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Original Research Article

Evaluation of the Effect of Pregabalin in Monosodiumiodo-Acetate (MIA) Induced Osteoarthritis in Wistar Rats

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Abstract

Background: Animal models of osteoarthritis (OA) have shown that sensory nerve fibers innervating the knee are significantly damaged with subchondral bone destruction, and induce neuropathic pain (NP). So, the investigators hypothesized, can drugs used in NP work in OA.

Methods: (Animal experimental study) After IAEC permission, Rats were randomly divided into 5 groups of 8 animals as Normal control (NC), MIA 2mg- (DC), Meloxicam 1mg/kg- (PC), Pregabalin 32.4 mg/kg- (TG-1), Pregabalin 16.2 mg/kg + Meloxicam 1mg/kg (TG-2). OA was induced by intraarticular MIA injection on day 0. After taking the baseline values, drugs treated groups were administered with respective drugs once a day subcutaneously for 28 consecutive days. Behavioral tests were compared from baseline, 7th, 14th, 21st & on 28th day. Histopathology and bone marker levels of COMP & MMP-13 were compared on the 28th day. ANOVA with post hoc Tukey's test was used for parametric data. Non-parametric data was analyzed using Kruskal Wallis test with post hoc Dunn's test.

Results: Rota rod, Hot Plate analgesiometer & Grip strength test showed significant results when TG-2 group compared to DC. Tail immersion & Acetone drop test showed significant results when TG-1 group compared to DC. In Histopathology grading, the scores in TG-2 group were significantly reduced compared to DC. MMP -13 & COMP levels in TG-2 group were significantly decreased as compared to the DC ($p<0.05$).

Conclusion: Meloxicam + Pregabalin was found to be chondroprotective & effective for pain relief which suggested OA pain is a combination of inflammatory & NP.

Keywords: Osteoarthritis, COMP, MMP-13, Grip strength.

Introduction

Osteoarthritis (OA) is the most common degenerative joint disease, affecting more than 25% of the population above 18 years and it is associated with pain and disability especially in the elderly. It is second most common rheumatologic problem with prevalence of 22-39 % in India. [1] The current pharmacological treatment of OA involves oral analgesics and anti-inflammatory agents, topical agents and intraarticular injections. [2] The large apparent variation in individual response to each drug, absence of clear clinical data regarding the

therapeutic potency, and the potential side effects are the limitations of the existing therapy. [3] Topical agents have only been proven useful for short-term use for mild to moderate pain in mild joint degeneration. [4] Intra-articular injections of corticosteroids, as indicated by a few studies, are only of short-term benefit for pain and function. [5] Commonly used medications such as non-steroidal anti-inflammatory drugs (NSAIDS) have serious dangerous side effects like gastrointestinal bleeding and ulcers can perforate if given along with steroids. Consequently, one long-standing focus of drug discovery has been the search for novel analgesics and disease modifying agents. [6]

Intra-articular injection of Monosodium-iodoacetate (MIA) into the femorotibial joint space of rodents induces a pathology with temporal similarities to OA, and this model of OA is now used for investigating the pathogenesis of knee pain. [7] It cause rapid induction of OA within seven days and clear data representing replication of cartilage lesions and functional joint impairment similar to human beings.

Chronic OA simulates neuropathic pain and drugs used for neuropathic pain are being tried for OA, one of them is pregabalin (others are Duloxetine and Gabapentin). [8] Pregabalin is the first drug to receive an approved labeling from Food and Drug Association (FDA) for the treatment of diabetic neuropathy and post-herpetic neuralgia. [9] Preclinical and clinical studies have shown the effectiveness of pregabalin in managing neuropathic pain. Pregabalin binding to α2-δ-1 subunit of voltage gated calcium channel (VDCC) is important in alleviating neuropathic pain. [10] Animal based studies have helped to describe the mechanisms for its anti-hyperalgesic and anti-allodynic action. Clinical studies have also shown the efficacy and dose dependent effects of pregabalin either as monotherapy or in combination with analgesics in relieving pain and related symptoms. [11,12] As pregabalin is used in NP, and as OA is thought to simulate NP, so pregabalin holds the potential to solve an unmet analgesic need [13,14] and it was of interest to test this drug in MIA model of OA.

Selection of Animals

Permission of the Institution Animal Ethics Committee (IAEC/13/2018) was obtained prior to the commencement of the study. The study was conducted according to CPCSEA guidelines. 40 swiss albino wistar rats of either sex ageing 6-8 weeks and weighing 150-250 gm was used in this study. 5 groups of wistar rats with 8 rats in each group was used. The females were nonpregnant, and all the animals were kept in the cages for 7 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

OA was induced by a single intra-articular injection of MIA 2mg dissolved in saline in left knee joint. Animals were anaesthetized with Ketamine (50mg/kg) and xylazine (10 mg/kg) intraperitoneal and MIA was injected into the left knee joint cavity using Hamilton syringe a 30 -gauge needle inserted through the intra patellar ligament. MIA induce OA in minimum of 7 days to maximum of 14 days.

Design of Experiment

40 swiss albino wistar rats of either sex ageing 6-8 weeks and weighing 150-250 gm was used in this study. 5 groups of wistar rats with 8 rats in each group was used. Animals were randomized by simple randomization method; animals were sequentially been randomized into test or control group. All behavioral tests were performed on day 0,7,14,21 and 28 days after administration as MIA induce OA from

minimum of 7 days to maximum of 14 days. The days were selected to see the progress of OA and to know the effect of drug as well. At the end of 28-day animals were sacrificed & the sample of left femur with tibia fibula was sent for knee joint histopathology testing and blood withdrawal for biomarker assessment by ELISA for Cartilage oligomeric matrix protein (COMP) & matrix metalloproteinase-13 (MMP-13). Brief overview of methodology is depicted in figure 1

Study drugs and doses as mentioned below:

Group 1: Normal control (NC) (Normal saline)

Group 2: Disease control (DC): 2mg MIA in saline I/A [15]

Group 3: Positive control (PC): Meloxicam 1mg/kg SC [16]

Group 4: Test group 1 (HD-P): 32.4 mg/kg SC [17,18]

Group 5: Test group 2(LD-P + PC): 16.2 mg/kg SC + Meloxicam: 1mg/kg SC [17,18]

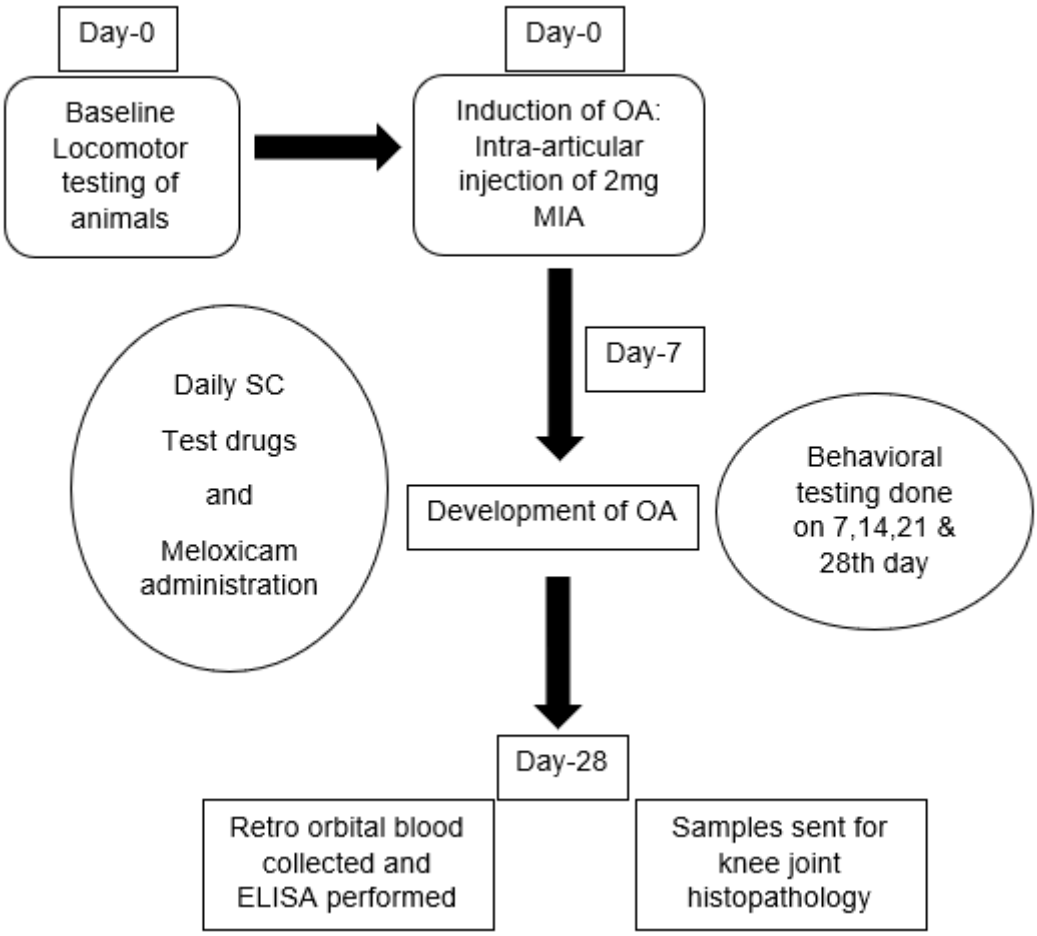


Figure 1: Brief overview of methodology

Results

Hot plate analgesiometer for thermal and neuropathic pain [19,20]

The latency of licking hind paw in seconds in hot plate test expressed as mean ± SD on days 0,7,14,21 and 28 after induction of OA in different groups are depicted in table below.

Table 1: Hot plate test results

Group name	Days	DC	NC	PC	HD-P	LD-P + PC
Latency of licking hind paw in seconds as mean ± SD	0	11.36875 ± 1.819572	11.8425 ± 2.563205	11.2 ± 1.366957	10.645 ± 2.986211	11.68375 ± 1.77624
	7	11.2 ± 1.366957	10.44375 ± 3.520596	10.2625 ± 2.154688	10.44375 ± 3.520596	10.52 ± 3.157015
	14	7.025 ± 3.234082	11.64375 ± 1.757774	7.65 ± 3.560096	7.175 ± 3.2884	8.6 ± 2.173871
	21	6.7875 ± 1.951876	11.36875 ± 1.819572	8.2 ± 1.916097	7.025 ± 3.234082	9.7125 ± 2.494243
	28	4.80 ± 0.91222	11.6188 ± 1.73867*	8.2 ± 1.9161*	7.45 ± 3.55608	11.3688 ± 1.81957*, #

* p< 0.05 vs DC and # p< 0.05 vs PC, using one-way ANOVA followed by post hoc Tukey's test

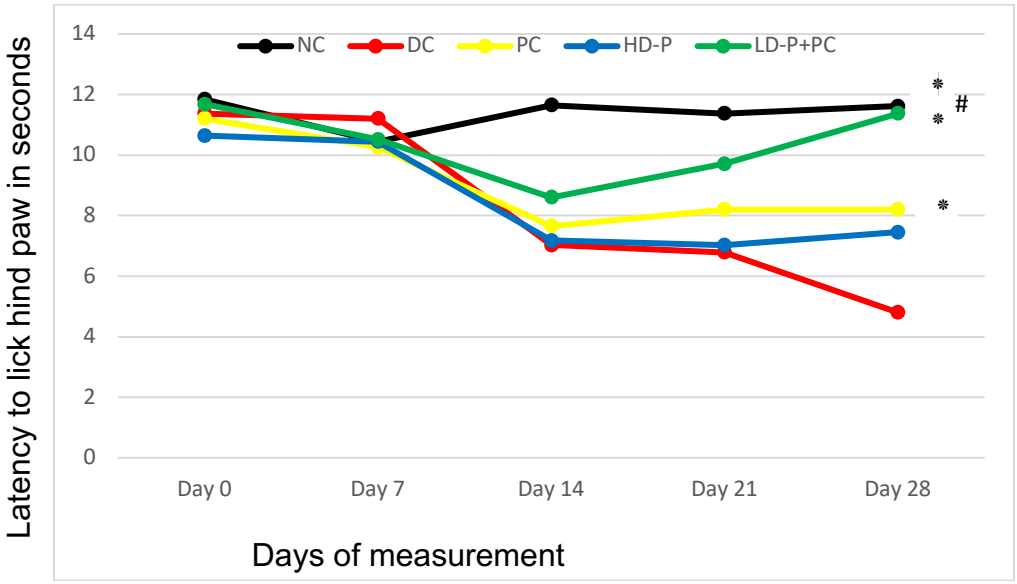


Figure 2: Hot plate test

*p< 0.05 vs DC and # p< 0.05 vs PC, using one-way ANOVA followed by post hoc Tukey's test.

The x-axis gives the days of measurement and the y axis Latency to lick hind paw and the five colours depicting the five groups.

The latency to lick hind paw i.e., pain threshold was all most same on 0 and 7th day in all the groups, Later the threshold decreased dramatically in DC till day 14 and stabilised later till day 21 which further decreased on day 28, The trend was similar with (HD-P) till day 21 but further showed some improvement on day 28 in threshold but statistically not significant. But pain threshold was dramatically improved in (LD-P + PC) which peaked for 10 and 11 seconds on day 21 and 28.

From the above time line graph the results interpreted as (LD-P + PC) and PC group both showed pain threshold improvement compared to DC group.

There was significant improvement in latency to lick hind paw were observed for NC, PC and LD-P + PC groups when compared to DC with $p< 0.05$.

There was significant improvement in latency to lick hind paw were observed for (LD-P + PC) when compared to PC with $p< 0.05$.

Rota rod test [19]

The number of falls per minute in Rota rod test expressed as mean ± SD on days 0,7,14,21 and 28 after induction of OA in different groups are depicted in table below.

Table 2: Rota rod test results

Group name	Days	DC	NC	PC	HD-P	LD-P + PC
Number of falls per minute in mean ± SD	0	1 ± 1.069045	1.5 ± 0.92582	1.25 ± 1.035098	1.625 ± 1.06066	1.375 ± 0.916125
	7	1.125 ± 0.834523	1.125 ± 0.834523	1.375 ± 0.916125	1.125 ± 0.834523	1.25 ± 0.707107
	14	5 ± 1.85164	1.5 ± 1.069045	2.625 ± 1.685018	4 ± 1.85164	2.625 ± 0.916125
	21	7.625 ± 2.66927	2 ± 1.603567	6 ± 2.44949	6.625 ± 2.66927	3.5 ± 1.603567
	28	7.75± 2.18763	2.125± 0.83452*	5.125± 1.45774*	6.75± 2.18763	2.625± 0.91613*,#

* $p< 0.05$ vs DC and # $p< 0.05$ vs PC, using one-way ANOVA followed by post hoc Tukey's test

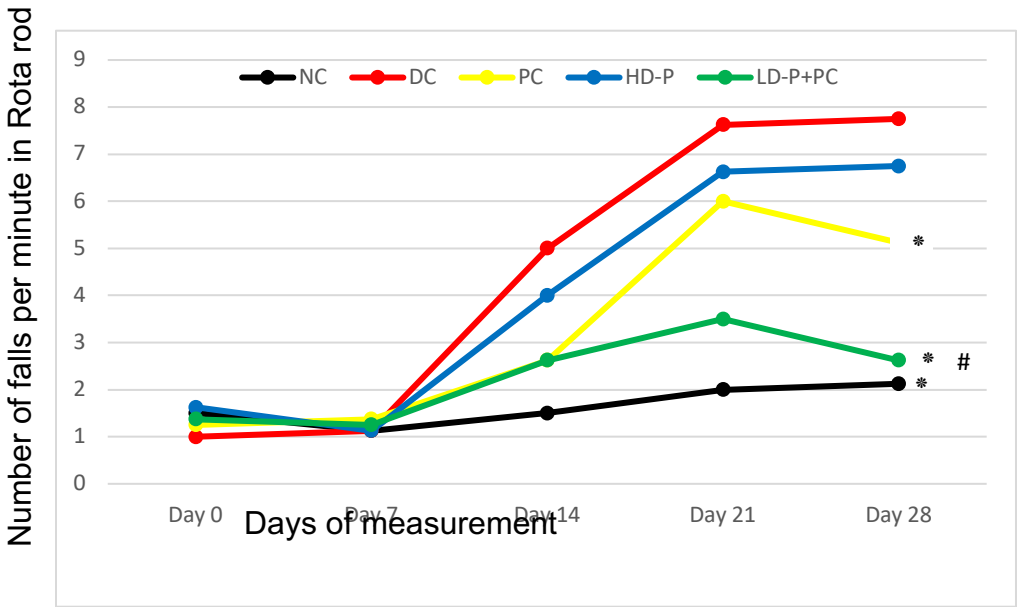


Figure 3: Rota rod test

*$p< 0.05$ vs DC and # $p< 0.05$ vs PC, using one-way ANOVA followed by post hoc Tukey's test

The x-axis gives the days of measurement and the y axis the number of falls and the five colours depicting the five groups.

The number of falls were same on 0 and 7th day in all the groups, Later the number of falls increased dramatically in DC till day 21 and

stabilised later till day 28, The trend was similar with (HD-P) and PC. But the falls were dramatically less in (LD-P + PC) which peaked for 3 falls on day 21 and later took a dip to 2 falls and it was consistent falls for NC groups.

From the above time line graph the results interpreted as (LD-P + PC) and PC group both showed preserved muscular strength compared to DC group.

There was significant reduction in number of falls were observed for NC, PC and LD-P + PC groups when compared to DC with $p< 0.05$.

There was significant reduction in number of falls were observed for LD-P + PC group when compared to PC with $p< 0.05$.

Grip strength meter test [21]

The force applied by hind paw in newton in grip strength meter test expressed as mean ± SD on days 0,7,14,21 and 28 after induction of OA in different groups are depicted in table below.

Table 3: Grip strength meter test

Group name	Days	DC	NC	PC	HD-P	LD-P + PC
The force applied by hind paw in newton as mean ± SD	0	4.355875 ± 0.447432	4.49675 ± 0.419706	4.355125 ± 0.274501	4.5195 ± 0.472488	4.5565 ± 0.576207
	7	3.1605 ± 0.135295	4.183 ± 0.672972	3.600125 ± 0.258187	3.111625 ± 0.077354	4.4525 ± 0.809299
	14	3.1515 ± 0.528862	4.517125 ± 0.775358	3.230875 ± 0.125949	3.00125 ± 0.597625	4.1055 ± 0.634766
	21`	2.26125 ± 0.188182	4.72875 ± 0.403217	3.5075 ± 1.222781	2.82975 ± 0.862741	4.4275 ± 0.82427
	28	2.32063 ± 0.79729	4.8 ± 0.91222	3.23088 ± 0.12595	2.26125 ± 0.18818	4.70064 ± 1.14172 *, #

* $p< 0.05$ vs DC and # $p< 0.05$ vs PC, using one-way ANOVA followed by post hoc Tukey's test.

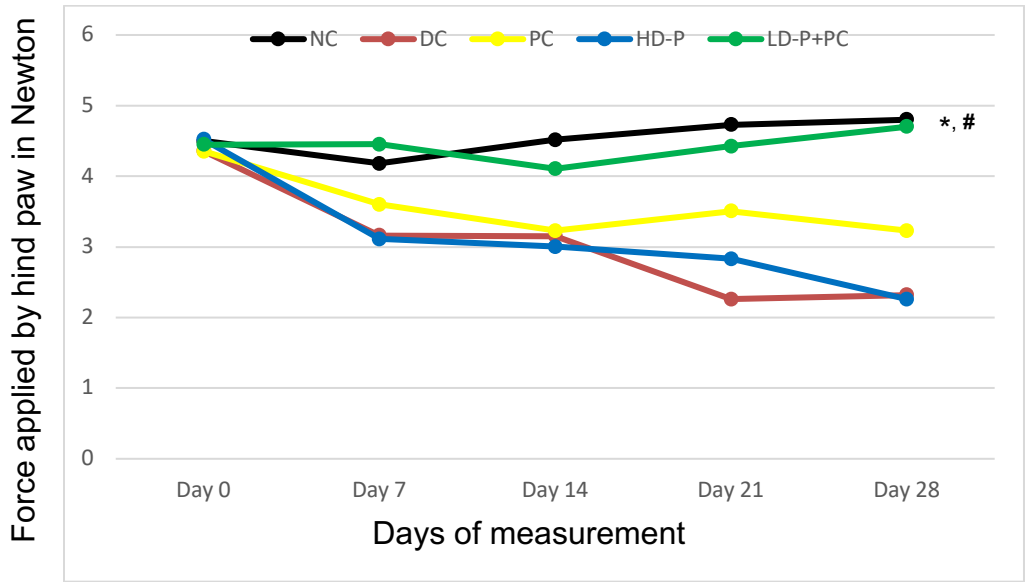


Figure 4: Grip strength meter test

*p< 0.05 vs DC and # p< 0.05 vs PC, using one-way ANOVA followed by post hoc Tukey's test.

The x-axis gives the days of measurement and the y axis the force applied by hind paw in Newton and the five colours depicting the five groups.

On day 0 groups were compared were all groups had similar force applied by hind paw i.e., muscular strength, later the muscular strength was decreased on day 7 and stabilised later till day 14 followed by decrease and stabilization at around force of 2 Newton on day 28 in DC group. The force applied by hind paw in LD-P + PC group improved dramatically till day 28 which was around 5 Newton and it was consistent improvement in force for NC group.

From the above time line graph the results interpreted as (LD-P + PC) showed improved force compared to DC group which suggest decrease osteoarthritic damage in (LD-P + PC) group.

There was significant increase in force applied by hind paw were observed for (LD-P + PC) group when compared to DC with p< 0.05.

There was significant increase in force applied by hind paw were observed for (LD-P + PC) when compared to PC with p< 0.05.

Tail immersion test [22]

The tail withdrawal latency in seconds in Tail immersion test expressed as mean ± SD on days 0,7,14,21 and 28 after induction of OA in different groups are depicted in table below.

Table 4: Tail immersion test

Group name	Days	DC	NC	PC	HD-P	LD-P + PC
The tail withdrawal latency in seconds as mean ± SD	0	7.4375 ± 0.682302	7.825 ± 0.681909	7.5 ± 0.542481	7.7875 ± 0.679154	7.5625 ± 0.592663
	7	7.58 ± 2.3051	9.48 ± 2.012809	7.4125± 0.53033	17.45 ± 1.288133	7.8375± 0.6255
	14	6.48 ± 1.923628	9.48 ± 2.824647	7.5 ± 0.542481	18.11 ± 0.760507	6.48 ± 1.923628
	21	4.8525 ± 1.738404	9.33 ± 2.658179	7.5 ± 0.542481	18.52 ± 0.370482	6 ± 1.690309
	28	6.48± 1.92363	8.58± 2.44204	7.8375± 0.6255	18.635± 0.37898 *, #, $	5.33± 1.17374

* p< 0.05 vs DC and # p< 0.05 vs PC and $ p< 0.05 vs LD-P + PC, using one-way ANOVA followed by post hoc Tukey's test.

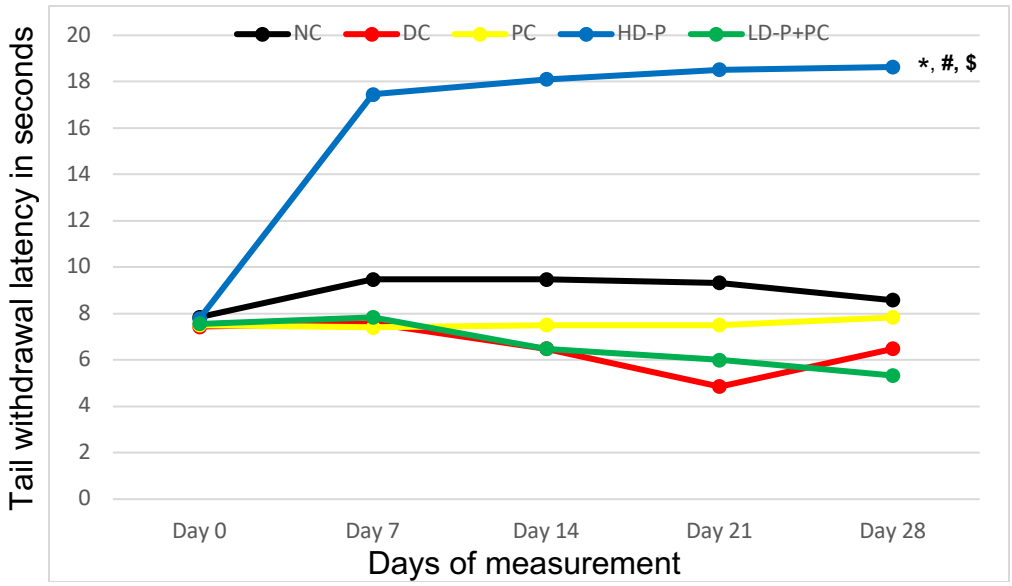


Figure 5: Tail immersion test

*p< 0.05 vs DC and # p< 0.05 vs PC and $ p< 0.05 vs LD-P + PC, using one-way ANOVA followed by post hoc Tukey's test

The x-axis gives the days of measurement and the y axis Tail withdrawal latency and the five colours depicting the five groups.

On day 0 groups were compared, later the tail withdrawal latency gradually decreased till day 21 of 5 seconds and day 28 of 6 seconds in DC group, the trend was similar for (LD-P + PC) group with latency of 5 seconds on day 28 and PC group with latency of 8 seconds on day 28. But the tail withdrawal latency was dramatically improved in (HD-P) group till day 7 of 17 seconds which gradually increased to 19 seconds on day 28.

From the above time line graph the results interpreted as (HD-P) group showed significant improvement in tail withdrawal latency i.e., neuropathic pain in the form of cold allodynia compared to DC, PC and LD-P + PC group.

There was significant increase in tail withdrawal latency were observed for HD-P group when compared to DC with p< 0.05.

There was significant increase in tail withdrawal latency were observed for HD-P group when compared to PC with p< 0.05.

There was significant increase in tail withdrawal latency were observed for HD-P group when compared to LD-P + PC with p< 0.05.

Acetone drop test [23]

The foot withdrawal latency in seconds in Acetone drop test expressed as mean ± SD on days 0,7,14,21 and 28 after induction of OA in different groups are depicted in table below.

Table 5: Tail immersion test

Group name	Days	DC	NC	PC	HD-P	LD-P + PC
The foot withdrawal latency in	0	2.875 ± 1.125992	3.25 ± 1.035098	2.625 ± 1.30247	2.75 ± 1.28174	3 ± 0.755929
	7	3.75 ± 0.707107	3.375 ± 0.916125	4.125 ± 0.834523	3.875 ± 0.834523	3.875 ± 1.125992

seconds as mean ± SD	14	20.25 ± 4.743416	5.25 ± 1.035098	17.75 ± 4.743416	10 ± 3.585686	12 ± 3.891382
	21	25.5 ± 7.010197	5 ± 2	22 ± 5.656854	12 ± 3.891382	24 ± 3.854496
	28	30± 3.11677	5.5± 2.32993	24± 3.8545	14± 2.67261*, #, $	28± 4.84031

* p< 0.05 vs DC and # p< 0.05 vs PC and $ p< 0.05 vs LD-P + PC, using one-way ANOVA followed by post hoc Tukey's test.

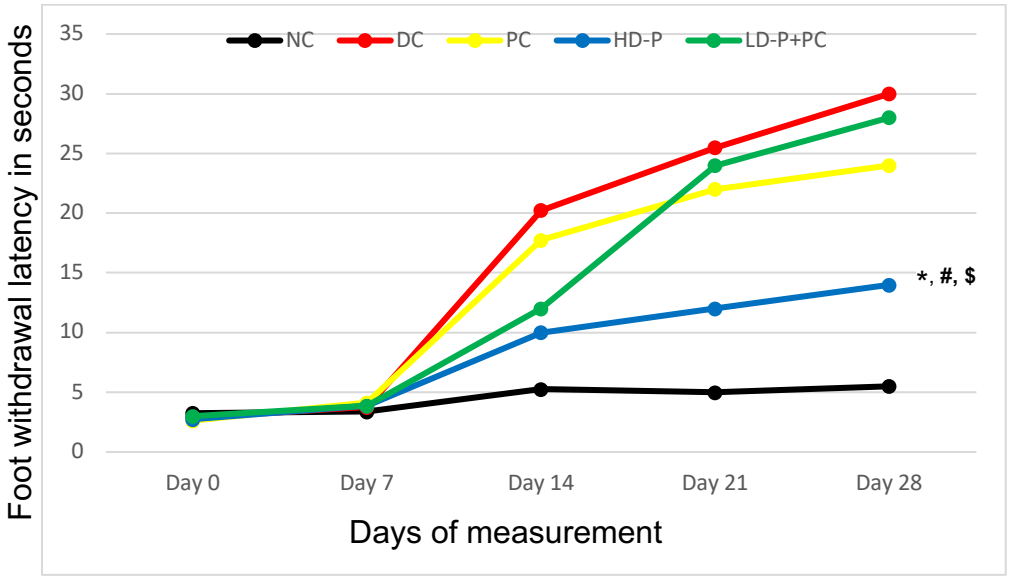


Figure 6: Acetone drop test

* p< 0.05 vs DC and # p< 0.05 vs PC and $ p< 0.05 vs LD-P + PC, using one-way ANOVA followed by post hoc Tukey's test

The x-axis gives the days of measurement and the y axis foot withdrawal latency and the five colours depicting the five groups.

The foot withdrawal latency was same for all groups from day 0 to day 7, later the foot withdrawal latency increased consistently till day 28 of 30 seconds in DC group, the trend was similar for (LD-P + PC) group with latency of 28 seconds on day 28 and PC group with latency of 24 seconds on day 28. But the foot withdrawal latency was dramatically improved in (HD-P) group from 4 seconds on day 7 to 14 seconds on day 28.

From the above time line graph the results interpreted as (HD-P) group showed significant improvement in foot withdrawal latency i.e., neuropathic pain in the form of cold allodynia compared to DC, PC and LD-P + PC group.

There was significant increase in foot withdrawal latency were observed for HD-P group when compared to DC with p< 0.05.

There was significant increase in foot withdrawal latency were observed for HD-P group when compared to PC with p< 0.05.

There was significant increase in foot withdrawal latency were observed for HD-P group when compared to LD-P + PC with p< 0.05.

Knee joint histopathology

The tissue samples were obtained from knee joint for histopathology of subchondral bone and cartilage and was stored in 6% formalin solution Then samples were decalcified in 10% EDTA and embedded in paraffin. The sections were stained with hematoxylin and eosin stain and are read by the veterinary pathologist blind to the study groups. Histological grading was done according to Gerwin et. al [24] scoring system for each rat which was expressed simply by the summation of individual score (0 is no changes, + is minimal, ++ is mild, +++ is moderate and ++++ is severe for each observation). This scoring system included parameters like Chondrocytes cellular changes, Chondrocyte's disorganization, Synovial cell infiltration, Subchondral bone exposure and Synovial membrane ulceration with maximum score of 24 and minimum score of 15 or more is required for standardization.

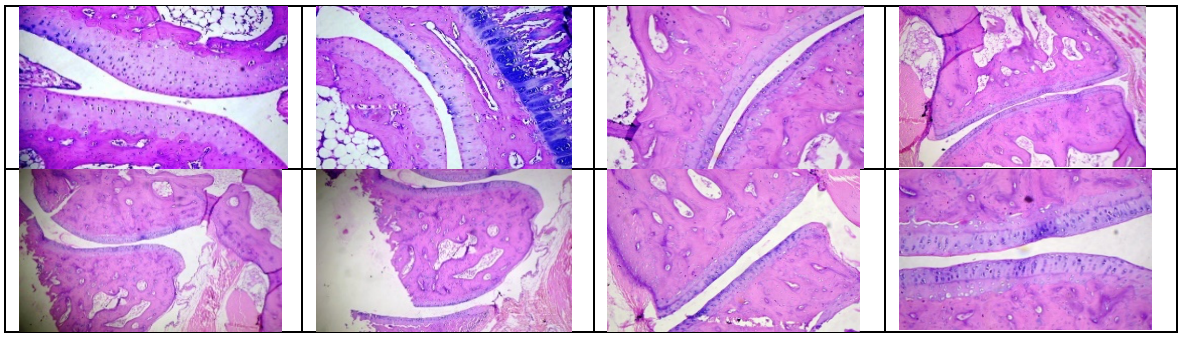
Table 6: Knee joint histopathology

Groups	Histopathology Score – Median (Inter quartile Range)
NC	0(0,0) $
DC	3(2,4)
PC	1.5(0,2) #
HD-P	2(1,1.5)
LD-P + PC	0.5(0,1)*

*, #, $ p< 0.05 vs DC, using Kruskal Wallis followed by post hoc Dunn's test

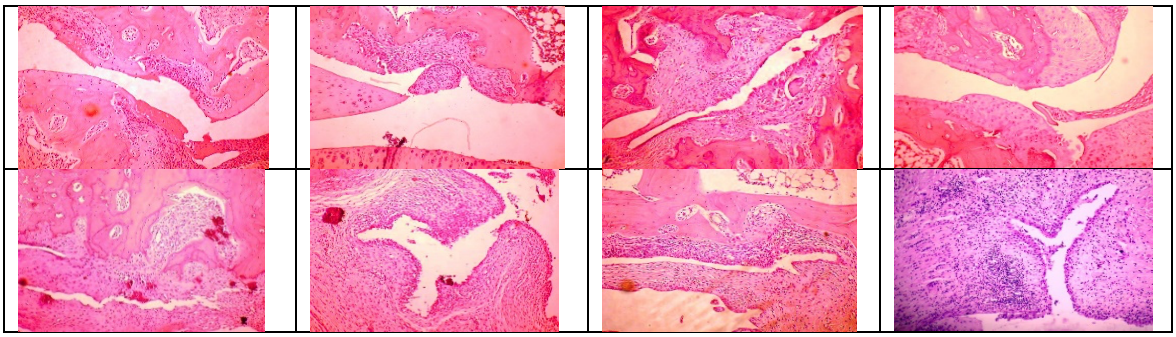
The histopathological photographs are given below

A. Normal control



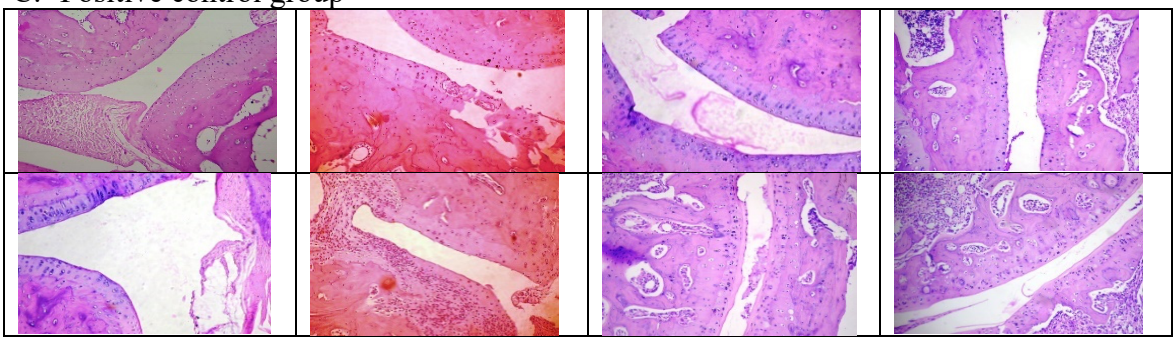
Showing knee joint histopathology slide of all rats of NC group(N=8) on 28 days post-induction with articular surface of the bone: Normal control group has shown the normal chondrocytes in the given slide. As we have given just intraarticular normal saline in this group. There were no osteoarthritic changes found in the given group. (Magnification 100X)

B. Disease control



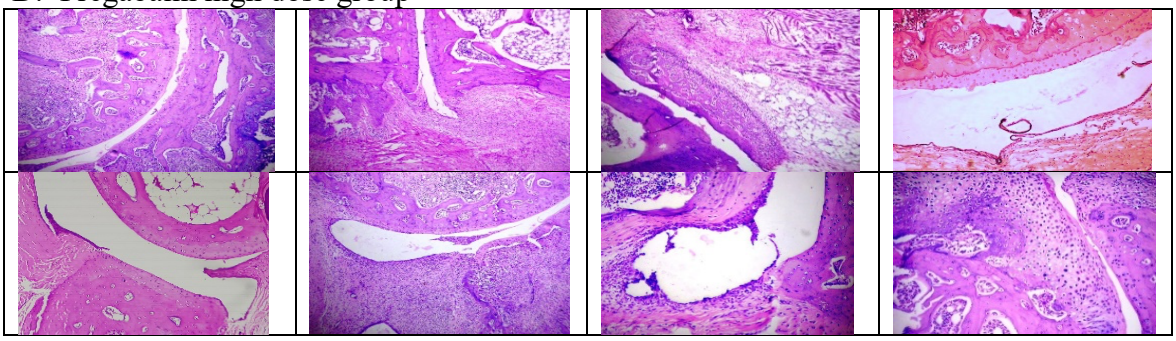
Showing knee joint histopathology slide of all rats of DC group(N=8) on 28 days post-induction with articular surface of the bone: Disease control group has shown clear loss and disorganization of chondrocytes with severe cartilage damage in the given slide. (Magnification 100X)

C. Positive control group



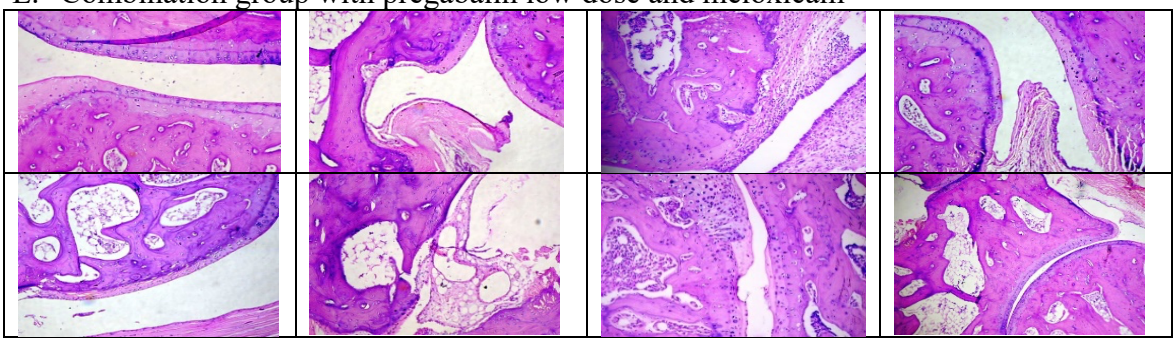
Showing knee joint histopathology slide of all rats of PC group(N=8) on 28 days post-induction with articular surface of the bone: positive control group has shown mild recovery of chondrocyte loss. (Magnification 100X)

D. Pregabalin high dose group



Showing knee joint histopathology slide of all rats with Pregabalin high dose group(N=8) 28 days post-induction with articular surface of the bone: Pregabalin high dose group has shown severe cartilage damage with inflammatory infiltrate. (Magnification 100X)

E. Combination group with pregabalin low dose and meloxicam

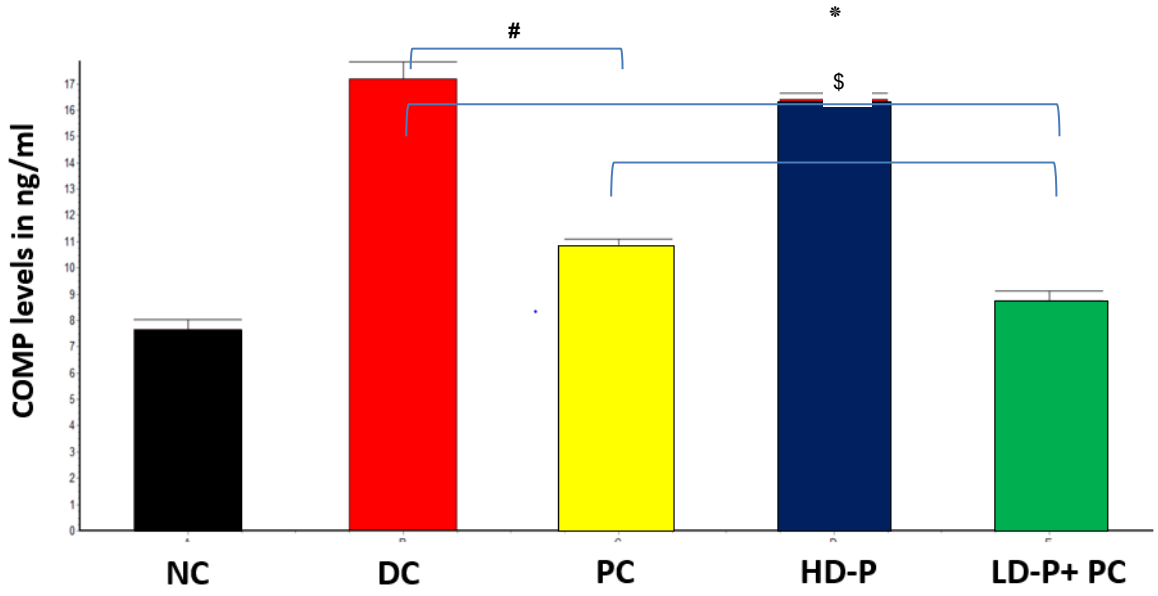


Showing knee joint histopathology slide of all rats of Combination of pregabalin low dose and meloxicam(N=8) on 28 days post-induction with articular surface of the bone: Combination group has shown almost normal recovery of chondrocytes and cartilage surface. (Magnification 100X)

So, from above all the finding we can conclude that Combination group of pregabalin low dose and meloxicam shown significant improvement in the histopathology as compare to disease control over meloxicam group alone.

Biomarkers level analysis by using ELISA:

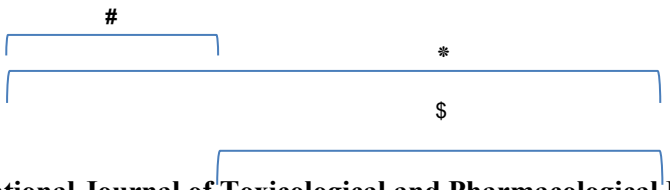
1. Cartilage oligomeric matrix protein (COMP) level

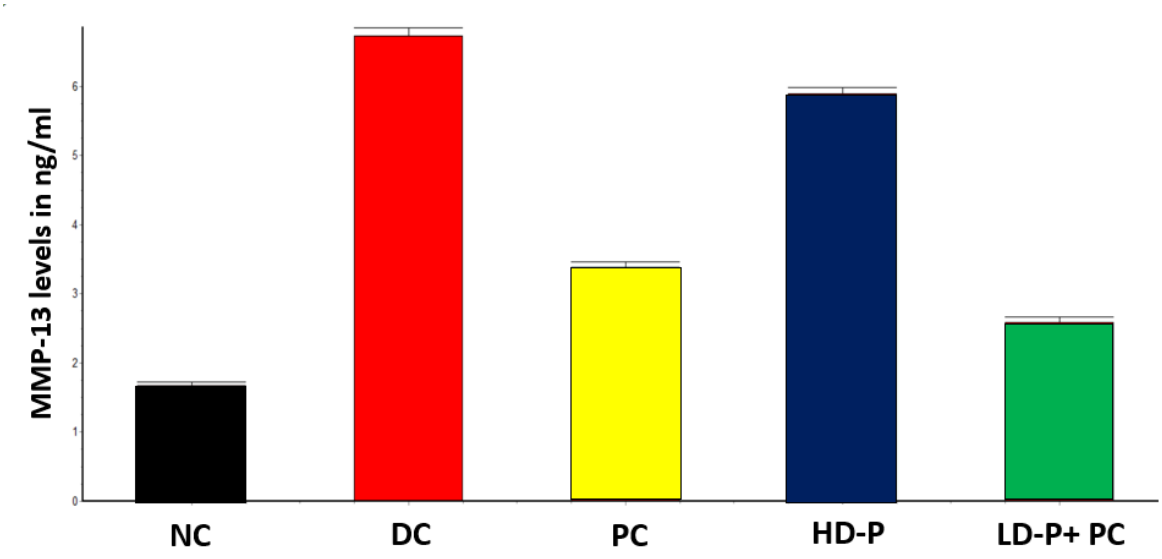


Group name	DC	NC	PC	HD-P	LD-P + PC
COMP levels as mean ± SD	17.201 ± 0.634022	7.674167 ± 0.361313	10.80833 ± 0.304186#	16.39417 ± 0.254466	8.760167 ± 0.365985*,$

* p< 0.05 vs DC and # p< 0.05 vs DC and $ p< 0.05 vs PC, using one-way ANOVA followed by post hoc Tukey's test.

2. Matrix metalloproteinase-13 (MMP-13) level





Group name	DC	NC	PC	HD-P	LD-P + PC
MMP-13 levels as mean ± SD	6.735833 ± 0.113019	1.6785 ± 0.041655	3.379167 ± 0.090711[#]	5.891833 ± 0.090711	2.587333 ± 0.074013 [*, $]

[*] p< 0.05 vs DC and [#] p< 0.05 vs DC and [$] p< 0.05 vs PC, using one-way ANOVA followed by post hoc Tukey's test.

So, from above all the finding on biomarkers, we can conclude that Combination group of pregabalin low dose and meloxicam shown significant decrease in biomarkers level in the ELISA as compare to disease control over meloxicam group alone.

Discussion

Our study primary objective was to find out whether Pregabalin have analgesic, anti-inflammatory and chondroprotective role in animal model of osteoarthritis, for that we have undertaken behavioral test, histopathology and biomarkers analysis.

In Rota rod we checked number of falls per minute as variable which indicate damage to the rat's joint, (LD-P + PC) and PC group both showed preserved muscular strength i.e., less joint damage as compared to DC group. The HD-P group was found to be ineffective and statistically non-significant.

In Hot plate test we checked latency to lick hind paw i.e., pain threshold as variable, (LD-P + PC) and PC group both showed increased pain threshold compared to DC group. The HD-P group was found to be ineffective and statistically non-significant.

The various behavioral tests i.e., Rota rod and Hot plate test used in our study were similar to those postulated by Mcllwain KL *et al* in arthritic model of pain. These tests correspond to the tests used by rheumatologists while assessing patients with OA.[25]

In Grip strength test we checked force applied by hind paw i.e., muscular strength as variable, (LD-P + PC) showed improved force compared to DC group which suggest decrease osteoarthritic damage in (LD-P + PC) group. The HD-P group was found to be ineffective and statistically non-significant.

For testing cold allodynia two standard animal models, namely Acetone drop test and Tail

immersion test were used in our study. (HD-P) group showed significant improvement in foot withdrawal latency and tail withdrawal latency i.e., neuropathic pain in the form of cold allodynia compared to DC, PC and LD-P + PC group. This finding showed that Pregabalin is effective in OA model of neuropathic pain. In one of the previous clinical study by Nidhi *et al*,[8] has shown Pregabalin decreases pain score in hand osteoarthritis in humans.

In histopathology analysis we found out that there was significant reduction in histopathological scoring in LD-P + PC group. Hence, we can confirm from our findings that combination of pregabalin and meloxicam is having antiarthritic potential and helps in decreasing the progression of OA. In one of the previous clinical study by Seiji *et al*, [17] that the combination of pregabalin and meloxicam is more effective than individual drug meloxicam in knee OA and basis of mechanism of action synergistically acting so this can be a line of treatment in chronic OA.

In ELISA for biomarkers analysis of COMP and MMP-13, The Combination group of pregabalin low dose and meloxicam showed significant decrease in biomarkers level as compare to disease control over meloxicam group alone. Hence, we can say from our findings that combination of pregabalin and meloxicam is having Chondroprotective potential and help in decrease the progression of OA.

MMP-13 levels were significantly decreased in combination group compared to the disease control group. MMP- 13 plays a central role in the degradation of articular cartilage in osteoarthritis. MMP-13 levels are upregulated in patients of osteoarthritis, which are otherwise undetectable. Inhibiting MMP-13 has emerged a new area of interest in the treatment of osteoarthritis.[26] Combination of pregabalin and meloxicam has reduced the levels of MMP 13, which are going hand in hand with histopathological findings. This reduction in MMP 13 levels show that combination of pregabalin and meloxicam may have a chondroprotective effect by inhibiting MMP-13.

COMP levels were significantly decreased in combination group as compared to the disease control. COMP is found in cartilage, synovial fluid and the serum in osteoarthritis. MMP -1, MMP -13 and ADAMTS play a major role in the elevation of COMP levels in OA.[27] The increase in COMP levels in disease control shows the osteoarthritic disease activity. The decrease of levels indicates chondroprotective effect and anti-arthritic potential of pregabalin and meloxicam combination.

So, the combination can be used as new line of treatment for chronic OA for better compliance, decrease pill count, cost-effective because it can lead to postponement of joint surgery for long time which can further lead to added benefits economically and for better QOL.

Summary

The combination of pregabalin and meloxicam has consistently worked better in Hot plate, Rota rod and grip strength test which suggest that combination has analgesic, anti-inflammatory and chondroprotective effect in OA.

Pregabalin high dose (HD-P) group showed significant improvement in neuropathic pain in the form of cold allodynia in tail immersion and acetone drop test compared to DC, PC and LD-P + PC group. This finding showed that Pregabalin is effective in OA model of neuropathic pain.

In histopathology analysis we found out that there was significant reduction in histopathological scoring in LD-P + PC group. Hence, we can say from our findings that combination of pregabalin and meloxicam is having antiarthritic potential and help in decrease the progression of OA.

In ELISA for biomarkers analysis of COMP and MMP-13, The Combination group of

pregabalin low dose and meloxicam showed significant decrease in biomarkers level as compare to disease control over meloxicam group alone. Hence, we can conclude from our findings that combination of pregabalin and meloxicam is having Chondroprotective potential and help in decreasing the progression of OA.

Conclusion

Combination has shown antiarthritic effect by virtue of its chondroprotective action along with its analgesic and anti-inflammatory effect.

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