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ANTI-ARTHRITIC, CHONDROPROTECTIVE, AND CHONDROGENIC POTENTIAL OF NUTRITIONAL FORMULA IN MONOIODOACETATE-INDUCED OSTEOARTHRITIS ANIMAL MODEL

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ABSTRACT

Introduction: Osteoarthritis (OA) is a degenerative joint disorder hallmarked by progressive cartilage loss, osteophyte formation, subchondral bone sclerosis, and synovial inflammation. These pathologic processes ultimately manifest as joint pain, structural deformities, and functional impairment. This preclinical study aimed to evaluate the anti-arthritic activity and chondrocyte regeneration potential of nutritional formula supplements in a monosodium iodoacetate-induced (MIA) rat model of osteoarthritis. **Materials & Methods:** In the preclinical study thirty-two Wistar rats were randomly divided into four groups, 8 rats in each group. Rats from sham control (SC) and disease control (DC) groups received oral Carboxymethyl cellulose (CMC) 1%, while oral Indomethacin (2 mg/ kg) in 1% CMC was administered to rats from PC group. The test group received an oral nutritional formula (218.24 mg/kg) in 1% CMC for 28 days. **Results:** Preclinical study of nutritional formula demonstrated 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. Radiological and histopathological analysis supported these findings, indicating less degeneration in the nutritional formula group compared to controls, suggesting potential for regeneration and chondroprotection. **Conclusion:** The preclinical findings conclude that nutritional formula exhibits multifaceted benefits, including anti-arthritic and anti-inflammatory effects, offering a promising alternative for knee osteoarthritis management. Additionally, nutritional formula shows promise in promoting joint cartilage regeneration, although further investigations are warranted to assess its chondrocyte protective effect in humans.

KEYWORDS: Knee osteoarthritis, Anti-inflammatory, Cartilage repair, Chondrocyte regeneration, Monoiodoacetate.

INTRODUCTION

Osteoarthritis (OA) is a degenerative progressive chronic joint disorder resulting from cartilage deterioration and results in pain and inflammation in major joints especially in knee joints. This causes bones to rub together, leading to stiffness, pain, and limited movement. Ligaments, menisci, and muscles can also be damaged. Cartilage fragments may float in the joint space, causing further pain, while osteophytes may develop, damaging surrounding tissues. The condition involves various joint changes, including cartilage loss, bone hypertrophy, subchondral sclerosis, and alterations in the synovial membrane and joint capsule.[1,2]

Osteoarthritis is a major cause of disability, impacting about 250 million people globally. WHO reports 9.6% of men and 18.0% of women over 60 years of age have symptomatic OA. 80% of OA patients face movement limitations, hindering daily activities. The prevalence of OA is rising due to aging, obesity, metabolic issues,

genetics, and lifestyle factors like sedentary habits and smoking. Physical disability from pain affects quality of life and raises the risk of additional health problems.[1,3]

OA is degenerative disease which usually affect weight bearing knee joints. The pathological alterations found at early stage of OA affect articular cartilage and underlying bones. These alterations are induced by a combination of metabolic, genetic, and biomechanical factors. Articular cartilage is made up of a single cell type, chondrocytes, encased in an abundant ECM, and absence of blood vessels, nerves, or lymphoid tissue. Any change in its components disrupts cartilage homeostasis. OA is often associated with changes in chondrocyte activities, such as proliferation, matrix deposition, inflammation cytokine production, and signaling molecules response, since cartilage ECM is produced by chondrocytes. In addition to these changes in subchondral bones and intraarticular inflammation also contribute significantly to the degeneration in OA. Bone degeneration in OA is characterized by two phases namely, biosynthetic phase and degradative phase. By targeting these changes, OA can be reversed and articular cartilage integrity can be maintained.[4,5,6]

The available treatment options for symptomatic OA, while providing pain relief and inflammation management, have significant limitations due to their side-effect profiles and lack of regenerative potential. Medications such as acetaminophen, NSAIDs, and opioids can offer relief but may lead to hepatotoxicity, gastrointestinal and cardiovascular adverse effects, and addiction risks. Slow-acting drugs like glucosamine and chondroitin sulfate have uncertain effects, while intra-articular treatments provide only short-term relief. Innovative biological treatments like platelet-rich plasma (PRP) and mesenchymal stem cell (MSCs) show promise, but standardization is needed.[7]

In response to the limitations of current treatments for OA, there is a growing demand for more holistic approaches that leverage the potential of phytoconstituents[8] in combination with vitamins and mineral supplements like nutritional formula. This integrated approach aims to enhance OA management effectively, providing a more holistic solution for patients suffering from this degenerative condition. Additionally, the study aimed to investigate the potential of nutritional formula in promoting chondrogenesis, with the hope of finding a way to enhance cartilage repair and regeneration in the context of osteoarthritis. By evaluating the effects of nutritional formula on cartilage tissue, researchers sought to gain insights into its potential as a therapeutic intervention for managing and possibly reversing the degenerative changes associated with osteoarthritis. By exploring the synergistic effects of phytoconstituents and essential vitamins and minerals in rats, we hope to pave the way for a safer antiarthritic and more regenerative-oriented treatment paradigm for OA.

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 permission of the Institutional Animal Ethics Committee (DYPIPSR/IAEC/sept/22-23/P-23) which is registered with CPCSEA, was taken before the commencement of the study. CPCSEA guidelines was followed for the conduct of the study. The study drug nutritional formula was procured from Cachet Pharmaceuticals Pvt. Ltd. (Mumbai, India). Thirty-two Wistar rats of either sex between 2 and 3 months of age weighing 200–250 g were selected and randomly divided into four groups viz. sham control (SC), disease control (DC), positive control (PC) and a nutritional formula (test) groups. Each group comprised of eight (8) animals. Animals were acclimatized for 7 days before the start of the study. For induction of OA, animals were anesthetized with ketamine (40–100 mg/kg) plus xylazine (5–13 mg/kg) (Anesthesia (Guideline) 2018). MIA 2 mg dissolved in 25 µl of saline was injected into the left knee joint cavity using a 26.5-G needle inserted through the patellar tendon. Rats from SC and DC groups received oral CMC 1% from day 0 to day 28, while oral Indomethacin (2 mg/ kg) in 1% CMC was administered to rats from PC group for 28 days. The test group after induction received an oral nutritional formula (218.24 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 capsule of 1750 mg containing approximately 1056 mg of active blend, the human dose was specified as one capsule twice daily (2112 mg) for a 60 kg adult, equating to 35.2 mg/kg/day. To translate this to an equivalent rat dose, the human equivalent dose of 35.2 mg/kg was multiplied by a conversion factor of 6.2, deriving an animal (rat) equivalent dose of 218.24 mg/kg/day. This calculated rat dose of 218.24 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.

Following tests were conducted to assess the efficacy variables.

The behavioral tests were carried out on days 0, 7, 14, 21 and 28.

Open field test: Measurement of locomotor activity was evaluated using open field test under video tracking (Maze master Software, VJ Instruments). All the animals were subjected to open field test before the induction of arthritis i.e. on day 0 and thereafter on day 7, 14, 21 and 28. The variables assessed were number of squares crossed and immobility time for a time period of 5 min each.

Joint swelling: Measurement of knee joint thickness/swelling was measured as thickness by vernier caliper (mm) at baseline day 7, 14, 21 and 28 in all the groups.

Biomarker assessment: For assessing the biomarkers TNF- alpha, cartilage oligomeric matrix protein (COMP) and MMP-13 levels, 2 ml blood was collected on day 28 through retroorbital puncture from anaesthetized rats. The blood samples were centrifuged at 3000 rpm to collect serum. Each of the separated serum samples was divided into two equal aliquots. All the aliquots were stored at −80 °C before performing ELISA for TNF-alpha, COMP and MMP-13 levels. Krishgen ELISA kits were used for the assay. These kits use a double-antibody sandwich enzyme linked immunosorbent assay (ELISA) to assess the Rat / TNF- alpha/MMP13/COMP level in samples by measuring absorbance at the particular wavelength. The concentration of Rat TNF- alpha/MMP-13/COMP is directly proportional to the color developed.

Histopathology analysis: Knee joint specimens were collected and fixed in 10% buffered neutral formalin for up to 5 days. All fixed specimens were washed in slowly running tap water for a minimum of 30 min. Excess soft tissue was stripped away from the knee joint to allow for greater surface area. Later, specimens were decalcified in 5% nitric acid in distilled water. After decalcification, specimens were rinsed in water briefly. To neutralize acids left in specimens, they were transferred to ammonia solution for 30 min. The samples were then washed in running tap water thoroughly up to 24 h. Following decalcification, the specimens were processed overnight in a Tissue Processor. Then they were embedded in molten paraffin wax at 60 °C. Sections were cut at 5 µm with rotary micro-tome. Paraffin ribbons were attuned in a water bath at 40 °C and collected onto microscope slides. Hematoxylin and Eosin (H&E) staining was used to evaluate morphological tissue structure preservation.

The slides were scored by an independent veterinary pathologist using following criteria: Cartilage degeneration score (0 to 5), Osteophyte score (0 to 4), Calcified cartilage and subchondral bone damage score (0 to 5), Synovial membrane inflammation score (0 to 4). The maximum cumulative score was 18.

Investigational product details

Table 1: Composition of nutritional formula.

Sr. No.	Name of the Ingredient	Amount per capsules
Natural Extracts:		
1.	Collagen Peptides (Marine Source)	150 mg
2.	Glucosamine Sulphate	500 mg
3.	Chondroitin Sulphate	100 mg
4.	Rose Hips Extract	40 mg
5.	Curcumin (Curcuma Longa)	50 mg
6.	Boswellia Serrata Extract	50 mg
7.	Omega-3-Fatty Acids (From natural source providing Alpha-Linolenic Acid)	150 mg
Vitamins:		
8.	Vitamin D-3	400 IU
9.	Vitamin E Acetate	10 mg
10.	Vitamin C	30 mg
11.	Folic Acid	200 mcg
12.	Vitamin B12	1 mcg
Minerals		
13.	Zinc	5 mg
14.	Copper	500 mcg
15.	Manganese	2.5 mg
16.	Selenium	40 mcg

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.

OBSERVATIONS AND RESULTS

Open field test

The table below depicts the number of squares crossed by rats of respective groups on respective time points on open field. As evident from data the OA disease control group animals showed significant and progressive reduction in number of squares crossed signifying diminishing joint mobility, which was significantly restored by administration of nutritional formula and standard Indomethacin (Table 2 & Figure 1).

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	72.63±7.35	71.75±6.43	70.63±6.16	71.63±6.39	71.38±7.33
DC	72.38±5.48	70.63±5.32	60.13±3.64$^{\#}$	53.13±6.27$^{\#}$	39.13±3.48$^{\#}$
PC	73.75±6.69	68.5±4.28	64.75±4.46*	60.25±5.78*	63.75±3.65*
Test	73.5±6.57	72.13±3.83	68.88±5.64*	64.88±5.54*	68.63±2.72*

Analysis done using one-way ANOVA test. Significant at p< 0.05.
SC- Sham control, DC- Disease control, PC- positive control.

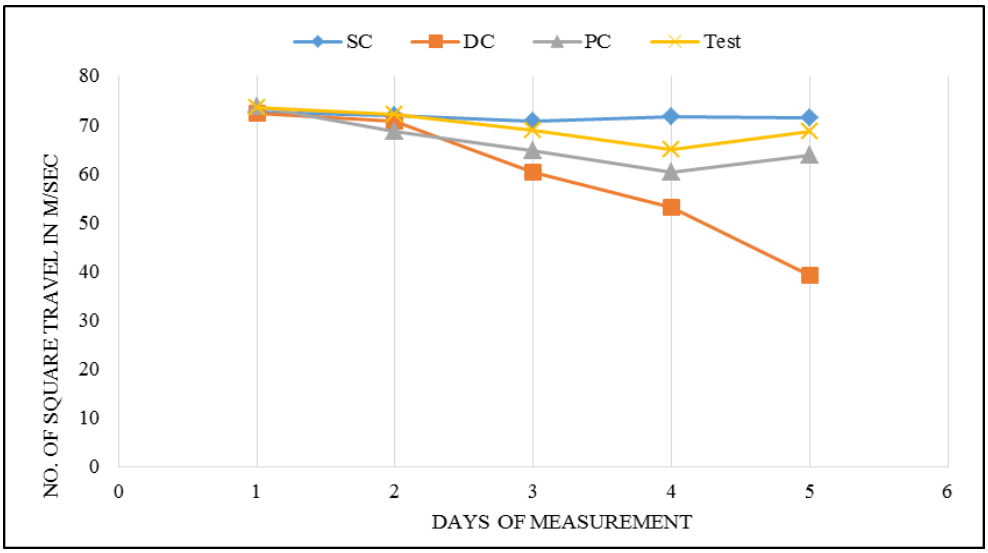


Figure 1: Number of squares crossed on Open field test.

Immobility time

If the animals with OA show a decrease in immobility time after treatment as compared to osteoarthritis model (DC), it suggests that the test (nutritional formula) is effective in providing pain relief and improving mobility.

The present data showed significant and progressive increased immobility time in OA disease control group. On the contrary, in standard and nutritional formula treated groups animals demonstrated significantly less immobility time. This indicates improved mobility due to reduced pain and swelling. (Table 3 & Figure 2).

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	54.63±3.66	55.25±3.88	53.13±2.70	53.63±2.67	53.38±2.83
DC	54.88±3.44	59.38±6.66$^{\#}$	65.38±2.62$^{\#}$	73.38±6.14$^{\#}$	86.75±4.80$^{\#}$
PC	52.25±4.92	60.25±5.78	64.75±4.46*	68.5±4.28*	69.63±6.97*
Test	53.38±2.83	56±2.51	60.75±3.85*	63.75±3.99*	62.75±4.33*

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

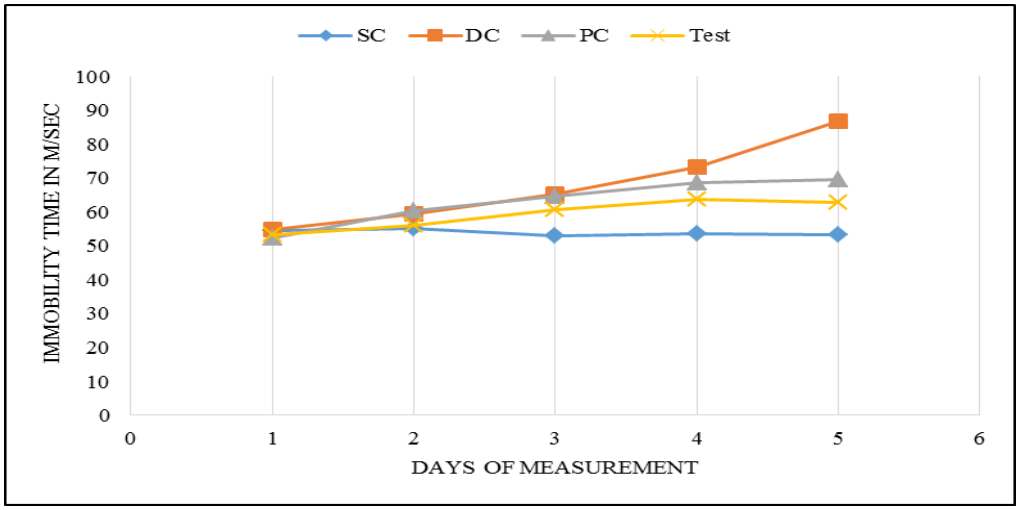


Figure 2: Immobility time on Open field test.

Joint swelling

The nutritional formula treated group showed a significant reduction in joint thickness (swelling) was observed on day 7 and continued to show significant reduction in swelling when compared to concurrent disease control group. There was 12.29% and 15.56% significant reduction in joint swelling in PC group and test group compared to DC group respectively. (Table 4 & Figure 3).

Table 4: Joint Swelling test.

Groups	Joint thickness (mm) (Mean±SD)				
	Day 0	Day 7	Day 14	Day 21	Day 28
SC	10.38±1.19	10.12±1.21	10.06±1.36	10.66±1.08	10.00±0.53
DC	10.13±0.99	10.88±1.05$^{\#}$	11.13±1.69$^{\#}$	11.44±1.99$^{\#}$	13.75±1.91$^{\#}$
PC	10.63±0.74	10.98±1.07*	11.77±0.84*	12.09±0.87*	12.06±1.09*
Test	10.13±0.99	10.71±0.18*	10.85±1.99*	10.45±1.02*	11.61±1.20*

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

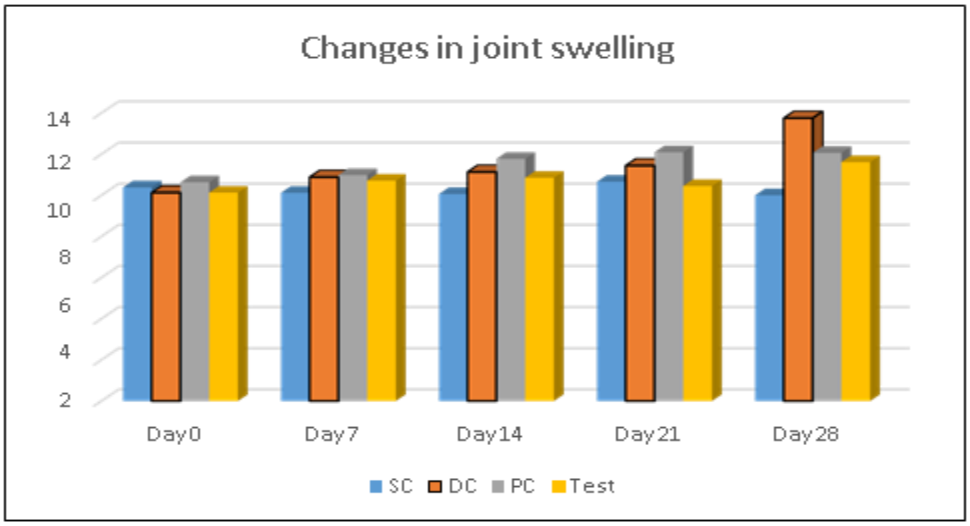


Figure 3: Joint Swelling test.

Biomarkers

From the data depicted it is evident that there was significantly elevated serum inflammatory markers like COMP, MMP-13, and TNF-alpha in the OA disease control group which got reduced after treatment of standard and nutritional formula for 28 days.

There was a 44.05% and 51.92% decrease in elevated COMP levels in the PC and test groups, respectively, compared to the DC group. (Table 5 & Figure 4).

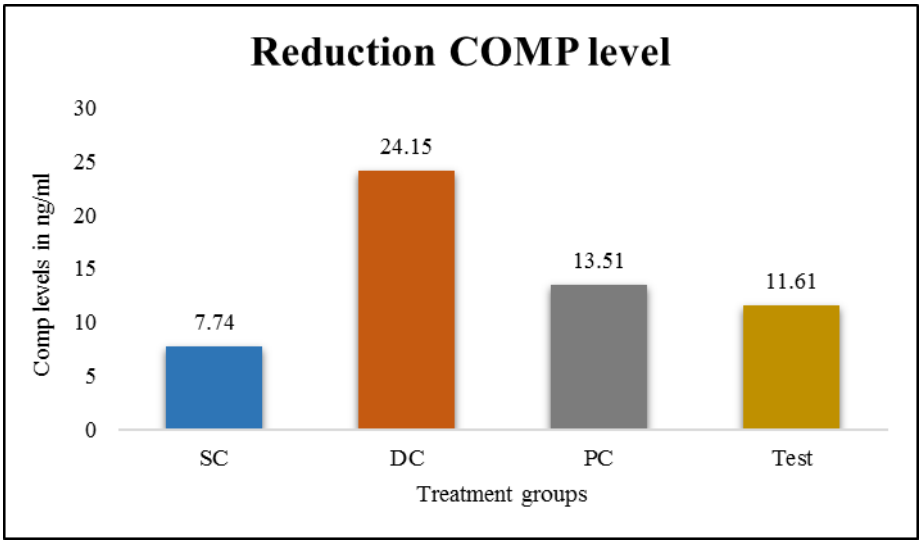


Figure 4: COMP levels.

There was a 62% and 64.83% decrease in elevated MMP-13 levels in the PC group and test group respectively, compared to the DC group. (Table 5 & Figure 5).

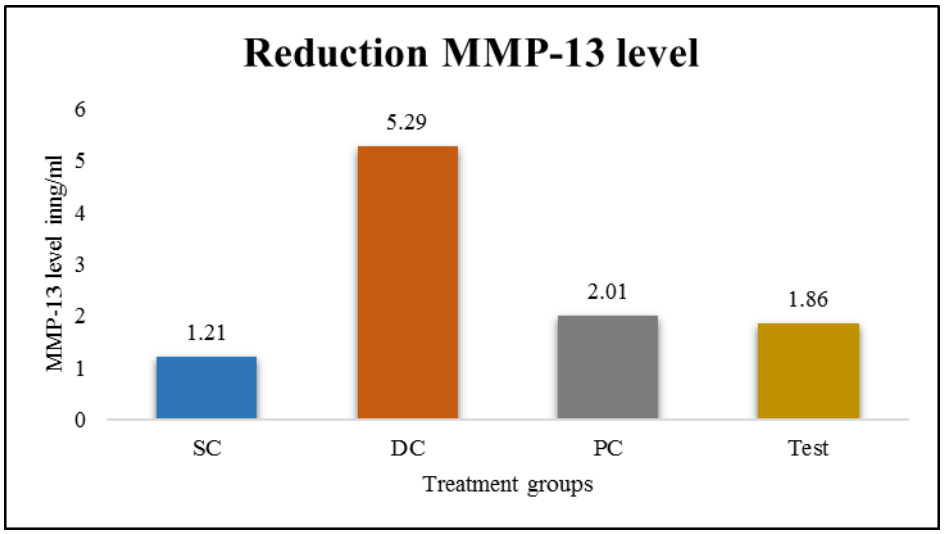


Figure 5: MMP-13 levels.

There was a 15.23% and 25.82% decrease in elevated TNF- alpha levels in the PC group and test group respectively, compared to the DC group. (Table 5 & Figure 6).

Table 5: Biomarker levels.

Groups	COMP level in ng/ml (Mean ± SD)	MMP-13 level in ng/ml (Mean±SD)	TNF- Alpha level inng/ml (Mean±SD)
SC	7.74±0.54	1.21±0.24	0.97±0.13
DC	24.15±3.47$^{\#}$	5.29±0.55$^{\#}$	1.51±0.27$^{\#}$
PC	13.51±2.53*	2.01±0.35*	1.28±0.13*
Test	11.61±1.67*	1.86±0.34*	1.12±0.12*

Analysis done using one-way ANOVA test. DC significant at $p< 0.05$ compared to SC, PC, and test group. PC not significant at $P> 0.05$ compared to test group.

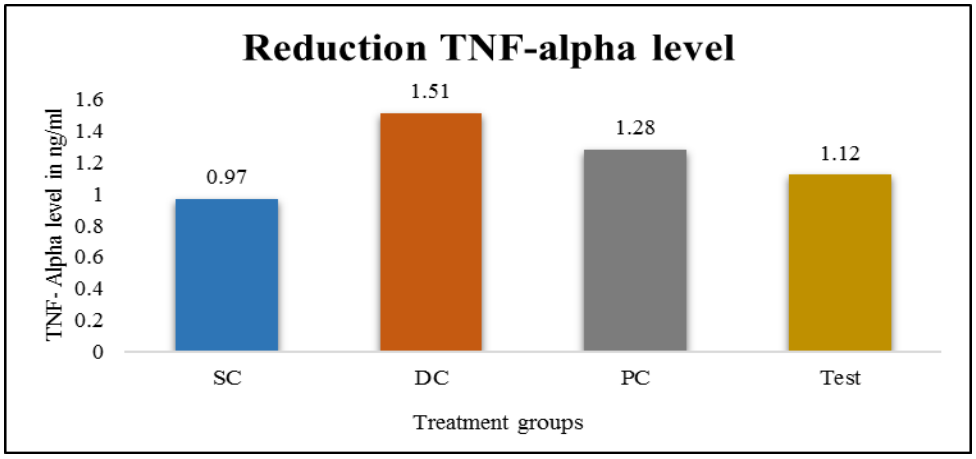


Figure 6: TNF- Alpha levels.

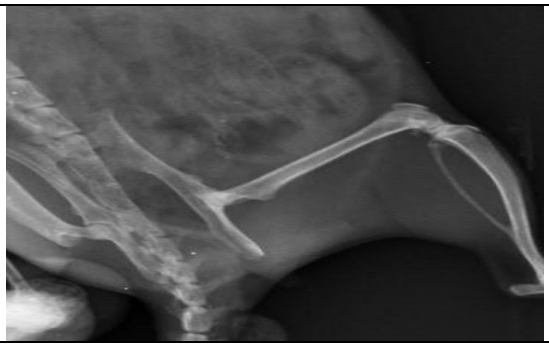
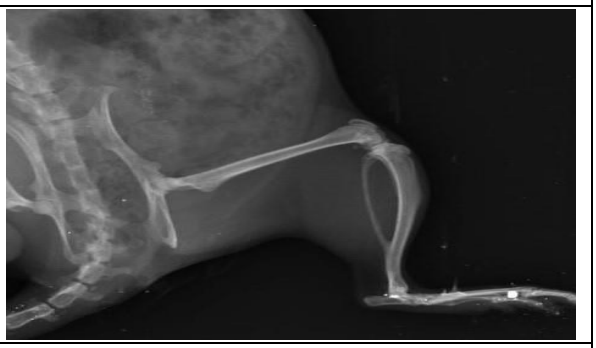
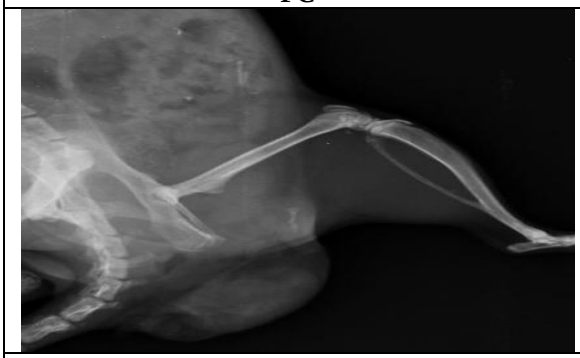
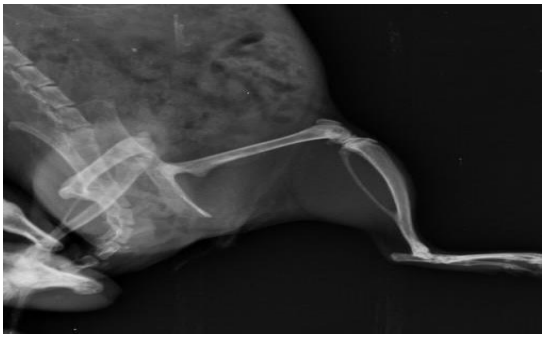
Radiological investigations

Digital X-ray images of hip and knee joints were evaluated for radiological abnormalities, including soft tissue swelling, periosteal reaction/hypertrophy, joint space narrowing, periarticular osteoporosis, and bone erosions. Abnormalities were graded from Normal (0) to Severe (3).

In the test group, knee joint and bone examinations showed no significant lesions. However, the disease control group exhibited moderate to severe abnormalities, including soft tissue swelling, periosteal reaction/hypertrophy, joint space narrowing, periarticular osteoporosis, and bone erosions over the course of treatment period (Table 6).

Rats treated with the test drug displayed mild osteoporosis and joint space narrowing in knee joints

Table 6: Radiological observations.

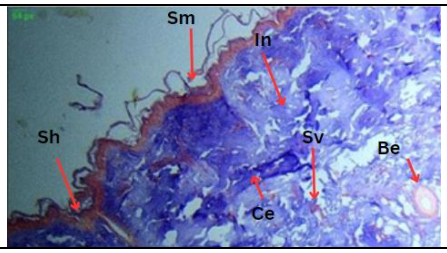
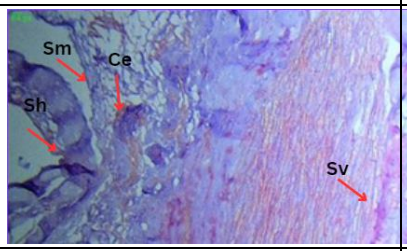
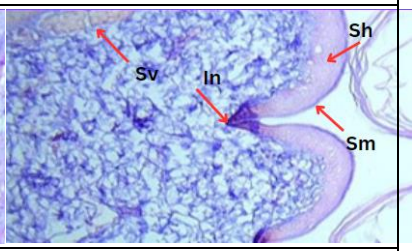
SC	**DC**
	
Showing normal morphology of bones viz., Femur (F), Tibia (Tb) and Fibula (Fb). Normal joint space with healthy cartilage at ankle joint (JsA) and periarticular soft tissue (ST)	*Showing bone erosion (B), soft tissue swelling (SS) and osteoporosis (OP), joint space narrowing (Js) and periosteal reaction (P) of moderate to severe grade.*
PC	**Test**
	
Showing soft tissue swelling (SS) and joint space narrowing (Js) and periosteal reaction (P) of mild grade.	*Showing soft tissue swelling (SS) and osteoporosis (OP), joint space narrowing (Js) of mild grade.*

Histopathological investigations

Histopathological examination of knee joint and bone of rats from normal control group did not reveal any lesion of pathological significance. Rats of the disease control group showed enlarged synovial membrane, hyperplastic synovium, increased synovial vascularity, inflammation, cartilage erosion.

The histopathological changes in knee joints of rats treated with test drug suggested mild inflammation. The histopathological changes in knee joints of rats treated with standard drug suggested mild inflammation with cartilage erosion (Table 7).

Table 7: Histopathological observations.

DC	PC	Test
		
Showing enlarged synovial membrane (Sm), hyperplastic synovium (Sh), increased synovial vascularity (Sv), inflammation (In),cartilage erosion (Ce)) and Bone erosion (Be) {H & E, 100X}	*Showing enlarged synovial membrane (Sm), hyperplastic synovium (Sh), increased synovial vascularity (Sv) and cartilage erosion (Ce) {H & E, 100X}*	*Showing enlarged synovial membrane (Sm), hyperplastic synovium (Sh), increased synovial vascularity (Sv), inflammation (In) {H & E, 100X}*

DISCUSSION

Osteoarthritis involves pathophysiology of the tissues of the joint, including the synovium, subchondral bone, periarticular muscle and supporting ligaments. Cartilage damage is a crucial and considered as irreversible feature of OA. Thus, drugs that block/prevent the destruction of cartilage will be of therapeutic value.[9]

Currently the primary approach in the clinical treatment of OA involves the use of the drugs which provide symptomatic relief but provide no apparent disease-modifying effect.[9] The increasing incidence of arthritis and the inadequacy of drug therapy underlines the need to improve the technical feasibility of discovering safe and effective anti-arthritic agents. The present study on animal model of Wistar rats was taken up to further obtain insight into the anti-arthritic and chondrocyte regeneration properties of nutritional formula supplement.

Chronic degenerative diseases and age-related tissue damage are major health challenges globally. There is a growing need for regenerative therapies that can enhance tissue repair, reduce inflammation, and promote overall tissue health.

Many of the individual ingredients in nutritional formula have been studied independently and have demonstrated promising regenerative potential.[8,11,12,13,14,15] This trial aims to explore whether the combination of these ingredients can produce synergistic effects, leading to more robust regenerative outcomes than any single ingredient alone.

The purpose of this clinical trial was to investigate the chondroprotective potential of nutritional formula in rats.

Each ingredient in nutritional formula has been carefully selected based on extensive preclinical and clinical research that supports its regenerative properties. The literature indicates that these ingredients play critical roles in promoting tissue healing, collagen synthesis, anti-inflammatory responses, antioxidant activities, and immune modulation.

Collagen from nutritional formula is having potential to support cartilage and joint health. The study showed that collagen peptides had an anabolic effect on chondrocytes, stimulating proteoglycan synthesis and supporting cartilage repair processes. Additionally, collagen peptides reduced inflammation in the joints, leading to observable and consistent reductions in inflammatory markers.[10,16]

Glucosamine Sulphate plays a role in the biosynthesis of proteoglycans and glycosaminoglycans, the building blocks of cartilage. The study indicated that glucosamine sulphate had anti-catabolic activities, inhibiting the expression of certain genes involved in cartilage degradation triggered by the pro-inflammatory cytokine IL-1β, TNFα and also reduces COMP levels.[11,12,17]

Research suggests that, chondroitin sulphate, a component of the extracellular matrix (ECM) and an essential part of cartilage, was found to have multiple beneficial effects. These effects included increased synthesis of glycosaminoglycans, enhanced hyaluronan production, significant reduction of inflammatory prostaglandin E2 (PGE2), protection against oxidative stress, and inhibition of apoptosis in chondrocytes.[14]

The standardized rose hip extract used in a subject with OA demonstrated inhibition of leukocyte functions that

cause cell injury in osteoarthritis. It also acted as a natural source of vitamin C with antioxidant potential, which could have contributed to its positive effects in reducing OA symptoms.[18,19]

Curcumin is known for its anti-inflammatory and antioxidant properties, and has been extensively used and studied in OA. It inhibits matrix metalloproteinases responsible for extracellular matrix degradation, reduces the synthesis of inflammatory cytokines, and improves joint mobility by relieving pain, swelling, and tenderness.[15,20]

Boswellia serrata extract effectively inhibits the synthesis of pro-inflammatory enzymes such as TNFα and matrix metalloproteinase (MMP-3), which contribute to inflammation and cartilage breakdown.[16]

The omega-3 fatty acids, particularly alpha-linolenic acid, demonstrated the ability to increase collagen synthesis and reduce inflammation mediator PGE2. The consumption of omega-3 PUFAs was associated with improvements in the symptoms of both osteoarthritis and rheumatoid arthritis.[21]

Naturally occurring minerals such as magnesium, copper, manganese, selenium and zinc have shown anti-inflammatory effects in both animal and human studies. Magnesium was demonstrated to enhance the amount of cartilage damage and also reduce the serum level of the pro-inflammatoryC-reactive protein.

The trace element copper is an essential cofactor in enzymes such as the collagen cross-linker lysyl oxidase and the anti-oxidant enzyme super oxide dismutase (SOD) that also requires zinc and manganese as cofactors. Recent evidence has suggested a role for oxidative stress in the pathogenesis of OA whereby an excess of reactive oxygen species arising from an imbalance in the antioxidant status of the joint (such as reduced levels of SOD) may result in cartilage degradation and joint remodeling.

Selenium is also an essential co-factor for glutathione peroxidase may have a role in reducing the incidence of osteoarthritic lesion. Positive roles have also been suggested for trace minerals in reducing the symptoms and slowing the pathogenesis of OA.[23]

Research suggests that antioxidants such as vitamin C could serve a protective function in preventing oxidative stress-induced chondrocyte dysfunction. Higher vitamin E intake is associated with better joint health. Vitamin D has been associated with cartilage regeneration in OA and deficiency is associated with an increased risk of developing OA.[23,24,25]

By combining these ingredients, we anticipate that their benefits may be amplified and contribute to a more comprehensive regenerative effect.[26]

The chondroprotective action of the nutritional formula can be explained by a dual mechanism

- ✓ The ingredients present in nutritional formula serve as fundamental components ofcartilage and synovial fluid. By promoting the anabolic pathways, nutritional formula canhelp to enhance the synthesis of important cartilage components, such as collagen and proteoglycans. This, in turn, supports cartilage regeneration and repair, ultimately contributing to the preservation of joint health and function.
- ✓ The anti-inflammatory properties of ingredients of the nutritional formula help to suppressinflammatory pathways, reducing the production of harmful mediators that contribute to cartilage damage. By mitigating inflammation, nutritional formula effectively slow down the progression of cartilage destruction, preserving the structural integrity of the joint. Their anti-inflammatory action can delay many inflammation-induced catabolic processes in the cartilage.

While animal studies have enhanced arthritis understanding, directly extrapolating findings to human arthritis requires caution due to inherent limitations. Animal studies are commonly used in preclinical research to study arthritis and provide only insights into potential treatments. However, it is important to note that arthritis in animals is typically artificially induced, which may not accurately represent the complex mechanisms and progression of human arthritis. In humans, arthritis is primarily an autoimmune disease. This means that the immune response mistakenly attacks healthy tissues, including cartilage, resulting in progressive joint damage.

Cartilage damage is a key characteristic of human arthritis. However, the artificial induction of arthritis in animal studies may not accurately represent the slow and gradual nature of cartilage degradation observed in human arthritis. The rapid destruction of cartilage observed in animal studies may not accurately represent the situation in humans, where cartilage damage occurs over time. Due to the limitations of animal models, a direct relationship between the activity observed in animal studies and the effect of potential treatments on human arthritis cannot be claimed.

Hence, we have conducted human clinical trials also to evaluate the effect of nutritional formula and by conducting clinical trials directly on human beings, we can overcome these limitations and gain direct evidence regarding the safety and efficacy of nutritional formula for arthritis treatment and management. In this manner, potential treatment benefits of nutritional formula are directly evaluated in the target population, resulting in more reliable conclusions regarding nutritional formula's potential in clinical settings.

CONCLUSION

There was a reduction in swelling and improved mobility in 7 days of treatment of nutritional formula which continued till day 28. On day 28, nutritional formula treatment showed significant anti-inflammatory activity by reducing elevated inflammatory markers like COMP, MMP-13, and TNF-alpha.

The findings are further substantiated by radiological and histopathological findings. These findings suggest minimal degeneration in the nutritional formula treated group compared to the negative control group. It may help to regeneration and chondroprotection action.

The findings of this study may have significant implications for the development of potential therapies in humans, providing a basis for clinical trials and potentially improving the treatment of chronic degenerative conditions and tissue damage in OA. By focusing on the safety, efficacy, and potential benefits of nutritional formula, this research aims to contribute to advancing antiarthritic regenerative and protective medicine and ultimately improving human health and quality of life in OA.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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