Original Article

Evaluation of the Chondroprotective Effect of an Ayurvedic Formulation Myostaal Forte Tablet in Experimental Model of Osteoarthritis in Rats

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Abstract

Background: Osteoarthritis is one of the prevalent and degenerative disorders of the joints that causes significant pain and functional disability. It is a disease in which not only the articular cartilage of the synovial joint is affected, but also the adjacent bone, ligaments, capsule, synovial membrane, and even peri-articular muscles are distressed.

Purpose: The purpose was to evaluate the chondroprotective effect of the formulation on the monosodium iodoacetate (MIA) induced arthritis in rats.

Materials and Methods: Osteoarthritis was induced in rats by giving a single intra-articular injection of 1 mg MIA. Three groups' viz. normal group, control group, and a test group were used to study the chondroprotective effect of myostaal forte in MIA induced osteoarthritis in rats. Each group had eight animals of either sex. Four animals from each treatment group were sacrificed and examined for the histopathological examinations on 14th day of treatment and remaining on the 28th day of treatment.

Results: In the myostaal forte treated group, the chondrocytes were present up to 50% and no synovial proliferation was observed which shows the protective effect of myostaal forte against chondrocytes damage. The swelling in the knee of the myostaal forte treated group was found significantly lower.

Conclusions: Myostaal forte has chondroprotective effect and palliates the inflammation and discomfort of the osteoarthritis.

Keywords: Chondrocyte, Myostaal forte, Osteoarthritis

INTRODUCTION

Osteoarthritis is a degenerative disorder of joints that causes significant pain and functional disability. Worldwide 630 million people or 15% population of the globe are affected with osteoarthritis. It is one of the most prevalent, disabling, chronic diseases affecting the elderly, estimated incidence of this severe radiographic disease is 30% in those aged 75 and above.[1]

Myostaal forte tablets (MFT) is a proprietary polyherbal formulation that is mainly recommended for pain relief in chronic disease conditions viz. arthritic disorders (osteoarthritis and rheumatoid arthritis), frozen shoulder, gout, lumbago, cervical spondylosis, lumbar spondylosis, etc. Its major ingredients include both Shallaki (*Boswellia serrata)* and Guggul (*Commiphora wightii). B. serrata* Roxb. is known as Kundur in Unani medicine, belongs to the family Burseraceae. It chiefly possesses anti-arthritic, anti-inflammatory, anti-hyperlipidemic, anti-cancer, hypoglycaemic, anti-asthmatic, analgesic, hepato-protective, etc., activities.[2] It is also reported that Guggul have the potential to relieve the osteoarthritic pain, regenerate the cartilaginous matrix and increase sub chondral bone components.[3]

The recommended dose of MFT is one tablet twice or thrice a day. In an *in vitro* study conducted on MFT in Department of Clinical Pharmacology, TNMC and BYL Nair Charitable Hospital, Mumbai to assess the anti-platelet and anti-inflammatory activities, MFT exhibited significant anti-inflammatory effect. Furthermore, Guggul (*C. wightii*, Syn. *Commiphora mukul*), one of the main ingredient of MFT exhibited chondroprotective effect in an experimental study.[4] Therefore, this study was planned to evaluate the chondroprotective effect of an ayurvedic formulation MFT in the experimental model of osteoarthritis in rats (Table 1).

Selection of Animals

Healthy young Wistar rats between 2 and 3 months of age (male and female) weighing 150-200 g were randomly selected and divided into the control and treatment groups. The females were nonpregnant, and all the animals were kept in the cages for 5 days prior to the start of the study to allow acclimatization.

Housing

The temperature in the experimental animal room was maintained at 22°C with relative humidity between 50% and 60%. Artificial lighting was provided which includes 12 h light, 12 h dark. All the animals were given complete standardized pelleted feed, and drinking water was supplied *ad-libitum*.

Induction of Osteoarthritis

The rats were anesthetized with ketamine hydrochloride, and osteoarthritis was induced by giving a single intra-articular injection of 1 mg monosodium iodoacetate (MIA) (crystal powder M = 185.96 g/mol, Germany, Sigma**)**. MIA was dissolved in physiologic saline and administered in a volume of 50 µL using a 30-gauge needle through the infra patellar ligament of the left knee.

Table 1: Composition of the MFT

Sanskrit name	Botanical name	Quantity/Tablet
Shallaki	*Boswellia serrata*	200 mg
Guggul	*Commiphora wightii*	100 mg
Ashvagandha	*Withania somnifera*	100 mg
Haridra	*Curcuma longa*	100 mg
Guduchi	*Tinospora cordifolia*	100 mg
Shunthi	*Zingiber officinale*	100 mg
Rasna	*Alpinia galangal*	75 mg
Musta	*Cyperus rotundus*	75 mg
Nirgundi	*Vitex negundo*	75 mg
Processed in Dashamoola kwatha	Generic ayurvedic formulation	150 mg
Eranda moola	*Ricinus communis*	75 mg
Punarnava	*Boerhaavia diffusa*	75 mg
Devdaru	*Cedrus deodara*	75 mg

MFT: Myostaal forte tablets

Design of Experiment

Three groups' viz. normal group, control group, and a test group were used to study the chondroprotective effect of MFT in MIA induced osteoarthritis in rats. Each group had eight animals of either sex. Four animals from each treatment group were sacrificed and examined for the histopathological examinations on 14th day of treatment and remaining on the 28th day of treatment. The dose for rats was calculated extrapolating the human therapeutic dose (HTD) using the following formula:

Dose in rats/200 g of body weight = HTD × 0.018

HTD = 1 tablet thrice a day (Average weight of tablet = 676 mg).

Administration of Doses

The test drug (MFT) was suspended in 1% carboxymethyl cellulose (CMC) solution in distilled water and administered using oral gavage. The normal control (NC) and the osteoarthritis control (OC) groups were given 1% CMC solution in distilled water as mentioned in the Table 2 for 28 days starting from day 1 after MIA injection.

Parameters under Study

Body weight

All animals were weighed on 1st, 7th, 14th, 21st, and 28th day of the study period.

Morphology

Morphology of the joint was observed on 1st, 7th, 14th, 21st, and 28th day by calculating the swelling of the joint based on synovial fluid volume with Vernier Calliper scale using the following formula:

Synovial fluid volume (mm^3) = $(a \times b^2)/2$

Where, a: Length in mm and b: Width in mm.

Histopathology

Animals were sacrificed in two batches, four number animals at 14th day and remaining four at 28th day by ether anesthesia at the time indicated. Soft tissues were removed from the left (osteoarthritic) legs, and patella was removed

Table 2: Study design

Groups	Number of groups	Therapeutic dose (mg/200 g of body weight)	Number of animals (8 animal/group)
NC	1	Saline water	8
OC	1	1% CMC	8
MFT treated	1	12.6	8

MFT: Myostaal forte tablets, OC: Osteoarthritis control, NC: Normal control, CMC: Carboxy methyl cellulose

from each knee to facilitate thorough fixation of the joint. Tissue samples were prepared for light microscopy using standard procedures.

The microscopic observations were done for chondrocytes damage area, chondrocytes necrosis, and inflammatory cells in synovial and synovial proliferation. The histopathological scoring was done according to the severity of the damage; the scores were given as slight = 1, moderate = 2, severe = 3.

RESULTS

Weekly Body Weight

The average body weight measured every week shows that after 14 days of the treatment, the rate of increase in the body weight of the OC group was lower than the weight of NC group animals whereas in the treated MFT group, the weight gain was equivalent to that of the normal rats as shown in Table 3 and Figure 1.

Morphology

The knee swelling was calculated based on synovial fluid volume using Vernier calliper scale. In the OC group, the swelling was more as compared to that of the MFT treated group during entire study period. In the MFT treated group, the swelling in the knee was observed till 14 day of the treatment which was gradually decreased to the normal levels equivalent to the volume of NC group animals on the 28th day of the study period. The swelling in the knee of the MFT treated group was found significantly lower than the OC group at the significance level of $P < 0.5$ when compared using one-way ANOVA *post-hoc* Tukey–Kramer test (Table 4 and Figure 2).

Histopathological Findings

Histopathological changes were assessed in all OC and MFT treated rats on 14th and 28th day of treatment and were compared with the knee histopathology of normal rat.

On 14th day of the treatment, the chondrocyte layer was found to be damaged more than 60% along with synovial proliferation and the presence of inflammatory cells in all the animals of OC group. On 28th day, the chondrocytes layer was completely damaged with synovial proliferation due to sepsis formation in the OC group rats, whereas in the MFT treated group on 14th day, the chondrocytes were damaged up to 40% with the presence of the inflammatory cells in only two animals and on 28th day, 50% of the chondrocytes were present and no synovial proliferation was observed. This difference can be contributed to the protective effect of MFT against chondrocytes damage.

The histopathology of OC group shows the presence of the inflammatory cells which were absent in the animals of MFT group on the 28th day of the treatment. The absence of the inflammatory cells indicates the suppression of the inflammatory response which can

Table 3: Weekly body weights of the animals with ±SE

Days	NC	OC	Myostaal forte
Day 1	128.37±0.8	128.75±1.05	128.25±1.4
Day 7	132±0.9	131.75±1.36	131.25±1.1
Day 14	136±0.6	133.12±1.67	135.12±1.04
Day 21	140.25±0.73	132.75±2.52	140±1.41
Day 28	143.25±1.1	135±2.85	142.5±0.95

NC: Normal control, OC: Osteoarthritic control, SE: Standard error

Table 4: Synovial fluid volume based on knee swelling

Days	NC	OC	Myostal forte
Day 1	1.85±0.056	1.95±0.11	1.83±0.05
Day 7	1.84±0.07	2.29±0.16	2.13±0.06
Day 14	1.85±0.005	2.46±0.17	2.07±0.05
Day 21	1.84±0.054	2.92±0.05	1.98*±0.056
Day 28	1.85±0.072	2.84±0.09	1.75*±0.9

*P<0.5 when compared with OC. NC: Normal control, OC: Osteoarthritic control

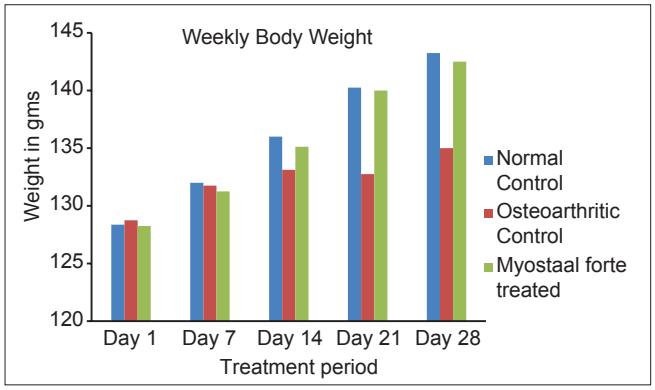

Figure 1: Average body weights

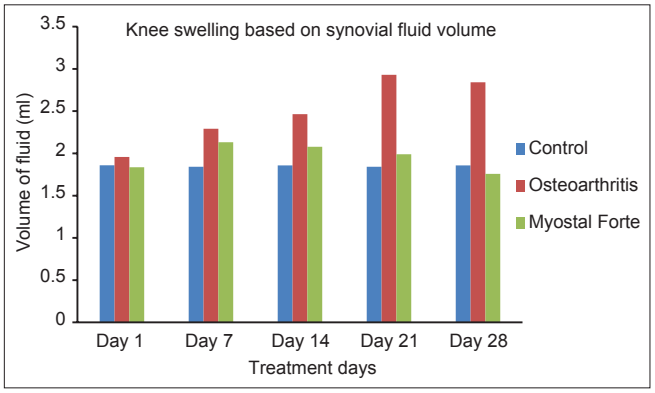

Figure 2: Synovial fluid volume based on knee swelling

be contributed to the anti-inflammatory activity of the MFT.

The total histopathological scoring for chondrocyte damage area, chondrocyte necrosis, inflammatory cells in the synovial and synovial proliferation for OC group was found to be 10 on 14th day and 12 on the 28th day of the treatment whereas it was 5.75 on 14th day and 2.75 on the 28th day of the myostaal forte tablet treatments shown in the Table 5 and Figures 3a and b.

DISCUSSION

Osteoarthritis is a degenerative disease associated with degradation of joint cartilage, structural changes, and pain in the knee joints. An experimental model of MIA induces osteoarthritis in rats in which the intra-articular injection of MIA inhibits glyceraldehye-3-phosphate resulting in disruption of chondrocyte metabolism and eventual cell death has shown a close resemblance to the pathophysiological conditions of the osteoarthritis in humans and helps in evaluation of various therapeutic agents.[5]

In this study, the MFT (*C. mukul* and *B. serrata*) a patent proprietary product of Solumiks Herbaceuticals has shown a significant action against chondrocytes damage in the rat model of MIA induce osteoarthritis. The animals of the osteoarthritic control group shown complete degradation of the chondrocyte layer, synovial proliferation, and the presence of inflammatory cells in synovial.[6,7]

Whereas, in the animals treated with MFT, chondrocyte layer was preserved up to 50% and inflammatory cells and synovial proliferation was absent on the 28th day of the treatment which reveals anti-inflammatory and chondroprotective action of MFT.

Of treated, control, and the normal groups, test substance has shown significant effect in the protection against chondrocyte damage in osteoarthritis. The absence of inflammatory cells with decreased synovial proliferation indicates the significant anti-inflammatory

Table 5: Histopathological findings

Areas	Score	Normal	OC		Myostaal forte	
			14 day	28 day	14 day	28 day
Chondrocyte damage area	+	0	1/4	0/4	1/4	4/4
	++	0	2/4	0/4	3/4	0/4
	+++	0	1/4	4/4	0/4	0/4
			2	3	1.75	1
Chondrocyte necrosis	+	0	0/4	0/4	2/4	4/4
	++	0	1/4	0/4	2/4	0/4
	+++	0	3/4	4/4	0/4	0/4
			2.75	3	1.5	1
Inflammatory cells in synovial	+	0	0/4	0/4	2/4	1/4
	++	0	1/4	0/4	0/4	0/4
	+++	0	3/4	4/4	0/4	0/4
			2.75	3	0.5	0.25
Synovial proliferation	+	0	0/4	0/4	0/4	2/4
	++	0	2/4	0/4	1/4	0/4
	+++	0	2/4	4/4	2/4	0/4
			2.5	3	2	0.5
Total score		0.00	10.00	12	5.75	2.75

Score: + Slight=1, ++Moderate=2, +++Severe=3. OC: Osteoarthritic control

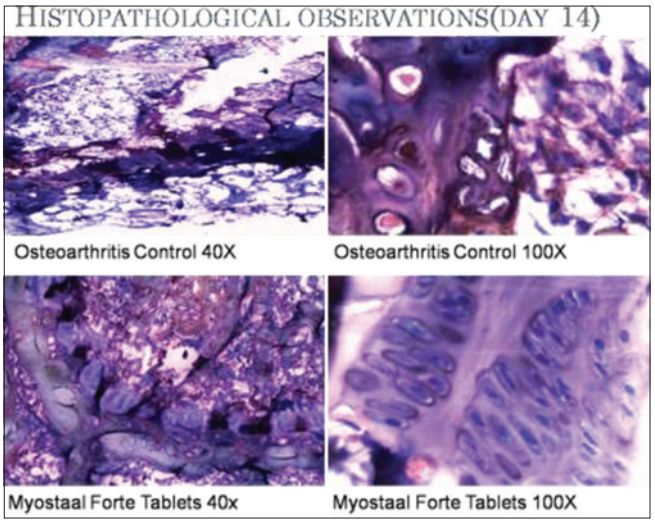

Figure 3a: Histopatological observations in the rat knee showing chondrocyte layer in the knee cartilage of osteoarthritic control, and myostaal forte treated rats, at ×40 and ×100 on 14th day of study

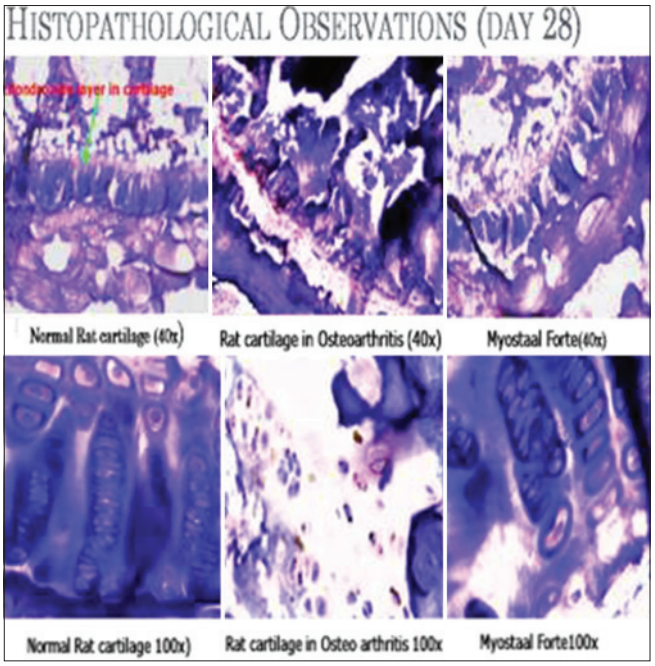

Figure 3b: Histopatological observations in the rat knee showing chondrocyte layer in the knee cartilage of normal control. Osteoarthritic control, and myostaal forte treated rats, at ×40 and ×100 on 28th day of study

and chondroprotective action of the MFT which can be helpful for the improvement of the quality-of-life in the osteoarthritic patients.

CONCLUSION

From the above results, it can be concluded that the MFT shows the time dependent protection against damage to the chondrocyte layer and palliate the inflammation and discomfort of the osteoarthritis.

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