ORIGINAL PAPERS

Evaluation of the Effect of Phytocomplex on Chondroprotective Biomarkers in an Experimental Model of Osteoarthritis in Rats

Shridhar PANDYA[1], Chetan SAVALIYA[1], Dheeraj NAGORE[2], Vaishali UNDALE[2], Shubhada KHADE[2], Nishant MANTE[3]

Abstract

Osteoarthritis (OA) is most prevalent joint disease and major contributor to non-fatal burden in India, with prevalence rates of 22% - 39%. The use of conventional medication can be associated with insufficient clinical management and serious side effects. The present study aims to evaluate the anti-arthritic activity and chondrocytes protection and regeneration potential of Joint Support Product (JSP) in monosodium Iodoacetate induced Osteoarthritis rat model. Pain threshold, knee joint swelling, Blood inflammatory parameters like Tissue necrosis factor, Interlukin-6, Leukotriene B 4, C- reactive protein and arthritic biomarkers Matrix metalloproteinase-13 and Cartilage Oligomeric Matrix Protein, were estimated. Also Radiographic and histopathological evaluation were done to estimate the severity of OA. Treatment of JSP demonstrated significant increase in pain threshold by 68 % and decrease in knee joint swelling by 92 %. The inflammatory markers decreased significantly ($p < .00001$) after treatment with JSP. The Tissue necrosis factor decreased by 55 %, Interlukin-6 by 61%, Leukotriene B 4 by 57%, C- reactive protein by 69 % and arthritic biomarkers - Matrix metalloproteinase by 88% and Cartilage Oligomeric Matrix Protein by 37% as compared to disease control rats. Also the radiological evaluation and gross histopathology of rats showed improved chondrocytes structure. Thus JSP's analgesic, anti-inflammatory, chondrocyte regeneration, and chondroprotective properties have therefore demonstrated an anti-arthritic impact.

Keywords: Osteoarthritis, MMP-13 (Matrix metalloproteinase), COMP (Cartilage Oligomeric Matrix Protein), chondrocytes regeneration,

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INTRODUCTION

Osteoarthritis is a debilitating joint illness marked by stiffness brought on by inactivity, changes in subchondral bone, and persistent discomfort. Joint injury, which continues to be the leading source of chronic disability in elder persons and is frequently idiopathic, can initiate OA and speed up its course.[1] Due to its gradual and incapacitating character, which causes high morbidity and a noticeable decline in quality of life, osteoarthritis (OA) is a serious global health problem. Advanced articular cartilage degeneration, remodeling of the subchondral bone, and synovitis are all symptoms of OA. It had been established that the main molecular players in OA were the signaling pathways controlling joint development and homeostasis.

A growing body of research shows that OA affects the entire joint, including alterations to the cartilage, meniscus, synovium, and subchondral bone. The latter shows a decline in the quantity and quality of bone minerals, an increase in turnover and resorption, the creation of cysts, and the growth of marginal osteophytes.[2] The biomarker cartilage oligomeric matrix protein (COMP) can be used to diagnose arthritis and to gauge the severity of the condition. COMP levels were discovered in all fluids from individuals with various forms of arthritis, although synovial fluids had ten times higher amounts than serum, indicating preferred release from the afflicted joints.[3] Leukotriene B4 (LT-B4) is a powerful chemoattractant of neutrophils that facilitates their adherence to vascular endothelium, which facilitates the development of arthritis.[4] As a result of phenotypic changes brought on by OA, chondrocytes exhibit aberrant growth, cell death, senescence, and major alteration in gene expression, like an uptick in the expression of matrix proteins, proteolytic enzymes and inflammatory cytokines. As a result, it causes osteoarthritis and a disruption of the articular cartilage's homeostatic equilibrium. IL-1β and TNF-α levels are increased in joint tissues of OA like the subchondral bone, articular cartilage, synovial fluid, and synovium. By inhibiting anabolic activity, promoting the breakdown of articular cartilage through catabolism, and raising the generation of inflammatory mediators and IL-1β, ROS (reactive oxygen species) and TNF-α alter the chondrocytes homeostatic balance.[5] MMPs -1, MMPs -3, MMPs -13, and ADAMTS (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs) has among the proteolytic enzymes that directly break the matrix of cartilage, and their expression is increased by IL-1β and TNF-α. The enzyme MMP-13 (collagenase 3) is crucial for the cleavage of type II collagen and is crucial for the destruction of cartilage in osteoarthritic joints.[6] Early-stage OA is characterized by persistent bone alterations. Bone changes vary between cortical, trabecular, and subchondral regions and are not all the same. Importantly, individuals with progressing OA experience discomfort that is correlated with the exposure of the subchondral bone plate, rapid subchondral bone turnover, cartilage loss in areas of subchondral bone attrition, and However, it is still unclear exactly how alterations in the subchondral bone relate to other osteoarthritic occurrences. Additionally, although pain is a prominent OA presenting feature, the precise origin and molecular causes of pain coming from an osteoarthritic joint are not fully understood.

India has received a great treasure of knowledge of various natural medicine and therapies with helps of Ayurveda. These medicine are used since ages for treatment for various aliments but sufficient clinical data is not available to justify their effectiveness.

Joint support product (JSP) is a poly herbal anti-inflammatory and analgesic formulation intended to be used in inflammatory conditions like osteoarthritis. It contains key ingredients like standardized and fortified extracts of Boswellia, Curcumin, Tinospora, Guggul, Nirgundi etc.[7] Boswellia and Guggul are recognised to have anti-inflammatory, anti-arthritic, and chondroprotective activity, according to established research. While Nirgundi, Tinospora, and Curcumin have an anti-arthritic and anti-inflammatory action.[8]

The present study aims to evaluate the anti-arthritic activity and chondrocytes regeneration potential of Joint Support Product (JSP) in a Monosodium Iodoacetate-Induced Osteoarthritis Rat Model.

MATERIAL AND METHOD

1.1. Chemicals and reagents:

The study drug, Joint support Product (JSP) (LIC No. 10718015000161) was procured from Gplife healthcare Pvt Ltd, Surat. Monosodium Iodoacetate (MIA) and indomethacin was procured from Sigma Aldrich, Hyderabad.

1.2. Animals:

For the study, 30 male and female young Wistar rats of weight among 150–200 g that were in good health

and were between 2 and 3 months old were employed. Animals were obtained from the D.Y. Patil Institute of Pharmaceutical Science and Research's animal house in Pune. The animals were all held in the cages for 5 days prior to the study's commencement to allow for acclimatization, and the females were not pregnant. Animals were kept in a standard environment at a temperature of 25±2°, with a relative humidity of 45–55% (each 12 hr of light and dark cycle). All of the rats had full access to food and water during an experiment. The CPCSEA (Committee for the Purpose of Control and Supervision of Studies on Animals) and the IAEC (Institutional Animal Ethics Committee) established ethical standards for the experiments' design and execution (DYPIPSR/IAEC/OCT/21-22 P-24).

1.3. Experimental model:

All 30 animals were divided randomly in 5 groups having 6 animals each. The groups were named as Normal control, Disease control, standard treatment, JSP 100 mg and JSP 200 mg. Rats has been given 50–60 mg/kg i.p. of ketamine to make them unconscious. The left knee was then shaved, and a 27G 0.5-inch needle was introduced into the intraarticular (IA) space through the patellar tendon. Saline was intravenously injected into healthy control rats (20 ml). All other groups of rats received a single injection of 3.0 mg MIA within a total volume of 20 ml saline for induction of osteoarthritis.[9] A test drug (JSP) was suspended within distilled water and injected at the dosage of 100 mg/kg and 200 mg/kg to JSP 100 mg group and JSP 200 mg group, respectively using oral gavage for the duration of 28 days. Distilled water was administered to the normal control (NC) and osteoarthritis control (OC) groups beginning on the first day after MIA injection for a total of 28 days.

1.4. Parameters assessed:

All the animals were observed for treatment-related clinical signs and mortality if any .The weekly body weights of all animals was done and used for dose calculation.

1.4.1 Open field test

Through the use of an open field test and video tracking (VJ Instruments, Maze master Software), locomotor activity was measured. Prior to the induction of arthritis (day 0), and afterward, on day 28, all the rats were put through an open field test.

1.4.2 Joint swelling

Morphology of the joint was observed at an interval of 7 days by calculating the swelling of the joint. The knee swelling was calculated using the Vernier caliper scale.

1.4.3. Hematological parameters

Blood inflammatory parameters like TNF α (Tissue necrosis factor), LT-β- 4 (Leukotriene β 4), Interlukin-6 (IL-6), CRP (C- reactive protein) and arthritic biomarkers MMP-13 (Matrix metalloproteinase) and COMP (Cartilage Oligomeric Matrix Protein), were estimated at the end of the study.

1.4.4. Radiological evaluations of hip and knee joints of arthritis in rats

On the 28th day radiography (03 sec Duration) was done to monitor the changes in the joint morphology and density. Digital X-Ray images of hip and knee joints were processed for radiological evaluations.[10]

The following parameters were considered for radiological evaluations of the bone and joint:

1) Soft tissue swelling around the joints
2) Narrowing of the joint space
3) Periosteal reaction/hypertrophy
4) Periarticular osteoporosis
5) Bone destruction/erosions
6) Any other lesions

• Grading of abnormalities is done according to the following severity level:

• 0-Normal, 1-Slight, 2- Moderate, 3-Severe

1.4.5. Histopathological evaluation

The animals were sacrificed by anesthesia. To enable complete fixation of the joint, the patella was removed from each knee and soft tissues were taken from the left (osteoarthritic) legs. The bones of the hind limb were collected and put in formalin. These tissues underwent standard trimming and processing. Prior to processing, bones were decalcified utilizing Gooding and Stewart solution. The tissue was dehydrated in various alcohol concentrations, clarified in xylene, then embedded in paraffin wax throughout processing. Slices of tissue blocks with embedded paraffin wax that were 4-5 µm thick were cut using the Rotary Microtome. Bone slide specimens has been stained with Hematoxylin and Eosin (H & E). Under a microscope, a pathologist examined the produced slides for any histopathological lesions.[11,12] The following factors were assessed: Pannus formation, bone erosion, infiltration of inflammatory cells, synovial vascularity, synovial lining cell layer, cartilage erosion, and synovial hyperplasia.

The observed lesions' severity is noted as follows:
0: No apparent change;
1: Minimal
2: Mild
3: Moderate
4: Severe

RESULTS

1.1. Evaluation of Pain Threshold as per open field test

In the disease control group, on Day 28, the rats in a diseased group showed a decrease in activity as represented by fewer distance traveled and higher immobility time. Meanwhile, it was found that the amount of distance traveled in those treated with Joint Support product was increased by 68 % which is significantly higher than those treated with disease control. (Figure 1). The fact that the immobility time of animals treated with Joint Support product was lower than that of control patients was also found to be significant.

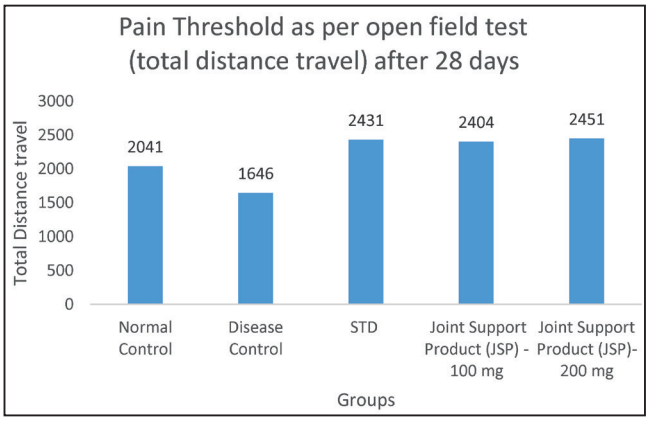


Figure 1. Evaluation of Pain Threshold as per open field test

1.2. Evaluation of Joint Thickness/Swelling

Knee joint thickness/swelling of all the rats was evaluated using Vernier caliper scale on day 0,7,14,21 and 28 days of treatment. (graph 2) On day 28 the normal control group showed the knee thickness of 10.4 mm whereas the disease control showed thickness of 11.7 mm indicating the development of arthritic swelling. The standard treatment group of indomethacin reduced the knee swelling to 10.8 mm whereas the JSP 100 mg and JSP 200 mg group rats showed knee thickness of 10.9 mm and 10.7 mm respectively. Thus the knee swelling reduced by almost 92 % as compared to disease control group. (Figure 2)

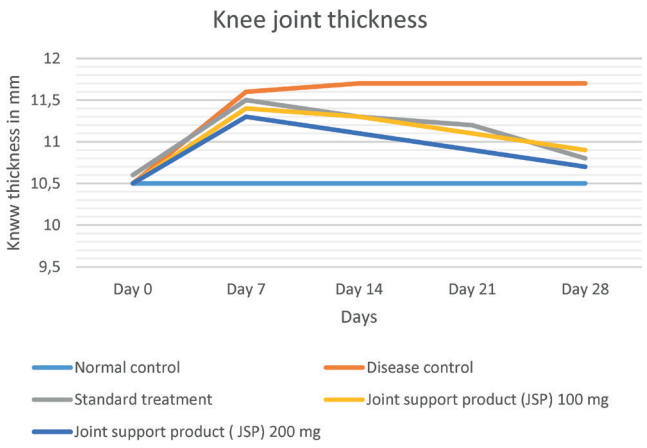


Figure 2. Trend in Knee Joint Thickness/Swelling till 28 days

1.3. Evaluation of inflammatory parameters

1.3.1. 1.3.1. Evaluation of Level of TNF-α.

On day 28 the TNF-α levels were found to be 14 pg/ml in normal control rats, whereas these levels increased to 98 pg/ml in disease control group indicating the inflammation of joints. The standard treatment group showed decrease in TNF-α value to 69 pg/ml and both the JSP treatment (100 mg/kg and 200 mg/kg) groups reduced the level to 63 pg/ml and 44 pg/ml, resp. When compared to the Disease Control group, TNF-α levels fell by 55% within the Joint Support Product group. When compared to the Disease Control group, the level of TNF-α has been noticeably lower within the Standard group and the Joint Support Product group. (Figure 3)

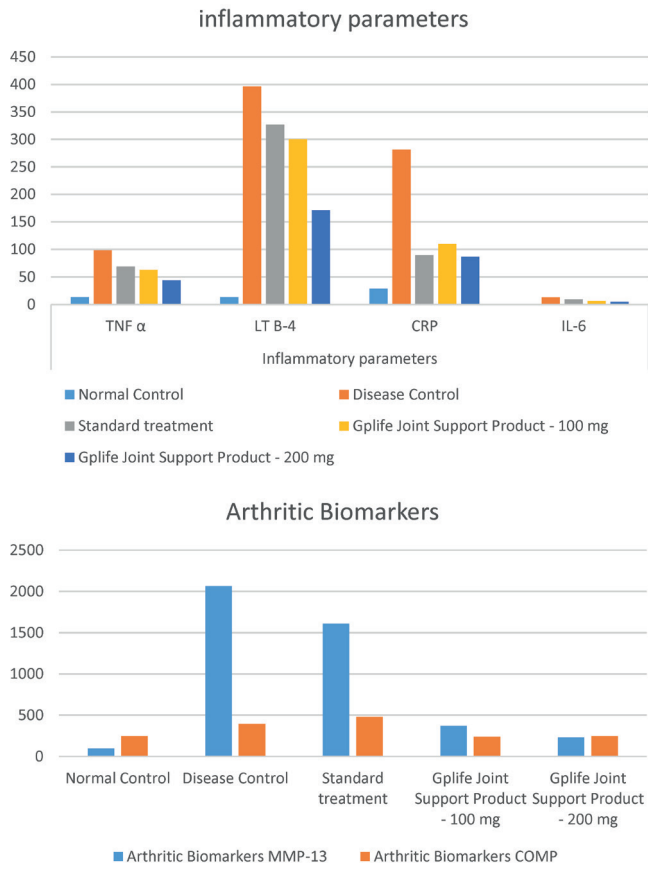


Figure 3. Evaluation of inflammatory markers and arthritic biomarkers. The p-value is < .00001. Analysis done by one-way ANOVA followed by post hoc tukey's test

1.3.2. Evaluation of Level of LT-B 4.

After treatment for 28 days , the normal control had LT-B4 levels of 14 pg/ml , whereas these levels increased significantly to 396 pg/ml in disease control group . The standard treatment group showed decrease in LT- B4 value to 327 pg/ml and both the JSP treatment (100 mg/kg and 200 mg/kg) groups reduced the level to 301 pg/ml and 171 pg/ml, respectively indicating better anti-inflammatory action in JSP groups. When compared to the Disease Control group, the Joint Support Product group's LT-B4 levels dropped by 57%. When comparing with the Disease Control group, the level of LT-B4 was significantly lower within the Standard group and the Joint Support Product group. (Figure 3) .

1.3.3. Evaluation of Level of CRP

On day 28 of treatment, the normal control showed CRP levels of 29 ng/ml , whereas these levels increased significantly to 281 ng/ml in disease control group . In standard treatment group the CRP level decreased to 90 ng/ml and both the JSP treatment (100 mg/kg and 200 mg/kg) groups reduced the level to 110 ng/ml and 87 ng/ml, resp. When compared to the Disease Control group, CRP levels fell by 69% in the Joint Support Product group. When compared to the Disease Control group, the level of CRP was noticeably lower in the Standard group and the Joint Support Product group. (Figure 3)

1.3.4. Evaluation of Level of IL-6 (pg/ml) after 28 days

The IL-6 levels were found to be 0.98 pg/ml in normal control rats, whereas these levels increased to 12.95 pg/ml in disease control group indicating the progression of inflammation of joints. The standard treatment group showed decrease in IL-6 value to 9.48 pg/ml and both the JSP treatment (100 mg/kg and 200 mg/kg) groups significantly reduced these level to 6.70 pg/ml and 5.01 pg/ml, resp. When compared to the Disease Control group, the Joint Support Product group's IL-6 levels dropped by 61%. When compared with the Disease Control group, the level of IL-6 was significantly lower within the Standard group and the Joint Support Product group. (Figure 3)

1.4. Evaluation of arthritic biomarkers

1.4.1.Evaluation of Level of MMP-13

The normal control rats had MMP-13 levels of 99 pg/ml , whereas these levels increased significantly to 2096 pg/ml in disease control group indicating development of osteoarthritic model . The standard treatment group showed decrease in MMP-13 value to 1611 pg/ml and both the JSP treatment (100 mg/kg and 200 mg/kg) groups prominently reduced the level to 371 pg/ml and 234 pg/ml, respectively indicating better chondroprotective action in JSP groups. When compared to the Disease Control group, MMP-13 levels fell by 88% in the Joint Support Product group. When compared to the Disease Control group, the level of MMP-13 has noticeably lower within the Standard group and Joint Support Product group. (Figure 3)

1.4.2.Evaluation of Level of COMP.

The TNF-α levels were found to be 247 pg/ml in normal control rats, whereas these levels raised to 396 pg/ml in disease control group indicating the inflammation of joints. The standard treatment group showed increase in COMP levels to 484 pg/

ml and both the JSP treatment (100 mg/kg and 200 mg/kg) groups reduced this level to 239 pg/ml and 246 pg/ml, resp. When compared to the Disease Control group, COMP levels fell by 37% in the Joint Support Product group. Comparing the Standard group and Joint Support Product group , the level of COMP has noticeably lowered after JSP treatment indicating non applicability of indomethacin in chondroprotection or regeneration activity. (Figure 3)

1.5. Histopathological evaluation

Rats from the normal control group's bone and knee joints underwent microscopic examination, but no pathologically significant lesions were found. Rats of the disease control group demonstrated synovial hyperplasia, enlarged synovial lining cell layer, increased synovial vascularity, pannus formation, cartilage erosion, bone erosion. and infiltration of inflammatory cells. Similar findings with less severity and number are observed in rats treated with test drug at different concentrations (100 mg and 200 mg) suggestive of mitigatory action of test drug and efficacy of the test drug to regenerate cartilage attributed to the regeneration of Chondrocytes. (table 1) (Figure 4)

Table 1. Individual Animal Microscopic Observations

GROUP	A	B	C	D	E	F	G
Normal Control	0	0	0	0	0	0	0
Disease Control	2	3	3	1	2	2	3
Healthcare Joint Support Product 100 mg	1	1	1	2	0	1	0
Healthcare Joint Support Product 200 mg	1	1	1	1	0	1	0

The above table enlist the following microscopic parameters ; A: Enlargement of the synovial lining cell layer, B: Synovial hyperplasia, C: Increased synovial vascularity, D: Infiltration of inflammatory cells in the synovial area, E: Pannus formation, F: Cartilage erosion,G: Bone erosion

Histopathology Photos (Figure 4)

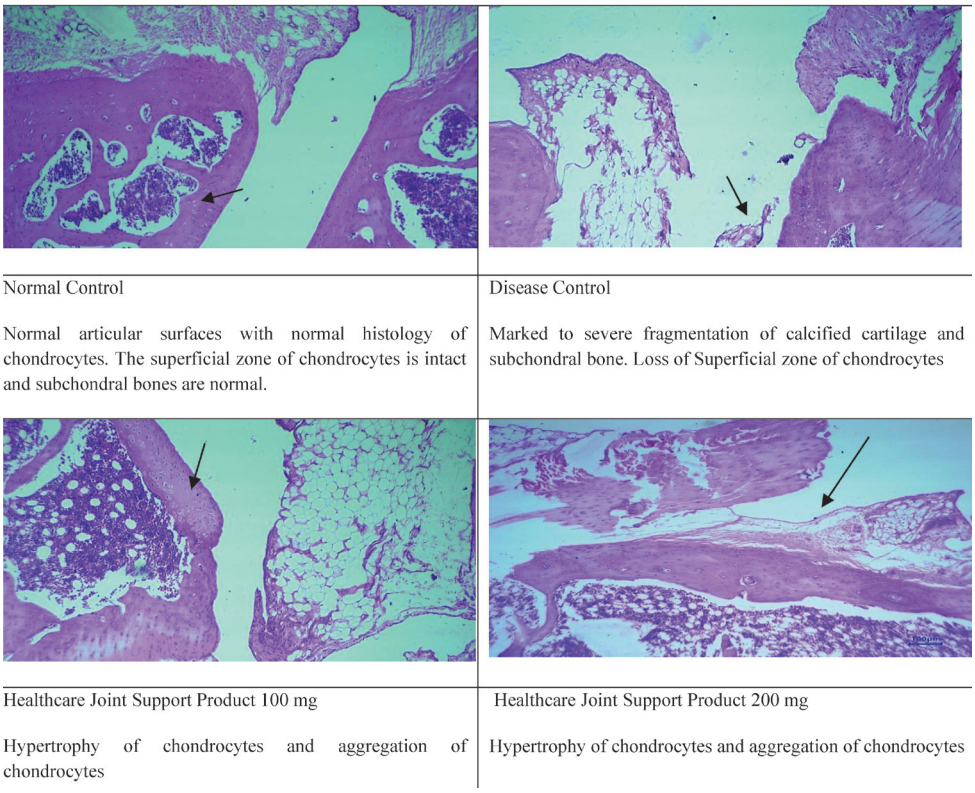
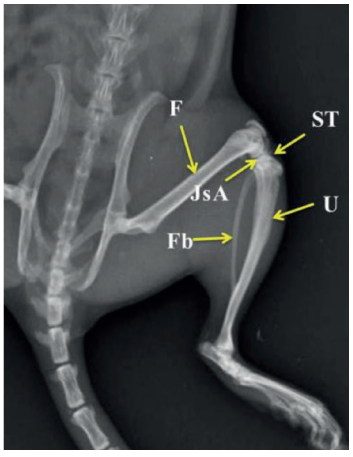


Figure 4. Histopathological evaluation of knee joint after 28 days

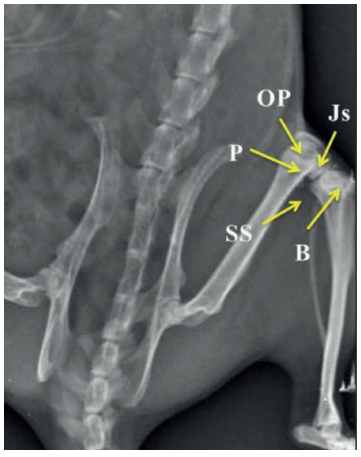
1.6. Radiological evaluations of hip and knee joints of arthritis in rats

Rats from the normal control group underwent radiological assessment of the knee joint and bone, but no pathologically significant lesions were found. Rats in the disease control group exhibited periarticular osteoporosis, bone erosions, periosteal reaction/hypertrophy, joint space narrowing, and soft tissue swelling surrounding the joints. Similar findings with less severity and number are observed in rats treated with standard and test drugs at different concentrations suggestive of mitigating action of test drug and/or efficacy of the test drug to regenerate cartilage. (Figure 5)

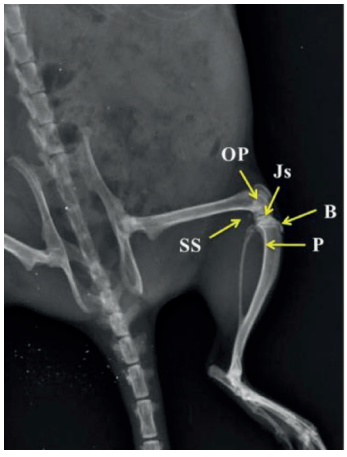
Keen joint description (Figure 5)



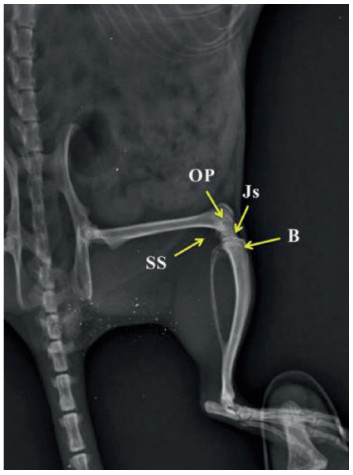
Normal Control



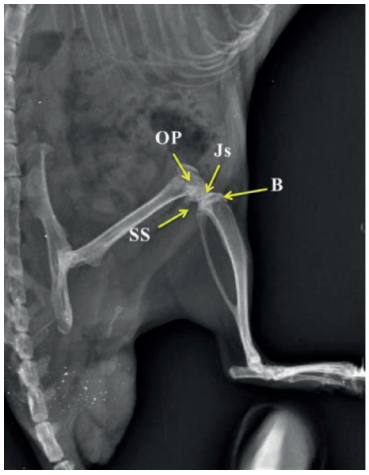
Disease Control
Swelling of the soft tissue around the joints (SS) +++, Periosteal reaction (P) +++, Joint space narrowing(Js) ++, Bone erosion (B) ++ , Osteoporosis(OP) ++



Standard Control
Swelling of the soft tissue around the joints (SS) +, Periosteal reaction (P) +, Joint space narrowing(Js) ++, Bone erosion (B) + , Osteoporosis(OP) +



Joint Support product Treatment 100 mg
Swelling of the soft tissue around the joints (SS) +, Joint space narrowing (Js) ++, Bone erosion (B) +, Osteoporosis(OP) ++



Joint Support product Treatment 200 mg
Swelling of the soft tissue around the joints (SS) +, Periosteal reaction (P) +, Joint space narrowing(Js) +, Bone erosion (B) ++ , Osteoporosis(OP) +

Figure 4. The above figure shows the radiological examination of knee joints . Here Normal Control Knee Joint : 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). Disease Control: Knee Joint : Showing bone erosion (B), soft tissue swelling (SS) and osteoporosis (OP), joint space narrowing (Js) and periosteal reaction (P). Standard control : Knee Joint : Showing bone erosion (B), soft tissue swelling (SS) and osteoporosis (OP), joint space narrowing (Js) and periosteal reaction (P). JSP 100 mg and JSP200 mg : Knee Joint : Showing bone erosion (B), soft tissue swelling (SS) and osteoporosis (OP), joint space narrowing (Js)

DISCUSSION

Osteoarthritis is the most common cause of damage to the joints. It is characterized by abnormal cartilage, inflamed and thickened synovium tissue, and altered bone structure that results in pain, diminished mobility, and disabilities. The current available treatments do not aid the delay or repair the degenerated cartilages in OA they only provide symptomatic relief.(13) Joint support product is a poly herbal anti-inflammatory and analgesic formulation intended to be used in inflammatory conditions like osteoarthritis. The present study aims to evaluate the anti-arthritic and chondroprotective and chondro regenerative ability of Joint support product in osteoarthritic wistar rats.

MIA induced osteoarthritic rats were assessed for various parameters like pain threshold as per open field test, joint swelling, blood inflammatory parameters and arthritic biomarkers, radiography and histopathological evaluation. Knee swelling is the most commonly seen symptom in OA. It restricts the patient's ability of movement and performing daily activities. The animals in the diseased group showed a reduction in activity as represented by fewer distance traveled and higher immobility time in open field test. Meanwhile, it was found that the amount of distance traveled in those treated with Joint Support product was significantly higher than those treated with disease control. Thus indicating analgesic activity of the product. The MIA induced OA rats showed significantly higher swelling than compared to both JSP treatment group, indicating the anti-inflammatory activity of the product. COMP has recently emerged as one of these potential biomarkers of arthritis(14)(3) COMP has shown promise as a diagnostic and prognostic indication, as well as a measure of the severity of the disease and therapy response, in a number of studies. In conclusion, our findings show that COMP is more prevalent in Diseases control animals than in the Standard and both JSP groups. The chondrocytes that make up the matrix of healthy articular cartilage also generate COMP. Furthermore, we were able to demonstrate that COMP production by type 1 and type 2 cells increased during the stages of OA in the region next to the primary defect. Because COMP has a broad binding repertoire, it may therefore play a role within the regeneration of cartilage tissue of OA as a substance secreted by chondrocytes to lessen matrix breakdown.

It has also been observed that pro-inflammatory markers like TNF-a, IL6, etc., upregulate the expression of MMP-13 in OA.(15) Therefore, aberrant MMP-13 induction in OA cartilage may function as a stress-related triggering event that releases chondrocyte maturational arrest and promotes differentiation into a hypertrophic-like state.[16] MMP-13 is a vital enzyme in osteoarthritis that plays a central role in the degradation of articular cartilage. Its degree of expression is increased in animals with OA and thus, inhibition of MMP-13 might be a new therapeutic target. MMP 13 levels has been significantly decreased within the joint support product group when compared to the disease control. This suggests that the joint support product showed anti-arthritic properties, as well as chondro-regeneration properties

In this investigation, we discovered that a decrease in MMP-13 stabilized the extracellular matrix of micro mass cultures, hindered the maturation of chondrocyte terminals, increased the survival of the cultures, and blocked a number of effectors and regulators of chondrocyte differentiation. The decreased levels of MMP-13 indicate the chondroprotective effect and anti-arthritic potential of joint support product. In X-rays evaluation, less severity and number of Joint space narrowing, soft tissue swelling around the joints and Bone erosion are observed in rats treated with test drugs (JSP) at different concentrations suggestive of mitigatory action of test drug and/or efficacy of the test drug (JSP) to regenerate cartilage[12] imaging plays an important role in clinical trials and epidemiological observational studies. In this narrative review article, we will describe recent developments in imaging of osteoarthritis in the research arena, mainly focusing on literature evidence published within the past 3 years (2014-2017. This indicates that Joint Support Product has chondroprotective and chondro-regeneration activity. In the current study's histopathology of joints, the damage to the cartilage and subchondral region in the Joint Support Product group was shown to have recovered, indicating that the damage had not as severe as in the disease control group in terms of the subchondral and cartilage regions.

CONCLUSION

JSP has shown improvement in the structure of cartilage and subchondral regions of knee joints in the MIA induced osteoarthritic rats. So it is clear that JSP has an anti-arthritic impact due to its analgesic, anti-inflammatory, chondro protective, and chondrocyte regeneration actions.

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Conflict of interest
Dr. Shridhar Pandya, Dr. Chetan Savaliya, are directors in GPlife Healthcare Pvt. Ltd**.** Other authors declare non competing interests.

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Compliance with ethics requirements The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2013, as well as the national law.

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