Original Research Article

Evaluation of the Anti-arthritic, Anti-inflammatory, and Chondroprotective effect of a Novel Nutritional Formulation in Monoiodoacetate-Induced Osteoarthritis Animal Model

ABSTRACT

Shallaki, Turmeric, and Ashwagandha are key ingredients commonly incorporated into nutritional formulations for the management of osteoarthritis (OA). This preclinical study aims to evaluate the efficacy of the nutritional Rhumaflex formulation in its antiarthritic, anti-inflammatory, and chondroprotective potential in osteoarthritis. In this study, 32 Wistar rats were evenly allocated to four groups of 8 each. SC and DC groups orally received 1% CMC, while the PC group was given oral Indomethacin (2 mg/kg) in 1% CMC. The test group orally received Rhumaflex (206.46 mg/kg) in 1% CMC over a 28-day period. The study demonstrated that the Rhumaflex treatment group reduced swelling and improved mobility within 7 days of treatment. By day 28, significant anti-inflammatory effects were observed, evidenced by lowered COMP, MMP-13, and TNF-alpha levels. Due to research-backed ingredients, chondroprotective potential and lower degeneration was indicated in the Rhumaflex group compared to controls, suggesting efficacy for regeneration and chondroprotection. Rhumaflex exhibits multifaceted benefits, including anti-arthritic and anti-inflammatory effects, offering a promising alternative, particularly for patients seeking alternatives to conventional treatments. The selective ingredients of the formulation have shown promising effects in promoting joint cartilage regeneration and chondroprotection. While these preclinical results are promising, further clinical trials are underway to confirm these benefits in human subjects and establish long-term safety and efficacy profiles.

Keywords: *Shallaki, Turmeric, Ashwagandha, Anti-inflammatory, Anti-arthritic, Nutrition, Osteoarthritis.*

1. INTRODUCTION

Knee osteoarthritis (OA) is a common progressive multifactorial joint disease characterized by chronic pain and functional disability [1]. Often referred to as degenerative joint disease, it typically results from the gradual deterioration of articular cartilage due to wear and tear. Osteoarthritis stands as one of the most prevalent conditions leading to disability, particularly

among the elderly population [2], and represents the most common articular disease in the developed world, contributing significantly to chronic disability primarily through hip and/or knee involvement [3].

The Global Burden of Disease Study's Prevalence Trends of Site-Specific Osteoarthritis 2019 report reveals a striking 113.25% increase in prevalent OA cases, rising from 247.51 million in 1990 to 527.81 million in 2019 [4]. The global prevalence of knee OA was found to be 16.0% (95% CI, 14.3%-17.8%) in individuals aged 15 and over, increasing to 22.9% (95% CI, 19.8%-26.1%) in those aged 40 and over. The incidence rate was recorded at 203 per 10,000 person-years (95% CI, 106-331) in individuals aged 20 and over, with both prevalence and incidence showing age-related increases, peaking at advanced age for prevalence and at 70-79 years for incidence [1].

Several risk factors contribute to the development of joint OA, including advanced age, female gender, overweight and obesity, knee injury, repetitive joint use, bone density, muscle weakness, and joint laxity [5,6]. As a leading cause of disability in the elderly, knee OA currently has no cure, with treatment focused on improving quality of life by addressing symptoms and maintaining or enhancing function. Current therapeutic options encompass non-pharmacologic, pharmacologic, and surgical interventions [7].

The pharmacological treatment paradigm begins with paracetamol (acetaminophen) as first-line therapy, followed by oral and topical nonsteroidal anti-inflammatory agents (NSAIDs) as second-line options, along with tramadol and intra-articular corticosteroid injections. When these initial treatments prove insufficient, alternatives such as opioids, duloxetine, and intra-articular hyaluronate injections are recommended [8]. While these treatments effectively address OA symptoms, they fail to modify the underlying disease process of chronic inflammation. Furthermore, nonselective NSAIDs, while capable of suppressing inflammation, are associated with gastrointestinal toxicity, and some medications like etoricoxib have been linked to acute myocardial infarction [9].

The management of OA remains unsatisfactory for certain patients who neither respond adequately to existing conservative treatments nor meet the criteria for joint replacement surgery. In this context, nutritional products have emerged as a promising alternative approach to potentially halt, delay, or reverse the degenerative process. These natural interventions offer several advantages, including minimal side effects suitable for long-term treatment and multi-target effects encompassing anti-inflammatory, anti-apoptotic, anti-catabolic, antioxidant, anabolic, and proliferative properties.

Osteoarthritis affects not only the cartilage but all joint tissues, including the synovium, subchondral bone, periarticular muscle, and supporting ligaments. While cartilage damage represents a crucial and traditionally considered irreversible feature of OA, drugs that can prevent or block cartilage destruction hold significant therapeutic potential. The current clinical approach primarily relies on symptomatic relief without apparent disease-modifying effects. Given the rising incidence of arthritis and the limitations of existing drug therapies, there is a pressing need to develop safe and effective anti-arthritic agents. This study, utilizing a Wistar rat animal model, aims to investigate the anti-arthritic and anti-inflammatory properties of Rhumaflex.

2. METHODOLOGY

MATERIALS AND METHODS

The study was conducted at Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research Centre (Sant Tukaram Nagar, Pimpri Colony, Pimpri-Chinchwad, Maharashtra

411018). The research adhered to ethical guidelines, obtaining approval from the Institutional Animal Ethics Committee and following CPCSEA protocols. The study utilized 32 Wistar rats, both male and female, aged 2-3 months and weighing 200-250 g. These were randomly allocated into four equal groups of eight animals each: Sham Control (SC), Disease Control (DC), Positive Control (PC), and Rhumaflex group. A 7-day acclimation period preceded the study's commencement. To induce osteoarthritis (OA), the rats were anesthetized using a combination of ketamine (40-100 mg/kg) and xylazine (5-13 mg/kg), as per 2018 anesthesia guidelines. OA was initiated by injecting 2 mg of monosodium iodoacetate (MIA) dissolved in 25 µl of saline into the left knee joint cavity, using a 26.5-G needle inserted via the patellar tendon. The SC and DC groups were administered 1% carboxymethylcellulose (CMC) orally from day 0 to 28. The PC group received oral Indomethacin (2 mg/kg) in 1% CMC for 28 days. Following OA induction, the test group was given an oral nutritional formula (206.46 mg/kg) in 1% CMC for 28 days.

The dose administered to the animals was calculated based on the human equivalent dose and converted to the appropriate amount for rats. With each tablet weighing 1000 mg, the human dose was specified as one tablet twice daily (2000 mg) for a 60 kg adult, equating to 33.3 mg/kg/day. To translate this to an equivalent rat dose, the human equivalent dose of 33.3 mg/kg was multiplied by a conversion factor of 6.2, deriving an animal (rat) equivalent dose of 206.46 mg/kg/day. This calculated rat dose of 206.46 mg/kg/day was then implemented for the study, adjusting the amount administered based on the weights of the individual rats to achieve the targeted dose level.

To evaluate the efficacy of the treatments, several tests were conducted throughout the study period. Behavioral assessments were performed on days 0, 7, 14, 21, and 28.

Open field test: Locomotor activity was measured using an open field test, employing video tracking software (Maze master Software, VJ Instruments). This test was conducted before OA induction and at regular intervals thereafter. The assessment focused on the number of squares crossed and immobility time over a 5-minute period. Joint swelling was monitored by measuring knee joint thickness with a Vernier Caliper at baseline and on subsequent test days.

Biomarker assessment: Biomarker analysis was performed at the study's conclusion. Blood samples were collected from anesthetized rats and processed to obtain serum. The serum was then analyzed for levels of TNF-alpha, cartilage oligomeric matrix protein (COMP), and MMP-13 using enzyme-linked immunosorbent assay (ELISA) kits. The concentration of these biomarkers was determined by measuring the absorbance of the samples, with the intensity of colour development directly corresponding to the biomarker levels.

Details of Investigational Medicinal Product:

Table 1: Each tablet (1000 mg) contains

Sr. No.	Name of the Ingredient & Plant Part Used	Scientific Name & Families	Quantity mg
1	Shallaki (Gum/Resin)	*Boswellia serrata*- Burseraceae	450 mg
2	Turmeric (Rhizomes)	*Curcuma longa* - Zingiberaceae	100 mg

3	Valerian (Roots)	*Valeriana wallichi*- Caprifoliaceae	100 mg
4	Guggul (Gum/Resin)	*Commiphora wightii* - Burseraceae	85 mg
5	Hadjod (Whole Plant)	*Cissus quadrangularis*- Vitaceae	20 mg
6	Ashwagandha (Roots)	*Withania somnifera*- Solanaceae	20 mg
7	Vitamin C	-	50 mg
8	Vitamin D3	-	7.50 mg
9	Magnesium	-	05 mg

Statistical analysis

Results were expressed as mean ± standard deviation. SPSS software version 10.00 was used for statistical analysis. Normality of data was assessed using Kolmogorov-Smirnoff test. Analysis of Variance (ANOVA) with post hoc Tukey's test was used to compare different variables amongst groups for parametric data.

3. RESULTS AND DISCUSSION

Open field test

The open field test revealed significant differences in locomotor activity among the groups by day 28. The analysis demonstrated an 84.04% reduction in squares crossed for the SC group compared to the DC group. The PC group showed a 63.91% improvement, while the Rhumaflex group exhibited a 75.74% improvement compared to the DC group. These results suggest that both the PC treatment and Rhumaflex were effective in mitigating the decline in locomotor activity associated with osteoarthritis, with Rhumaflex showing a slightly higher efficacy in restoring joint mobility.

Table 2: Number of squares crossed on open field test.

Groups	No. of squares crossed duration (Mean±SD)				
	Day 0	Day 7	Day 14	Day 21	Day 28
SC	74.5±8.298	72.37±9.425	71.5±7.502	73±5.477	72±9.457
DC	73.5±8.668	63.62±6.163	56.62±5.370*	46.75±8.924*	39.12±6.621*
PC	70.5±4.899	67.25±7.146	64.25±3.495	62.12±5.668*#	64.12±4.155#
Rhumaflex	71.25±5.007	70.25±4.464	65.62±6.022#	67±9.134#	68.75±3.882#

Analysis done using one-way ANOVA test. Significant at $P< 0.05$.
***** indicates the significant p value of SC/DC and SC/PC.
# indicates the significant p value of DC/PC and DC/Rhumaflex.

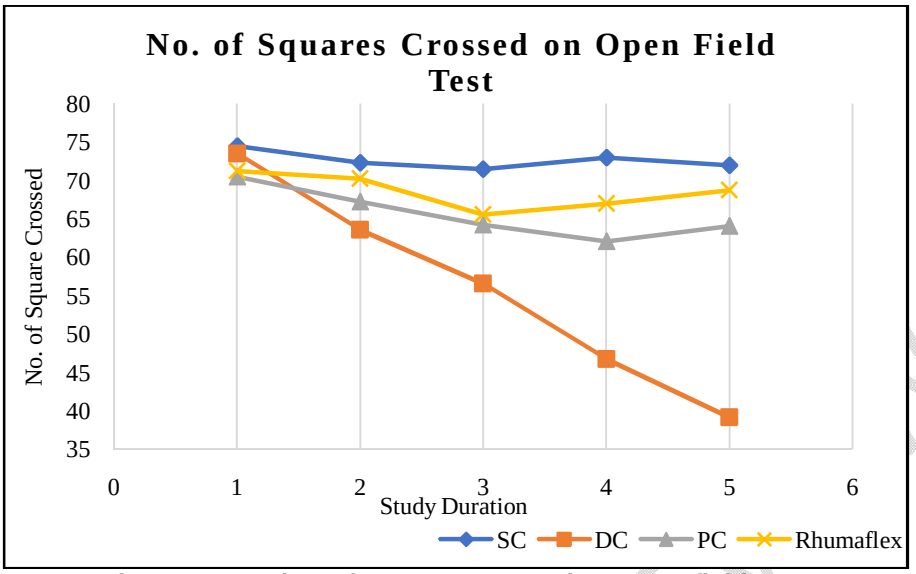

Figure 1: Number of squares crossed on open field test.

Immobility time

The data suggest that, the DC group exhibited a 39.37% increase in immobility time compared to the SC group. This indicates a substantial reduction in mobility due to OA progression.

On the contrary, both the PC and Rhumaflex treated groups demonstrated significantly less immobility time compared to the DC group. The PC group showed a 24.50% improvement in mobility (decrease in immobility time) compared to the DC group, while the Rhumaflex group exhibited a 28.89% improvement. These results indicate that both treatments were effective in mitigating OA-induced mobility impairment, with Rhumaflex showing a slightly superior effect.

The reduction in immobility time observed in the Sham and Rhumaflex treated groups suggests improved mobility, likely due to reduced pain and swelling associated with OA. This improvement was statistically significant ($p<0.05$) compared to the DC group.

Table 3: Immobility time on open field test

Groups	No. of squares crossed duration (Mean±SD)				
	Day 0	Day 7	Day 14	Day 21	Day 28
SC	54.12±3.871	55.25±3.882	52.87±2.900	53.12±2.949	53.5±2.878
DC	53.5±3.338	62.5±5.398*	68.37±5.927*	79.75±9.498*	88.25±5.445*
PC	53.25±5.092	61.75±6.065*	62.62±4.658*	64.7±4.528*#	66.62±4.470*#
Rhumaflex	52.37±4.373	56±2.507*#	60.75±3.845*#	63.75±3.991*#	62.75±3.655*#

Analysis done using one-way ANOVA test. Significant at $P < 0.05$.

***** indicates the significant p value of SC/DC, SC/PC and SC/Rhumaflex.

# indicates the significant p value of DC/PC and DC/Rhumaflex.

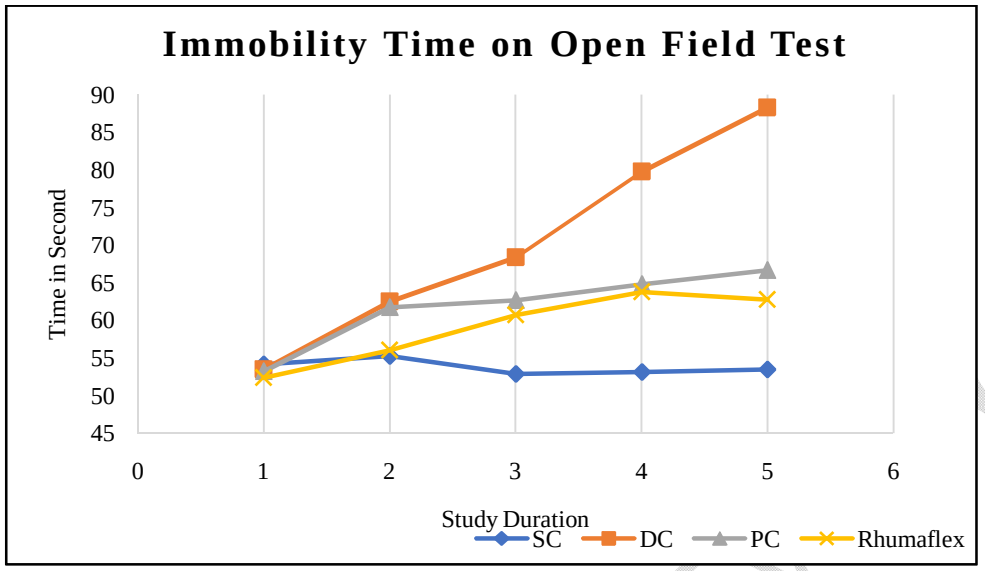

Figure 2: Immobility time on open field test

Joint swelling

The Rhumaflex group demonstrated a significant reduction in joint thickness (swelling) compared to the DC group. By day 28, there was a 15.63% reduction in joint swelling in the Rhumaflex group compared to the DC group. The PC group also showed improvement, with a 14.48% reduction in joint swelling compared to the DC group. Both Rhumaflex and PC groups exhibited significantly lower joint thickness measurements (11.60 mm and 12.40 mm, respectively) compared to the DC group (14.50 mm) on day 28. Notably, the Rhumaflex group showed significant reduction in swelling compared to the PC group.

Table 4: Joint Swelling test

Groups	Joint thickness (mm) (Mean±SD)	
	Day 0	Day 28
SC	10.13±1.46	10.06±1.37
DC	10.38±1.41	14.50±2.39
PC	10.13±0.64	12.40±1.28*
Rhumaflex	10.38±0.92	11.60±0.94*#

Analysis done using one-way ANOVA test. Significant at *P*< 0.05.
***** indicates the significant p value of SC/DC, SC/PC and SC/Rhumaflex.
# indicates the significant p value of DC/Rhumaflex.

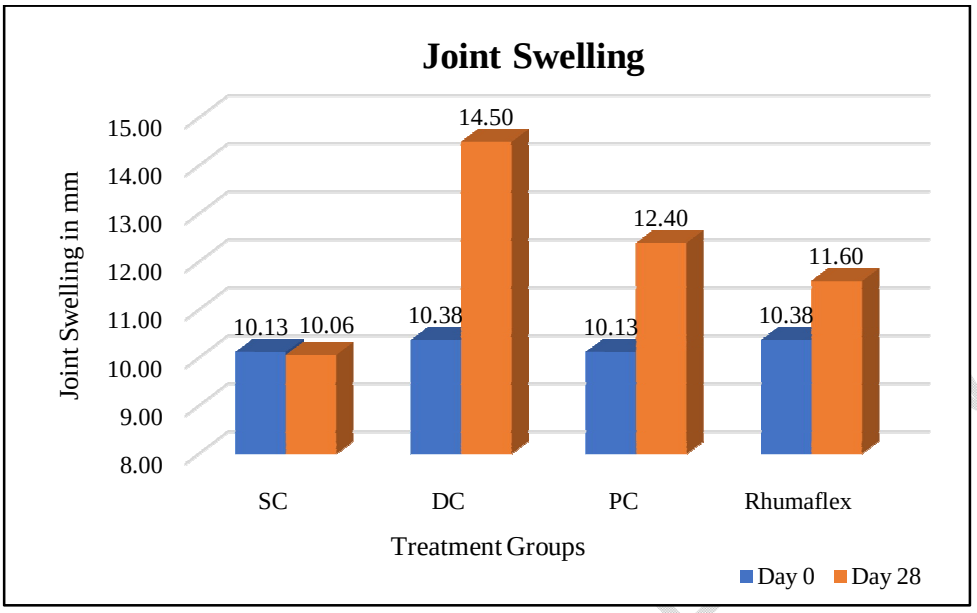

Figure 3: Joint Swelling

Biomarkers

COMP

The DC group showed significantly elevated levels of COMP (5.55 ng/ml) compared to the SC group (1.2 ng/ml), representing a 78.37% increase. Treatment with Rhumaflex resulted in a substantial reduction of COMP levels (1.75 ng/ml), demonstrating a 68.46% decrease compared to the DC group. The PC group also showed improvement, with COMP levels of 2.22 ng/ml, indicating a 60% reduction compared to the DC group. These results suggest that Rhumaflex may be more effective than the PC group in normalizing COMP levels, bringing them closer to those observed in the SC group.

MMP-13

MMP-13 levels were markedly elevated in the DC group (23.89 ng/ml) compared to the SC group (7.65 ng/ml), showing a 67.97% increase. The Rhumaflex-treated group exhibited a significant reduction in MMP-13 levels (11.06 ng/ml), representing a 55.56% decrease compared to the DC group. The PC group also showed improvement, with MMP-13 levels of 13.85 ng/ml, indicating a 44.35% reduction compared to the DC group. These findings suggest that Rhumaflex may be more effective than the PC group in reducing MMP-13 levels, potentially indicating better management of the disease process.

TNF-Alpha

TNF-Alpha levels were considerably higher in the DC group (1.87 ng/ml) compared to the SC group (0.8 ng/ml), representing a 57.21% increase. Treatment with Rhumaflex led to a notable reduction in TNF-alpha levels (1.09 ng/ml), showing a 41.71% decrease compared to the DC group. The PC group also demonstrated improvement, with TNF-alpha levels of 1.3 ng/ml, indicating a 30.48% reduction compared to the DC group. These results suggest that Rhumaflex may be more effective than the PC group in reducing TNF-alpha levels, potentially indicating a stronger anti-inflammatory effect.

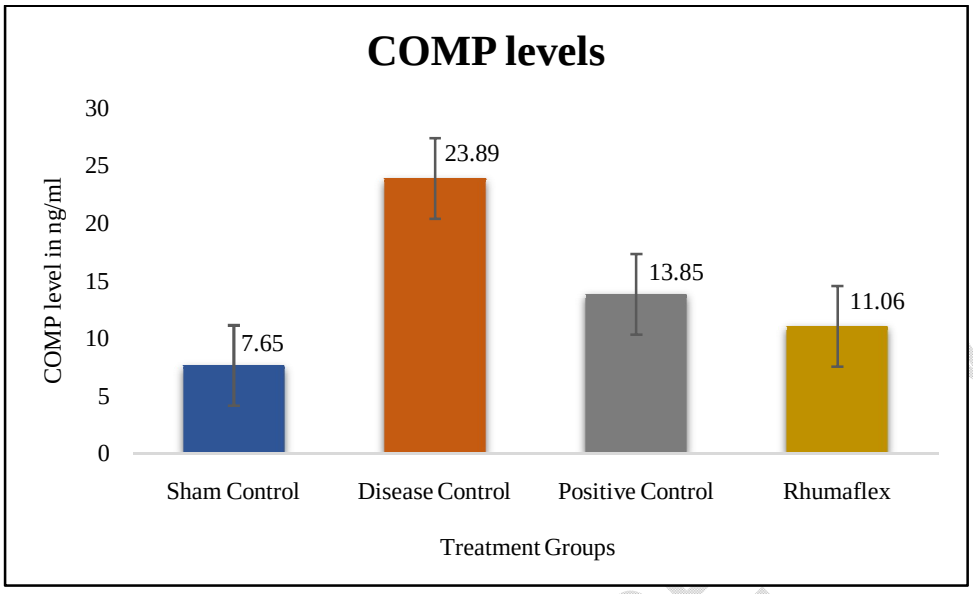

Figure 4: COMP levels

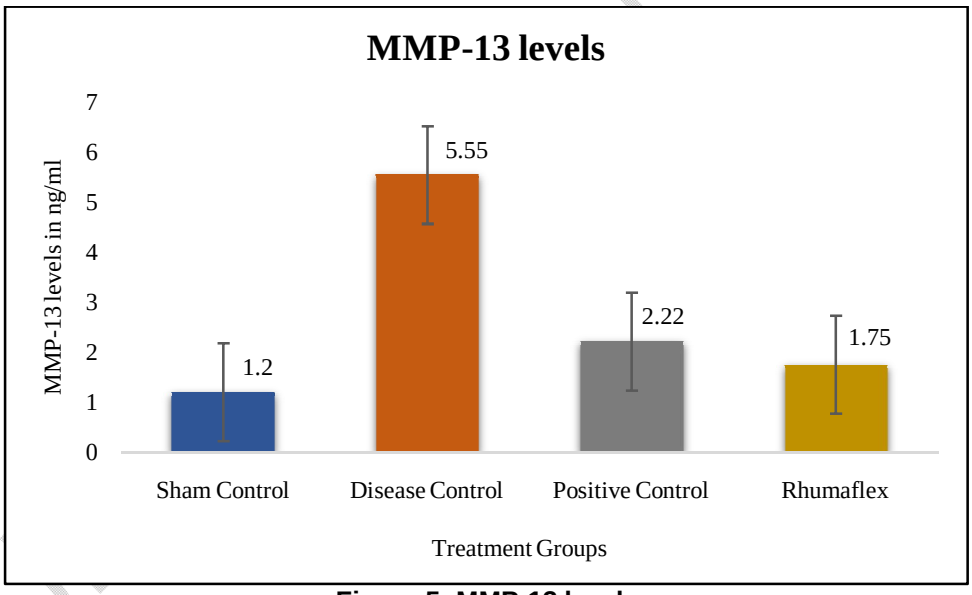

Figure 5: MMP-13 levels

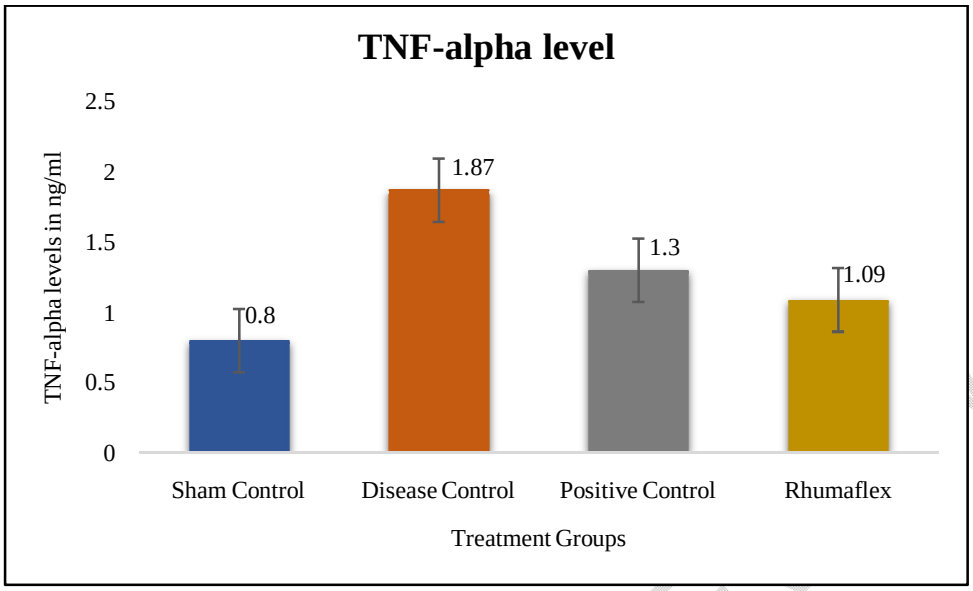

Figure 6: TNF-alpha levels

4. DISCUSSION

The present preclinical study on Wistar rats demonstrates the therapeutic potential of Rhumaflex, a novel nutritional formulation, in managing osteoarthritis (OA) through multiple mechanisms of action. The results reveal significant improvements in joint mobility, joint swelling, and key inflammatory biomarkers (COMP, MMP-13, TNF-alpha), supported by the synergistic effects of its research-proven ingredients. By leveraging the unique mechanisms of action of each ingredient, it is possible to achieve enhanced OA reducing potential that goes beyond what can be achieved by any single component alone.

The open field test results showed remarkable improvement in locomotor activity in the Rhumaflex treated group, demonstrating a 75.74% increase in squares crossed compared to the disease control (DC) group. This improvement surpassed the positive control (PC) group's 63.91% enhancement, suggesting superior efficacy in restoring joint mobility. Similarly, the immobility time data revealed that Rhumaflex treatment resulted in a 28.89% reduction compared to the DC group, outperforming the PC group's 24.50% improvement. These findings are particularly significant as they reflect meaningful functional recovery in daily activities, a crucial aspect of OA management.

The assessment of joint swelling provided further evidence of Rhumaflex's anti-inflammatory effects, with a 15.63% reduction in joint thickness compared to the DC group by day 28, slightly superior to the PC group's 14.48% reduction. These results align with previous studies on *Boswellia serrata*, a key component of Rhumaflex. The use of *Boswellia* in the treatment of OA has been studied extensively in China and India. These studies have shown that it not only has anti-inflammatory properties but also relieves pain and improves physical function [10,11].

The biomarker analysis revealed substantial improvements across all measured parameters. Rhumaflex significantly reduced COMP levels by 68.46%, MMP-13 by 55.56%, and TNF-alpha by 41.71% compared to the DC group, consistently outperforming the PC group. These results suggest comprehensive disease-modifying effects, particularly in cartilage protection and inflammation reduction. The superior performance of Rhumaflex can be attributed to the synergistic effects of its constituents. A study demonstrated that derivative of shallaki, *3-O-Acetyl-11-keto-β-boswellic acid* (AKBA), has an inhibitory effect on TNF-α

production and blocks MAPK/NFκB activation, elucidating the molecular mechanisms underlying shallaki 's therapeutic effects [12].

Research studies demonstrate *curcumin*, *Curcuma longa's* primary component, alleviates arthritis and OA symptoms. Its anti-inflammatory actions involve shielding chondrocytes from IL-1β-induced apoptosis, mitigating early cartilage degeneration, and suppressing cytoplasmic phospholipase A2 and COX-2 [13-15]. The 2019 OARSI guidelines endorse shallaki and turmeric as grade 3 treatments for OA management [16].

Numerous preclinical and clinical studies have proven that ashwagandha has anti-inflammatory and analgesic effects. It has been found to inhibit the production of TNF-α, IL-1β, and IL-12 by diminishing the activation of NF-κB and activator protein 1 (AP-1) signaling pathways [17]. Ashwagandha also slowed the degradation of bovine achilles tendon type I collagen by inhibiting the activity of collagenase [18]. Treatment with ashwagandha decreased swelling, redness, deformity, and ankylosis in a collagen-induced arthritis rat model [19]. The anti-arthritic activity of ashwagandha may be attributed to its ability to reduce biomarkers such as TNF-α, IL-1B, IL-6, MMP-8, NF-κB activation, and increase IL-10 secretion [20].

Sumantran et al. (2007) demonstrated that ashwagandha showed a significant chondroprotective effect on damaged human OA cartilage via diminishing the gelatinase activity of collagenases [21]. Another study has demonstrated its analgesic effects in patients with knee OA. In this 12-week clinical trial, treatment with 125 or 250 mg of ashwagandha was associated with significant reductions in the mean WOMAC and Knee Swelling Index. A significant reduction in pain, stiffness, and disability was also observed. The higher dose showed efficacy earlier, better physician global assessments, and less need for rescue medication [22].

The formulation's effectiveness is enhanced by *Commiphora wightii* (Guggul). Guggul contains bioactive compounds such as guggulsterol, guggulsterone, flavonoids, and coumaric acids. In OA, guggul prevents chondrocyte breakdown [23]. The results of several studies confirm the anti-inflammatory and anti-arthritic activities of guggul [24].

Dinesh Kumar et al. (2020) observed the anti-osteoarthritic activity of Cissus quadrangularis (CQ) stem for systemic and physiological recovery. The status of the antioxidative mechanism in synovial fluid is balanced by the natural enzymatic antioxidant system. The dysfunction in the production of these enzymatic antioxidants plays a crucial role in OA pathogenesis, which has been addressed by numerous reports. Recovery from the loss of SOD and GPx levels could dampen the severity of OA. Accordingly, the effectiveness of CQSE for the promotion of these enzymes in synovial fluid was demonstrated in this study [25].

According to a study conducted by Cemal Orhan et al., the therapeutic impacts of a joint health formula (JHF) containing bisdemethoxycurcumin-enriched *curcumin*, *3-O-Acetyl-11-eto-beta-Boswellic acid*-enriched shallaki, and ashwagandha in OA rats were assessed. JHF ameliorated the signs and symptoms of OA in rats by preventing oxidative stress, inflammation, and damage to the joints in a dose-dependent manner [26]. Samarasinghe et al. investigated the therapeutic potential of Lakshadi Guggul and *Cissus quadrangularis* nano-formulations. In vivo experiments using collagen-induced arthritis mice models revealed that this nano-formulation effectively promoted cartilage regeneration, chondroprotective, reduced joint inflammation, and suppressed the expression of matrix metalloproteinases and inflammatory cytokines [27].

Research suggests that antioxidants such as vitamin C could serve a protective function in preventing oxidative stress-induced chondrocyte dysfunction. Vitamin D has been associated with cartilage regeneration in OA and deficiency is associated with an increased risk of developing OA [28]. Magnesium was demonstrated to reduce the amount of cartilage damage and also reduce the serum level of the pro-inflammatory C-reactive protein [29].

By demonstrating the synergistic efficacy of a multi-component nutritional formulation, this research provides a strong foundation for future clinical trials. The comprehensive approach

of Rhumaflex, addressing multiple aspects of OA pathophysiology, suggests its potential to improve current treatment strategies for chronic degenerative conditions and tissue damage. Future research should focus on conducting large-scale clinical trials to confirm the efficacy and safety of Rhumaflex in human OA patients, investigating the long-term effects of Rhumaflex treatment on disease progression and joint health, exploring potential synergies between Rhumaflex and conventional OA treatments, and elucidating the precise molecular mechanisms underlying the observed effects of Rhumaflex and its individual components.
In conclusion, this preclinical study provides compelling evidence for the therapeutic potential of Rhumaflex in managing osteoarthritis. By harnessing the synergistic effects of its carefully selected nutritional components, Rhumaflex demonstrates promise in addressing multiple aspects of OA pathophysiology, potentially offering a comprehensive approach to improving joint health.

5. CONCLUSION

This preclinical study provides compelling evidence for Rhumaflex's therapeutic potential in managing knee osteoarthritis. The formulation demonstrated significant multi-targeted benefits, including marked improvements in joint mobility (increase in locomotor activity), reduction in immobility time, and decreased joint swelling. The anti-inflammatory and anti-arthritic effects were further validated through substantial reductions in key biomarkers: COMP (68.46%), MMP-13 (55.56%), and TNF-alpha (41.71%). The synergistic action of carefully selected ingredients, including *Boswellia serrata, Curcuma longa, and Withania somnifera,* appears to promote joint cartilage regeneration and chondroprotection through multiple mechanisms. These findings suggest that Rhumaflex could offer a comprehensive therapeutic approach for osteoarthritis management, particularly beneficial for patients seeking alternatives to conventional treatments. While these preclinical results are promising, further clinical trials are undertaking (unpublished) to confirm these benefits in human subjects and establish long-term safety and efficacy profiles.

COMPETING INTERESTS:

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL APPROVAL

The research adhered to ethical guidelines, obtaining approval from the institutional animal ethics committee and following CPCSEA protocols

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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