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Evaluation of the effect of an ayurvedic formulation Myostaal forte tablets on chondroprotective biomarkers in an experimental model of osteoarthritis in rats

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ABSTRACT

Background: Osteoarthritis is a chronic progressive disease commonly affecting the hip and knee joints. Although many drugs are available and afford symptomatic relief, their side effects pose limitations to their continuous use.

Introduction: Myostaal forte (MF) is a poly herbal Ayurvedic formulation that has shown protection against damage to the chondrocyte layer on histopathological examination in previous studies. But biomarkers which are indicative of chondroprotection have not been assessed. So, the present study was planned to reconfirm the protective effect of MF in osteoarthritic rats by histopathology and create a more substantial evidence by assessing the levels of Cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-13 (MMP-13).

Methods: 32 rats were divided into four groups (n = 8 each group); sham control (SC), disease control (DC), positive control (PC) and a MF group. Behavioural tests were compared from baseline to 7th day, 14th day, 21st day and on 28th day. Histopathology and bone markers were compared on the 28th day. $p < 0.05$ was considered as statistically significant. Analysis of Variance (ANOVA) with post hoc Tukey's test was used for parametric data. Non-parametric data was analysed using Kruskal Wallis test with post hoc Dunn's test.

Results: On measurement of locomotor activity, number of squares crossed was significantly higher in MF group when compared to DC group & there was a significant decrease in the immobility time in MF group when compared DC group. Number of falls on Rota rod test was significantly lower in MF group when compared to DC on day 28. Hot Plate Analgesiometer showed no significant difference in the MF group compared to DC group but over the period of time till day 28, the latency time to lick hind paw was higher in the MF group compared to DC group. In histopathology grading, the scores in MF group were significantly reduced compared to DC group. MMP-13 levels and COMP levels in MF group were significantly decreased as compared to the DC and were statistically significant ($p < 0.05$).

Conclusion: Myostaal Forte has shown antiarthritic effect by virtue of its chondroprotective action.

Introduction

Osteoarthritis (OA) is one of the most common forms of degenerative joint disease and a major cause of pain and disability affecting the ageing population (de Lange-Brokaar, 2012). Genetic susceptibility, injuries and obesity are various factors which are considered as important risk factors (Sowers, 2001). With a prevalence of 22% to 39% in India, it is a common joint inflammatory disease. It is a chronic degenerative disorder of multifactorial aetiology characterised by the loss of articular cartilage, hypertrophy of bone at the margins, subchondral sclerosis, and range of biochemical and morphological alterations of the synovial membrane and joint capsule (Pal, 2016). The major goal in treating OA is to improve the quality of life and extend the years of athletic activity in patients. Current treatment options for OA such as topical agents have

Abbreviations: MF, Myostaal forte; COMP, cartilage oligomeric matrix protein; MMP, matrix metalloproteinase; SC, sham control; DC, disease control; PC, positive control; OA, osteoarthritis; HA, hyaluronic acid; ECM, extracellular matrix; MIA, monosodium iodoacetate; CPCSEA, committee for the purpose of control & supervision of experiments on animals; CMC, carboxymethyl cellulose; ELISA, Enzyme linked immunosorbent assay; OARSI, osteoarthritis Research Society International; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs.

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only been proven useful for short-term use, for mild to moderate pain in mild joint degeneration. While systemic drugs like NSAIDs have serious side effects such as gastrointestinal bleeding and perforated ulcers. Even locally acting intra-articular drugs like steroids give only short-term benefit for pain and function and may even accelerate disease progression (Anandacoomarasamy and March, 2010; Ayhan, 2014). Intra articular Hyaluronic acid (HA) induces chondrogenic differentiation of embryonic mesenchymal cells suggesting a potential role in favouring cartilage regeneration (Akmal, 2005). But there are meta-analyses suggesting lack of its efficacy in treatment of OA (Wu, 2017; Zhang, 2019). Also, the current medications inadequately address the pathophysiological and biochemical mechanisms involved with cartilage degeneration and the induction of pain in arthritic joints. So, there is need for a newer, more effective and possibly safer agents that would control the pain, perhaps by targeting different pain pathways.

In India, since ancient times, Ayurveda is practiced as a Traditional System of Medicine. Plant drugs advocated in Ayurveda are being predominantly used to treat a wide variety of clinical diseases, though their mechanism of action cannot be interpreted in terms of contemporary scientific language. Hence, increased research is being focused on validating the claims made in Ayurveda about these drugs and if they demonstrate potential, then conducting studies to delineate their pharmacological profiling.

Myostaal Forte is a proprietary poly-herbal analgesic and anti-inflammatory formulation for chronic disorders like osteoarthritis. Each coated tablet contains the powder of Shallaki (*Boswellia serrata*) and Guggul (*Commiphora wightii*) as the main ingredients, which have been reported to possess anti-inflammatory, anti-arthritic and chondroprotective effects (Nikam, 2013; Lahkar, 2014). Along with these, other constituents include extracts derived from Ashvagandha (*Withania somnifera*), Haridra (*Curcuma Longa*), Guduchi (*Tinospora cordifolia*), Shunthi (*Zingiber officinale*), Kulanjana (*Alpinia galanga*), Musta (*Cyperus rotundus*) and Nirgundi (*Vitex negundo*) having anti-inflammatory and anti-arthritic effects (Mishra, 2000; Kertia, 2012; Ghosh and Saha, 2012; Van Breemen, 2011; Pothacharoen et al., 2006; Singh, 2012; Kulkarni, 2008). The composition of Myostaal Forte tablet is illustrated in Table 2. Myostaal forte showed protection against damage to the chondrocyte layer on histopathological examination in a study done by Lahkar (2014). However, biomarkers which are indicative of chondroprotection or cartilage damage like matrix metalloproteinase – 13 (MMP-13) and cartilage oligomeric matrix protein (COMP) have not been assessed so far. A meta-analysis has shown that serum COMP is elevated in patients with knee osteoarthritis and it correlates to osteoarthritis disease severity (Hoch, 2011). MMP-13 is rarely detected in normal tissues and is usually seen in the joints and articular cartilage in OA patients. It is known to function as an extracellular matrix (ECM)-degrading enzyme in OA joints (Li, 2017).

The present study was planned to confirm the chondroprotective effect of MF against monosodium iodoacetate (MIA) induced OA in rats by histopathology and substantiate the existing evidence by assessing the levels of biomarkers.

Materials and methods

The permission of Institutional Animal Ethics Committee (IAEC/GSMC/4/2018) which is registered with CPCSEA, was taken before commencement of the study. CPCSEA guidelines was followed for the conduct of the study. The study drug Myostaal forte (Batch No. SF071801) was procured from M/s Shree Dhootapapeshwar Limited (Mumbai, India). Indomethacin and Monosodium Iodoacetate (MIA) were procured from Sigma Aldrich (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 Myostaal Forte (MF) groups. Each group comprised of 8 animals. Animals were acclimatised for 7 days prior to the start of the study. For induction of OA, animals were anesthetised 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 (Lahkar, 2014). Rats from SC and DC groups received oral CMC 1% from day 0 to day 28, while oral Indomethacin (2 mg/ kg) (Ashraf, 2011) in 1% CMC was administered to rats from PC group for 28 days. The MF group after induction received oral Myostaal forte (185.22 mg/kg) in 1% CMC for 28 days.

Following tests were conducted to assess the efficacy variables:
The behavioural 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.
- Rota rod Test: The rats were placed on the Rota rod at the speed of 4,5 rotations per minute (Piel, 2014) The variable measured was number of falls in one minute. The readings were taken at days 0, 7, 14, 21 and 28.
- Hot Plate Analgesiometer: It was used to assess the pain threshold of the rats. The rats were placed in hot plate Analgesiometer at a temperature of 55 ˚C with a cut off period of 15 s . Time to hind paw licking was taken as the variable in the experiment. The readings were taken at days 0, 7, 14, 21 and 28.

Biomarker assessment

For assessing the biomarkers COMP and MMP-13 levels, 2 ml blood was collected on day 28 through retroorbital puncture from anaesthetised rats. The blood samples were centrifuged at 3000 rpm to collect serum. Each of the separated serum sample was divided into two equal aliquots. All the aliquots were stored at −80 ˚C before performing ELISA for COMP and MMP-13 levels. KinesisDx ELISA kits were used for the assay. These kits use a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Rat MMP-13/COMP in samples. MMP-13/COMP were added to wells pre-coated with monoclonal MMP-13/COMP antibody. After incubation, MMP-13/COMP secondary antibodies labelled with biotin were added followed by Streptavidin-HRP to form immune complex. Unbound immune complex was removed by washing step. Then addition of Chromogenic Solution *A* and *B*, developed blue colour. Stop solution was added to stop the reaction. The concentration of Rat MMP-13/COMP is directly proportional to the colour 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 (Gerwin, 2010).

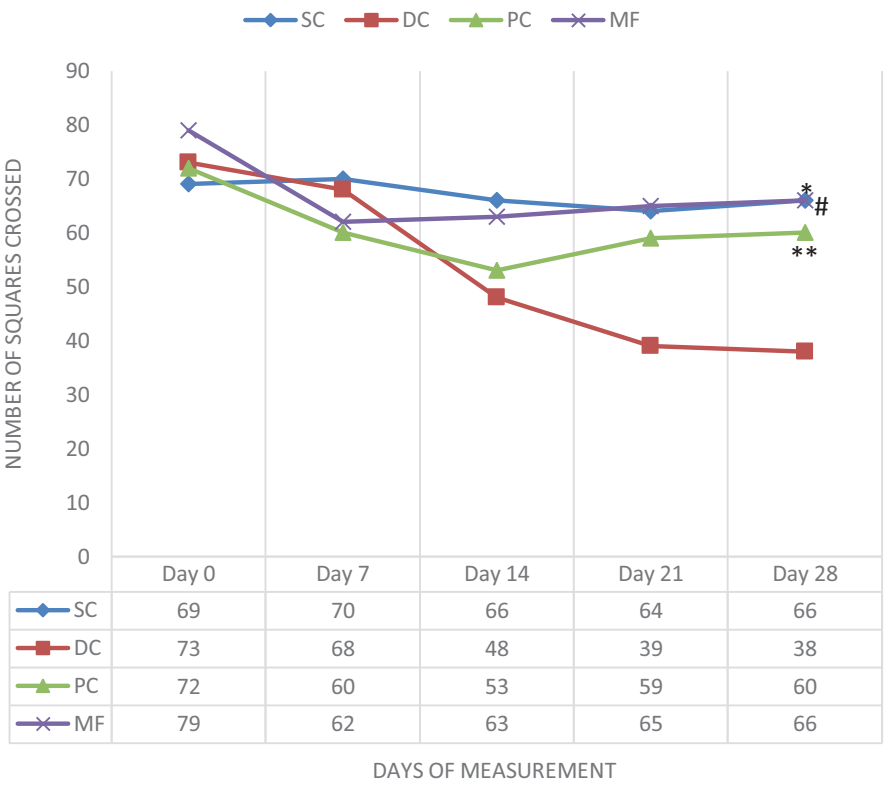


Fig. 1. Number of squares crossed on Open field test $^{*}p < 0.001$ - SC v/s DC; $\#p < 0.001$ - MF v/s DC; $^{**}p < 0.001$ - PC v/s DC. Analysis done using one-way ANOVA followed by post hoc Tukey's test.

Statistical analysis

Results were are expressed as mean ± standard deviation. GraphPad in stat software (version 3.10) 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. Non-parametric data was analysed using Kruskal Wallis test with post hoc Dunn's test.

Results

Behavioural tests

Open field test

Number of squares crossed. On day 0, the number of squares crossed was comparable in all the groups. On day 28, the number of squares crossed remained higher in the SC group compared to all other groups. There was a significant difference between SC group and DC group. Also, the number of squares crossed was significantly higher in PC group & MF group when compared to DC group. The measured results and also the trend over different days are expressed in Fig. 1.

Immobility time. On day 0, intergroup comparisons showed that the immobility time were comparable amongst all groups. On day 28, the immobility time was the least in SC group. There was a significant difference between SC group and DC group. There was significant decrease in the immobility time in MF group when compared DC group. But there was no such difference between PC group and DC group. The measured results at various interval days and the trend over different days are expressed in Fig. 2.

Rotarod test

On day 0, all the groups were comparable. On day 28, the number of falls remained least in the SC group when compared to all the other groups. There was a significant difference in the number of falls between SC and DC. The number of falls was significantly lower in PC and MF group when compared to DC on day 28. Though statistically not significant, the number of falls was decreased in MF group than PC group. The measured results and trends over different days are expressed in Fig. 3.

Hot plate analgesiometer

On day 0, all the groups were comparable. On day 28, the latency time to heat was higher in the SC group when compared to all other groups. There was significant difference in the latency time between SC and DC. There was no significant difference in the MF group or PC compared to DC group. The measured results and trend over different dates are expressed in Fig. 4.

Biomarkers -COMP and MMP −13 levels

COMP levels

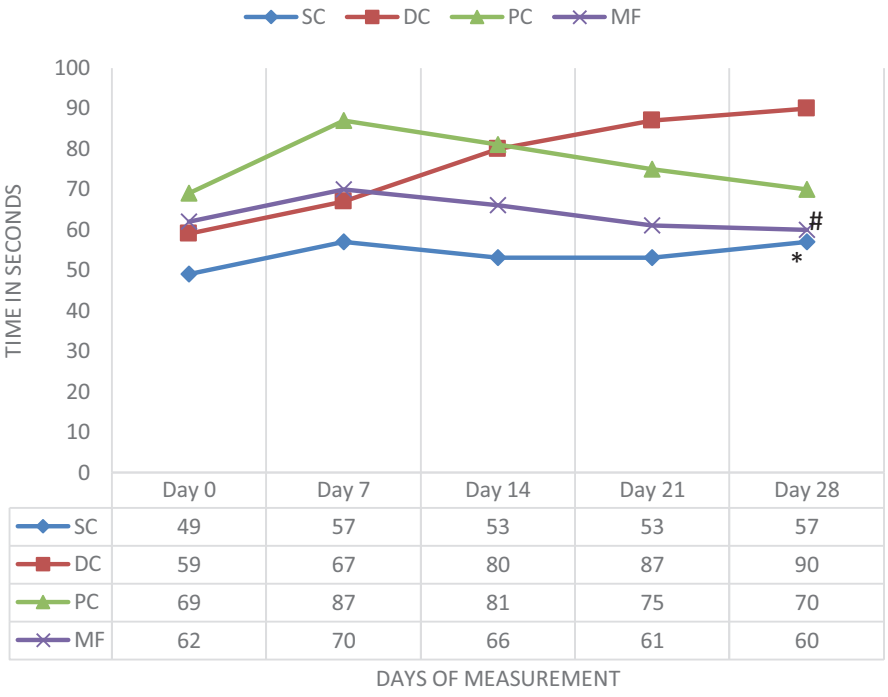
There was significant increase in COMP levels in the DC group compared to the SC group. The level of COMP was significantly low in PC group and MF group when compared to DC group. COMP level was comparable between PC and MF group (Fig. 5).

MMP-13 levels

The MMP-13 levels were higher in DC compared to the SC group. However, the difference was not statistically significant. There was significant difference in PC group and MF group when compared to DC group (Fig. 6). MF group and the PC group were comparable.

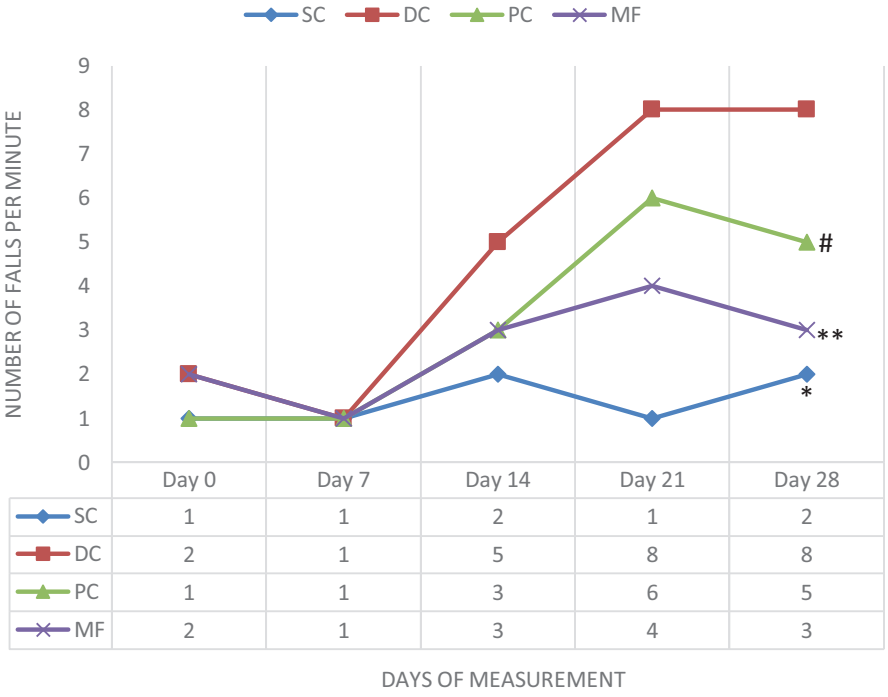
Histopathology analysis

There was a significant difference in the histopathology score of DC group when compared to SC group. The histopathological scores of DC, PC and MF group were comparable. No significant change was observed between histopathological scores of PC and MF group. Histopathology scores are summarised in Table 1. Histopathological images are represented in Fig. 7a and b.



	Day 0	Day 7	Day 14	Day 21	Day 28
SC	49	57	53	53	57
DC	59	67	80	87	90
PC	69	87	81	75	70
MF	62	70	66	61	60

Fig. 2. Immobility time on Open field test $^{*}p < 0.05$ - SC v/s DC; # $p < 0.05$ - MF v/s DC; Analysis done using one-way ANOVA followed by post hoc Tukey's test.



	Day 0	Day 7	Day 14	Day 21	Day 28
SC	1	1	2	1	2
DC	2	1	5	8	8
PC	1	1	3	6	5
MF	2	1	3	4	3

Fig. 3. Number of falls per minute on Rota rod test $^{*}p < 0.001$ – SC v/s DC; $^{**}p < 0.001$ - MF v/s DC; $\#p < 0.01$ – PC v/s DC. Analysis done using one-way ANOVA followed by post hoc Tukey's test.

Discussion

Till date no treatment is available for osteoarthritis and therefore there remains an unmet need for a new drug that can offer cure. Various disease modifying therapies are tried from modern medicine, but none has found success (Watt and Gulati, 2017). Myostaal forte is a multi-ingredient formulation developed based on knowledge and practice from traditional system of Indian medicine i.e. Ayurveda. This formulation is available in the Indian market and has sales of 3 to 4 crores annually. It is prescribed widely by the practitioners and is also available as an over the counter product. An experimental study reported previously has demonstrated its protective effects on the chondrocyte layer (Lahkar, 2014). The present study illustrates its effects on the various behavioural activities and bone biomarkers along with histopathology.

Osteoarthritis models have been established in various animals including mice, rats, rabbits, guinea pigs, dogs and horses. Mouse and rat models are most commonly used. Rats have cartilage which is thick enough to induce both partial and full-thickness cartilage defects. Methods to induce OA in rats include medial meniscus tear, anterior cruciate ligament tear, partial medial meniscectomy and iodoacetate injections. The iodoacetate model has been used most often amongst rat models (Gregory, 2012).

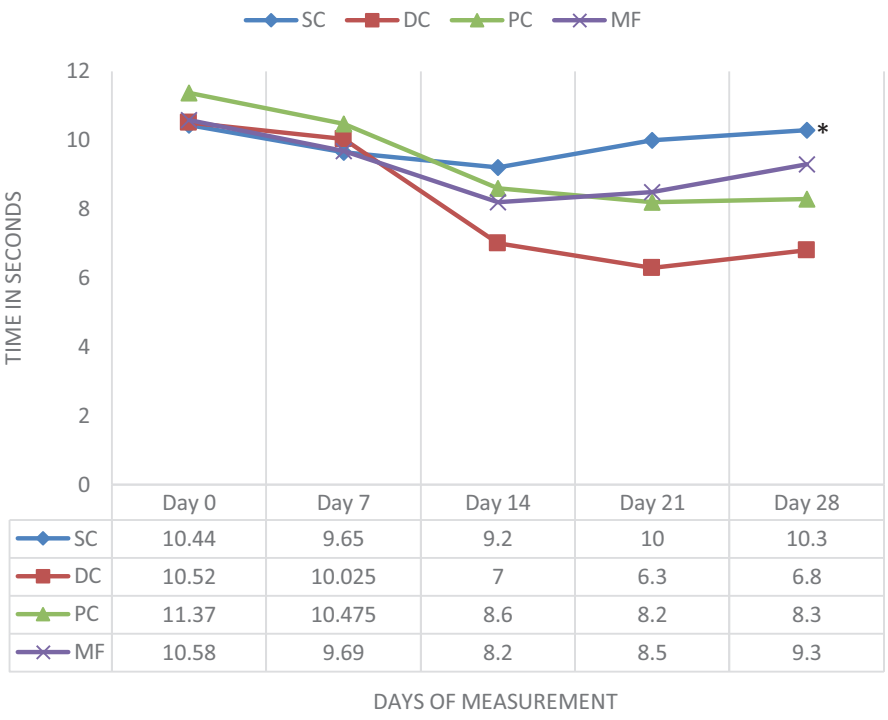


Fig. 4. Hot plate Analgesiometer $^{*}p < 0.05$ –SC v/s DC – Analysis done using one-way ANOVA followed by post hoc Tukey's test.

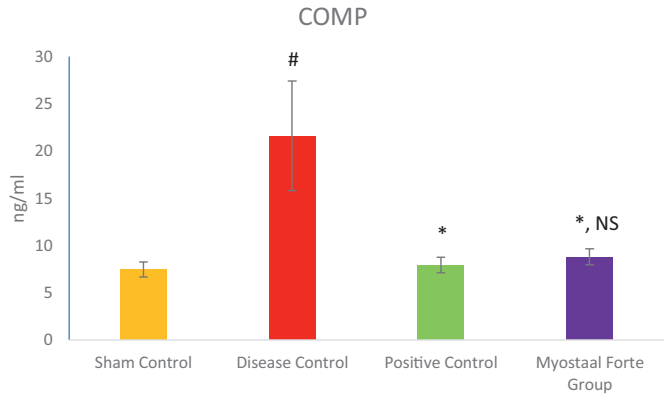


Fig. 5. COMP Levels COMP – Cartilage Oligomeric Matrix Protein; $\#p < 0.05$ compared to SC, $^{*}p < 0.01$ compared to DC, Analysis done using one-way ANOVA followed by post hoc tukey test. NS- Not significant; compared PC.

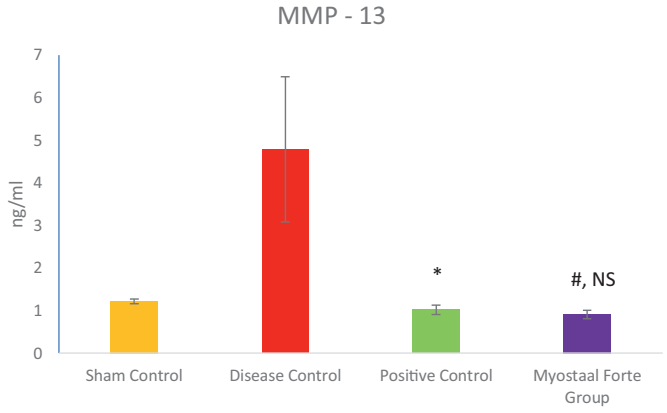


Fig. 6. MMP 13 Levels MMP-13 – Matrix Metalloproteinase 13; $^{*}p < 0.01$ vs DC compared using Kruskal Wallis test followed by post hoc Dunn's test. $\#p < 0.001$ vs DC compared using one-way ANOVA followed by post hoc tukey test. NS – Not significant; compared to PC.

Table 1
Histopathology scores.

Groups	Histopathology Score (Median, Range)
SC	0 (0,3)
DC	12.5 (11,14) *
PC	11.5 (11,13) NS1, NS2
MF Group	9 (8,11) #

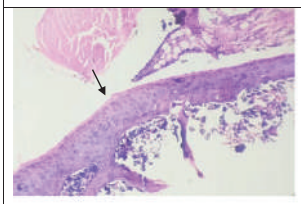
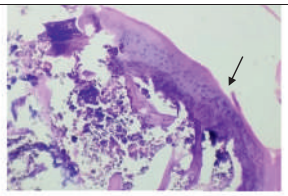
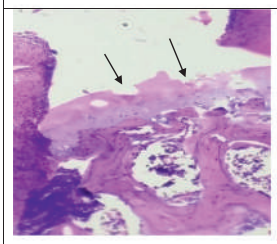
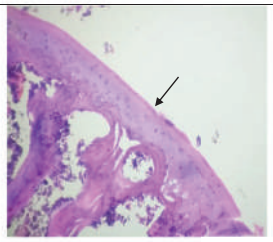
$^{*}p < 0.05$ compared to SC, $\#p < 0.05$ compared to DC, NS1 – Not Significant compared to DC, NS2 – Not significant compared to MF group. Analysis done using Kruskal Wallis test followed by post hoc Dunn's test.

The behavioural tests were carried out on days 0, 7, 14, 21 and 28 to roughly determine the time of onset and duration of action of the study drug. The behavioural tests selected in the present study included open field test, rota rod test and hot plate Analgesiometer test. They simulate various clinical tests used by the rheumatologists to evaluate patients of

Table 2
Myostaal forte ingredients & compositions.

Each coated tablet contains powder of:	
Kunduru [Shallaki] (*Boswellia serrata*)	200 mg
Shodhit Guggulu (*Processed Commiphora wightii*) and extract derived from:	100 mg
Ashvagandha (*Withania somnifera*)	100 mg
Haridra (*Curcuma longa*)	100 mg
Guduchi (*Tinospora cordifolia*)	100 mg
Shunthi (*Zingiber officinale*)	100 mg
Kulanjana (*Alpinia galanga*)	75 mg
Musta (*Cyperus rotundus*)	75 mg
Nirgundi (*Vitex negundo*)	75 mg

Processed in decoctions of: Dashamoola, Eranda moola (*Ricinus communis*), Rakta Punarnava (*Boerhaavia diffusa*) & Devadaru (*Cedrus deodara*).

Sham Control (H&E100X)	Positive Control (H&E100X)
	
Focal cartilage damage (+)	Cartilage damage (+++), calcified cartilage & subchondral damage (++)
	
Cartilage damage (++++), calcified cartilage & subchondral damage (++)	Cartilage damage (++), calcified cartilage & subchondral damage (++)
Disease control (H&E100X)	Myostaal Forte group (H&E100X)
Arrow indicate - cartilage damage	

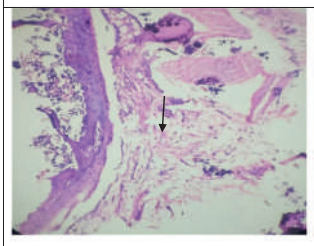
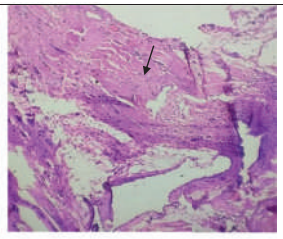
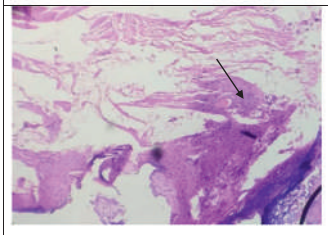
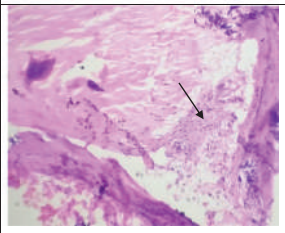
Sham Control (H&E100X)	Positive control (H&E100X)
	
Slight proliferation of synovial tissue	Proliferation of synovial tissue(++++) & infiltration of MNC.
	
Proliferation of synovial tissue(+++) & infiltration of MNC.	Proliferation of synovial tissue(++) & infiltration of MNC.
Disease control (H&E100X)	Myostaal Forte group (H&E100X)
Arrow indicate - proliferation of synovial tissue, MNC(Mononuclear cells)	

Fig. 7. (a) Comparative Chart (b) Comparative Chart.

osteoarthritis (Little and Smith, 2008) and were similar to those used in animal models of arthritis by other research workers in the field. For example, open field test was used to assess the mechanical pain sensitivity and locomotor behaviour (McIlwain, 2001). The animals were expected to restrict their locomotion to minimize discomfort depending on the severity of osteoarthritis. As seen from the Results, on Day 28, the animals in the disease control group exhibited reduction in activity which was reflected in the smaller number of squares crossed and higher immobility time. On the other hand, number of squares crossed were found to be significantly increased in Myostaal forte group compared to the disease control group. The immobility time was also significantly lower in the Myostaal forte group compared to the disease control group. Though statistically not significant, the number of squares crossed were higher and immobility time was lesser in the Myostaal forte group than found for rats in the positive control group. The number of falls on the rota rod was also lesser in the former group. These findings point towards better locomotion in the rats administered Myostaal forte than those given indomethacin, as a positive control.

The rota rod test was used to find out ability of rats to maintain balance (Shiotsuki et al., 2010). In osteoarthritis of knee there is a loss of proprioception reflex, which is responsible for loss of postural balance (Knoop et al., 2011). In a similar way, a rat when placed on a rotating rod would be expected to find difficulty in maintaining balance, more so if it has an osteoarthritic joint. With a greater degree of the disease, inability to balance on the rotating rod for a longer duration results in early fall offs. In this test, there was a significant difference in the number of falls between Myostaal group and disease control group on day 28. Though statistically not significant, the number of falls were lesser in the Myostaal forte group than observed in the positive control group.

Osteoarthritis lowers the pain threshold (Kuni, 2015). This was assessed using the hot plate method. The rats with osteoarthritis would be expected to have more pain when placed on a hot plate and therefore, to withdraw their paw within a short span. In this test, there was no significant difference observed between the Myostaal forte and disease control group on the 28th day but the trend over the days shows improvement when compared to disease control.

From these results it is evident that Myostaal forte has a trend towards increasing locomotor activity, ability to maintain balance despite constant change in surface position and latency to heat. This may be secondary to improvement in arthritic changes and implies that Myostaal forte may have analgesic and chondroprotective effect.

The time points included were to find out the effect kinetics of Myostaal forte. All the behavioural testing pertaining to locomotor activity (open field test and rota rod test) point out that the onset of action of Myostaal forte was around 14th day with peak action reaching at 21st day after initiation of treatment. The effect was seen till 28 days. As the model was of 28 days, we cannot comment on the total duration of action of this formulation beyond this time point.

Though no one particular standard offers exceptional correlation to OA, histopathology is currently the gold standard for assessing of OA in animal models (Kuyinu, 2016). It is used in experimental settings to evaluate involvement of all the structures of the entire knee. By using various scoring systems for numerous variables, the degree of damage can also be evaluated (Thysen, 2015). In the current study, the histopathology scores of Myostaal forte group were found to be significantly lower compared to the disease control but the positive control group was comparable to the disease control group. There was no statistically significant difference between the Myostaal forte and positive control groups. When the results were further analysed it was found that —- of 6 rats had score of 11 whereas —- from the positive control group had score of 11.

The histopathology scores of the present study suggest that the damage to the cartilage and subchondral region in Myostaal forte group was not as severe as in disease control. This indicates that Myostaal forte may have chondroprotective and anti-inflammatory activity. Our study re - confirms the findings of Lahkar (2014). A simpler grading system was used by Lahkar et al. to assess the histopathological findings whereas in the present study we used the grading pattern given by the OARSI (Osteoarthritis Research Society International) specific to OA in rats (Lahkar, 2014; Gerwin, 2010)

In the present study both the biomarkers, COMP and MMP-13 were found to be significantly lower in the Myostaal forte treated rats. Of

these, COMP is found in cartilage, synovial fluid and the serum in osteoarthritis. The increase in COMP levels in disease control shows the osteoarthritic disease activity. MMP −1, MMP −13 and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) play a major role in the elevation of COMP levels in osteoarthritis (Nguyen, 2017). MMP −13 levels were significantly lower compared to the disease control group. MMP-13 play a central role in the degradation of articular cartilage in osteoarthritis. Its level is upregulated in patients of osteoarthritis, which are otherwise undetectable. Inhibition of MMP-13 has emerged as a new target of interest in the treatment of osteoarthritis (Li, 2017).

In the present study, Indomethacin also showed significantly low levels of COMP and MMP-13 compared to the disease control group. Although studies have shown that Indomethacin is not chondroprotective, the reduction in the levels of biomarkers may be attributed to the anti-inflammatory action of the NSAID. In the study by Gallelli (2013) NSAID reduced the levels of proinflammatory cytokines in the synovial fluid. Proinflammatory cytokines have shown to upregulate the expression of MMP-13 in the knee joint (Chen, 2017; Chan, 2017). Similarly, COMP levels have been shown to positively correlate with the proinflammatory cytokine IL-1ß in osteoarthritis (Verma and Dalal, 2013).

Myostaal Forte has reduced the levels of MMP 13, which are going hand in hand with histopathological findings. This reduction in MMP 13 levels show that Myostaal Forte may have a chondroprotective effect by inhibiting MMP-13. COMP levels in Myostaal forte group were significantly decreased as compared to the disease control, and comparable with the positive control. The decrease of levels indicates chondroprotective effect and anti-arthritic potential of Myostaal Forte.

Conclusion

Myostaal Forte has shown antiarthritic effect by virtue of its anti-inflammatory effect and chondroprotective action.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Shetty YC: Formal analysis, Writing - original draft, Writing - review & editing. **Singh VK:** Formal analysis, Writing - original draft. **Manjesh PS:** Formal analysis, Writing - original draft. **Vetrivel Babu:** Formal analysis, Writing - review & editing. **Patil P:** Writing – original draft. **Rege NN:** Writing – review & editing.

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