic inflammatory model (Freund's Complete Adjuvant injection) both RG and RF have shown significant activity in reducing the long term (21 days) inflammatory responses. The injection of Freund's Complete Adjuvant produces an acute inflammatory response that peaks at about 24 hours which is maintained for 21-day post-injection. This effect may be due to the sustained release of inflammatory mediators and their chemotactic responses.[15] The data generated during the study indicates that the tested preparations are having potential to reduce the prostaglandins secretions and their repeated dose can be useful in chronic inflammatory conditions. It is also interesting to observe that, administration of a higher daily oral dose of RF (300 mg/kg) did not cause any gastric injury unlike the same observed with diclofenac (10 mg/kg). Thus, it can be interpreted that RF tablet is having a potential to be one of the safe probable alternative in management of inflammatory conditions.

In *in vitro* assay, RF tablet produced a dose dependent protection against heat induced protein denaturation. Denaturation of proteins is one of the well-defined causes of inflammation as proteins lose their secondary and tertiary structures and activity with application of external heat.[24] This suggest that RF is potential to protect the tissue proteins against injurious substances and thereby can be an effective choice in acute inflammatory conditions.

Conclusion

The results of the acute and chronic inflammation models suggest that both Rhumayog forte and Rhumasyl gel, the different dosage form based on traditional Ayurvedic wisdom, have anti-inflammatory potentials. These

formulations may be useful in management of musculoskeletal inflammatory conditions.

Acknowledgments: The authors express their gratitude to Emami Limited, Kolkata for providing the sample formulations and bio-assay laboratory facility to carry out these studies. The authors are also thankful to the Principal and Department of Pharmacology, R. G. Kar Medical College and Hospital, Kolkata for providing the experimental facilities.

Conflicts of interest: There are no conflicts of interest

References

1. Deb L, Dey A, Sakthivel G, Bhattamishra SK, Dutta A. Protective effect of *Clerodendrum colebrookianum* Walp., on acute and chronic inflammation in rats. Indian J Pharmacol 2013;45(4):376-80.
2. Snyderman R. Mechanisms of inflammation and leukocyte chemotaxis in the rheumatic diseases. Med Clin North Am 1986;70(2):217-35.
3. Bhanu N, Mishra NK, Panda JR, Patra S. Anti-Inflammatory Activity of Polyherbal Formulation by Using Cotton Pellet Granuloma in Rat and Xylene Induced Mice Ear Edema Model. Journal of Engineering, Computers & Applied Sciences 2014; 3(3): 13 – 21.
4. Govinda DS. Vatavyadhirogadhikar (*Mahamash Taila*). in: Bhaishajya Ratnavali. Varanasi: Chaukhamba Krishnadas Academy; 2014. pp 455.
5. Ayurvedic formulary of India. Part I. 2nd ed. Chapter 8:48 (*Vishgarbha Taila*). New Delhi, India: Department of Indian System of Medicine and Homeopathy, Ministry of Health and Family Welfare, Government of India; 2003.
6. Sharma P. Sharangdhar Samhita, Madhyamkhand (*Narayana Taila*). Chapter 9. Varanasi: Chaukhamba Series Office; 1958.
7. Chunekar KC. Bhavprakash Nighantu (Indian Meteria Medica) of Sri Bhavamishra, Pandey GS (Ed). Varanasi: Chaukhamba Bharati Academy; 2015.
8. The Ayurvedic Pharmacopoeia of India, Part II. Volume II (Formulations). New Delhi, India: Department of Ayurveda, Yoga & Naturopathy, Unani and Homoeopathy, Ministry of Health and family Welfare, Government of India; 2008.
9. Deshpande SV, Deshpande VS, Potdar SS. Effect of panchakarma and Ayurvedic treatment in postpartum rheumatoid arthritis (amavata): A case study. J Ayurveda Integr Med 2017;8(1):42-4.
10. Sharma MR, Mehta CS, Shukla DJ, Patel KB, Patel MV, Gupta SN. Multimodal Ayurvedic management for *Sandhigatavata* (Osteoarthritis of knee joints). Ayu 2013;34(1):49-55.
11. Ayurvedic formulary of India. Part 1. 2nd ed. Chapter 4:28 (*Maharasnadi Kwath*). New Delhi, India: Department of Indian System of Medicine and Homeopathy, Ministry of Health and Family Welfare, Government of India; 2003.
12. Gopal K. Rastantrasar Ebam Siddhprayogsangraha. Part 1. Ajmer, Rajasthan: Krishna Gopal Ayurveda Bhavan; 2006.
13. Pal D, Sahu CK, Haldar A. Bhasma/ : The ancient Indian nanomedicine. J Advanced Pharm Tech Res 2014;5(1):4-12.
14. Whiteley PE, Dalrymple SA. Models of Inflammation: Carrageenan Induced Paw Edema in the Rat. Current Protocols in Pharmacology. Unit 5.4. New Jersey, USA: John Wiley & Sons, Inc.; 2001.
15. Fehrenbacher JC, Vasko MR, Duarte DB. Models of inflammation: Carrageenan- or complete Freund's Adjuvant (CFA)-induced edema and hypersensitivity in the rat. In: Current Protocols in Pharmacology. Chapter 5. Unit 5.4. New Jersey, USA: John Wiley & Sons, Inc.; 2012.
16. Govindani H, Dey A, Deb L, Rout SP, Parial SD, Jain A. Protective role of methanolic and aqueous

extracts of Cucurbita moschata Linn. fruits in inflammation and drug induced gastric ulcer in Wister rats. Int J PharmTech Res 2012; 4(4): 1758–65.

17. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharmacol Sci 2010;2:146-55.
18. Kumar A, Nair AG, Reddy AV, Garg AN. Bhasmas: unique ayurvedic metallic-herbal preparations, chemical characterization. Biol Trace Elem Res 2006;109(3):231-54.
19. Ota S, Singh A, Srikanth N, Sreedhar B, Ruknuddin G, Dhiman KS. Chemical Characterization of an Ayurvedic Herbo-Mineral Formulation - Vasantakusumâkara Rasa: A Potential Tool for Quality Assurance. Ancient Sci of Life 2017;36(4):207-14.
20. Sharma R, Bhatt A, Thakur M. Physicochemical characterization and antibacterial activity of *Rajata Bhasma* and silver nanoparticle. Ayu 2016;37(1):71-5.
21. Shailajan S, Menon SN, Tiwari BR, Singh AS. Standardization of *Shadbindu Taila*: An Ayurvedic oil based medicine. Ayu 2013;34(1):103-7.
22. Mangal A, Shubhasree MN, Devi P, et al. Clinical evaluation of Vatari guggulu, *Maharasnadi kwatha* and *Narayan taila* in the management of osteoarthritis knee. J Ayurveda Integr Med 2017;8(3):200-4.
23. Thabrew MI, Dharmasiri MG, Senaratne L. Anti-inflammatory and analgesic activity in the polyherbal formulation *Maharasnadhi Quatha*. J Ethnopharmacol 2003;85(2-3):261-7.
24. Leelaprakash G, Dass SM. In-vitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*. Int J Drug Dev Res 2011;3:185-96.

Source of Support : Nil
Conflict of Interest : None

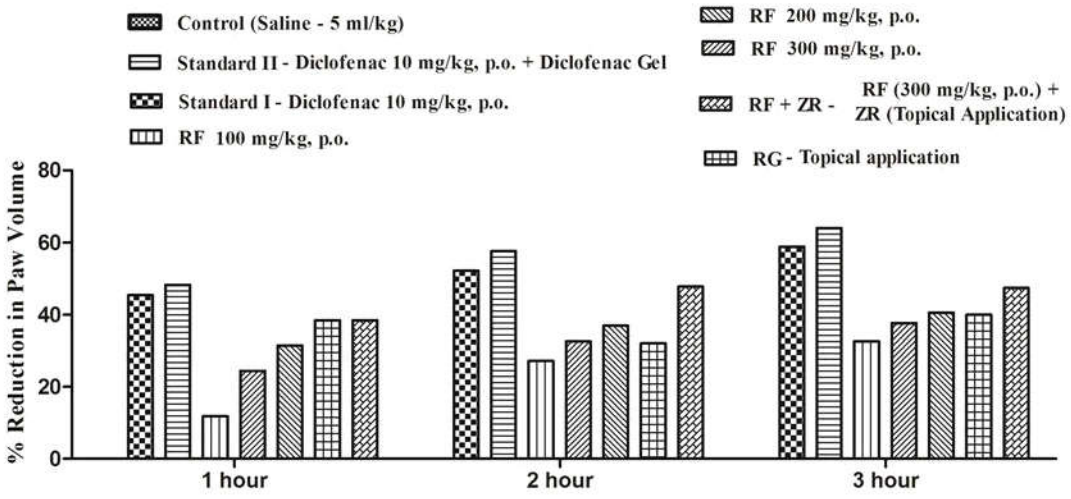

Figure 1: Effect of test formulations on percentage inhibition in paw volume after carrageenan injection. RF: Rhumayog Forte; RG: Rhumasyl Gel.

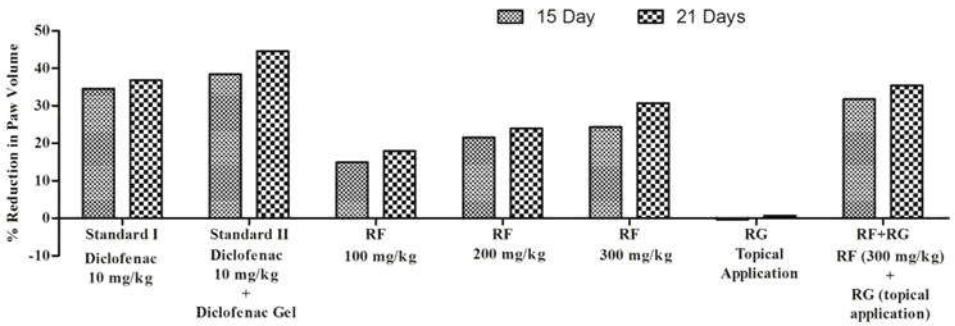

Figure 2A

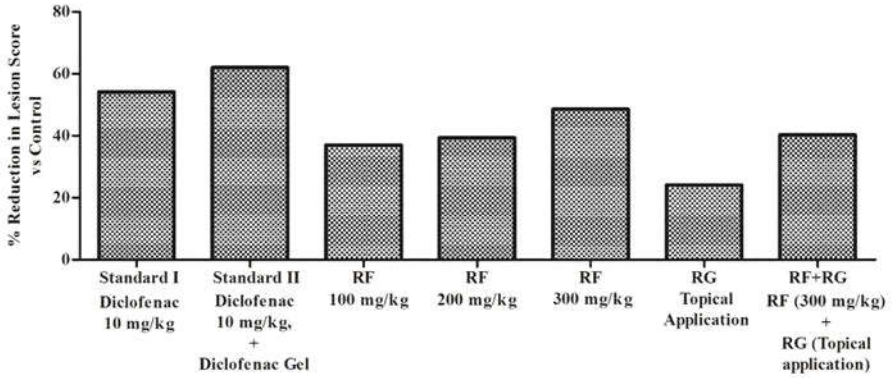

Figure 2B

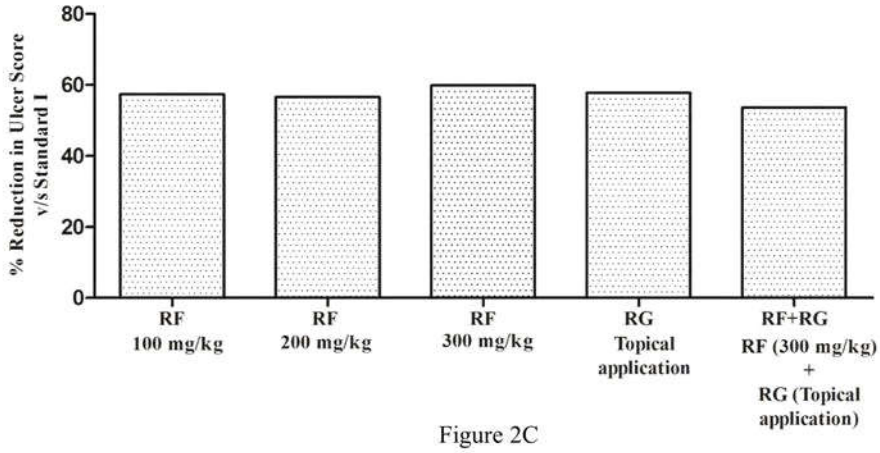

Figure 2C

Figure 2: Effect of test formulations on Percentage inhibition in paw volume (A), lesion score (B) and ulcer score (C) after FCA-injection. RF: Rhumayog Forte; RG: Rhumasyl Gel.

Table 1: Effect of test formulations on carrageenan-induced acute paw inflammation.

Treatment Groups	Mean paw volume (ml)			
	0 hr	1 hr	2 hr	3 hr
Control (Saline – 5 ml/kg, p.o.)	0.54±0.004	1.43±0.054	1.84±0.089	1.75±0.061
Standard I (Diclofenac 10 mg/kg, p.o.)	0.52±0.006	0.78±0.084	0.88±0.071	0.72±0.039
Standard II (Diclofenac 10 mg/kg, p.o. + Diclofenac Gel)	0.56±0.003	0.74±0.038	0.78±0.049	0.63±0.026
RF (100 mg/kg, p.o.)	0.56±0.007	1.26±0.068	1.34±0.084	1.18±0.092
RF (200 mg/kg, p.o.)	0.51±0.003	1.08±0.052	1.24±0.091	1.09±0.075
RF (300 mg/kg, p.o.)	0.53±0.004	0.98±0.058	1.16±0.072	1.04±0.083
RG (topical application)	0.52±0.008	0.88±0.020	1.25±0.031	1.05±0.029
RF (300 mg/kg, p.o.) + RG (topical application)	0.54±0.003	0.88±0.043	0.96±0.055	0.92±0.057

Values are expressed as Mean ± SD. *=$p<0.05$ vs. the corresponding vehicle (Saline – 5 ml/kg, p.o.) treated control groups (Two-way ANOVA followed by Bonferroni post hoc test).

Table 2: Effect of test formulations on FCA-induced chronic paw inflammation (volume and lesion) and gastrointestinal ulcer score

Treatment Groups	Mean paw volume (ml)			Lesion Score	Ulcer Score
	0 day	15 Day	21 Day		
Control (Saline – 5 ml/kg, p.o.)	0.53±0.003	3.15±0.098	2.96±0.083	21.6±2.28	12.4±1.12[b]
Standard I (Diclofenac 10 mg/kg, p.o.)	0.56±0.006	2.06±0.046	1.87±0.052	9.9±1.07	24.2±2.64[a]
Standard II (Diclofenac 10 mg/kg, p.o. + Diclofenac Gel)	0.52±0.008	1.94±0.172	1.64±0.259	8.2±1.98	22.7±2.91
RF (100 mg/kg, p.o.)	0.55±0.004	2.68±0.041	2.43±0.055	13.6±1.92	10.3±1.26[b]
RF (200 mg/kg, p.o.)	0.53±0.006	2.47±0.036	2.25±0.028	13.1±1.74	10.5±1.88[b]
RF (300 mg/kg, p.o.)	0.52±0.005	2.38±0.018	2.05±0.068	11.1±1.36	9.7±1.92[b]
RG (topical application)	0.55±0.007	3.16±0.354	2.94±0.342	16.4±1.93	10.2±2.80[b]
RF (300 mg/kg, p.o.) + ZR (topical application)	0.56±0.007	2.15±0.301	1.91±0.226	12.9±2.15	11.2±1.75[b]

RF: Rhumayog Forte; RG: Rhumasyl Gel. Values are expressed as Mean ± SD. *=$p<0.05$ vs. the corresponding vehicle (Saline – 5 ml/kg, p.o.) treaded control groups (Two-way ANOVA followed by Bonferroni post hoc test). a=Control group compared with standard I group within groups; b= standard I group compared with test groups within groups.

Table 3: Effect of Rhumayog Forte (RF) on heat induced protein denaturation.

Test formulation	Concentration (µg/ml)	% Inhibition
RF	25	17.83
	50	25.16
	100	40.36
	200	57.58
Diclofenac sodium	25	45.60
	50	66.09
	100	83.59
	200	90.05