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Research Article

Bioactive Collagen Peptides Ameliorate Monoiodoacetic acid Induced Osteoarthritis in Rats

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Abstract

This study was conducted to evaluate the efficacy of different doses of Bioactive Collagen Peptide (BACP) in Monoiodo Acetic Acid (MIA) induced Osteoarthritis (OA) rats in comparison with conventional Collagen Peptide (CP). Animals were supplemented with daily doses of CP (1033 mg/kg) and BACPs with a dosage of 1033mg/kg, 517mg/kg, 258 mg/kg, 103mg/kg which are equivalent to human dosage of 10g, 5g, 2.5g and 1g respectively. Etoricoxib (10mg/kg) was used as reference drug. Different efficacy parameters such as Rotarod fall latency, heat hyperalgesia, cold hypersensitivity, knee thickness and IL -6 levels were measured at different time intervals. All doses of BACP and CP showed significant improvement ($p<0.05$) in Rotarod fall latency, heat hyperalgesia, cold hypersensitivity and knee thickness when compared to vehicle control on days 14, 21 and 28. CP 1033mg/kg, BACP 1033 mg/kg and 517 mg/kg supplementation showed significant improvement ($p<0.05$) in all above mentioned parameters on day 28 when compared to day 7. All test groups except BACP 103 mg/kg group showed significant decrease in IL-6 levels when compared to the vehicle control on the day 28. Based on the study results under tested condition, BACP at 517 mg/kg showed potent anti-osteoarthritis effect similar to that of conventional Collagen Peptide (CP) at a dosage of 1033 mg/kg against MIA induced osteoarthritis in rats. It can be concluded that 517 mg/kg dose of bioactive collagen peptide is equivalent to 1033 mg/kg of CP supplementation in the management of osteoarthritis in the tested conditions.

Introduction

Osteoarthritis (OA) is one of the most prevalent diseases in the world, with estimates projecting that >300 million people are affected globally [1]. The characteristic features of this chronic, progressive and degenerative disorder of the entire joint include, variable inflammation and changes in the structure of bone bordering the joint and in the protective cushion called articular cartilage. Clinical manifestations include joint pain, tenderness, limitation of movement, effusion and varying degrees of inflammation, and finally induce disability in many patients [2]. Recent pathological understandings elaborate that OA is not restricted to the articular cartilage but involves entire joint including the subchondral bone and synovium and thus it is defined as a collection of overlapping distinctive joint disorders which result in similar biological, morphological and clinical outcomes [3]. The quality of life is impaired due to persistent pain and difficulties in routine activities like stair climbing, squatting, etc [4]. Therefore, effective management of OA is necessary to improve the quality of life. OA is treated mainly by exercise along with the use of analgesics like Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). These medications though relieve the symptoms, do not play disease modifying role and are associated with increased risk of adverse effects over long term use. Studies show that supplementation with collagen hydrolysates may induce the synthesis of cartilage matrix, by stimulating the chondrocytes [5], after intestinal absorption and accumulation in articular cartilage through blood circulation. In fact, experimental studies have demonstrated that peptides from orally administrated collagen hydrolysates accumulated in cartilage tissue a few hours

after administration [6-8]. Investigating the efficacy of exogenous administration of collagen derivatives in treating OA in animal models, recent preclinical studies have reported promising results [9,10] suggesting some potential for cartilage repair in OA patients.

In our previous study we have demonstrated Collagen Peptide (conventional) as an effective supplement for the improvement in overall physical problems associated with OA and thereby help to improve the quality of life. It is hypothesized that the supplementation of collagen peptide regulates chondrocyte differentiation and stimulates synthesis of proteoglycans, resulting in the initiation of repair processes in cartilage tissue [2]. Etoricoxib, the reference drug used in the study is a Non-Steroidal Anti-Inflammatory Drug (NSAIDs) that has anti-inflammatory effect. The drug acts by inhibiting the Cyclo-Oxygenase-2 (COX-2) enzyme. Pain and inflammation are caused by prostaglandins, which are released at sites of injury or damage. Since COX-2 enzymes are inhibited, fewer prostaglandins are released, reducing pain and inflammation. New Bio Active Collagen Peptide (BACP) is developed by Nitta Gelatin India Ltd, with enhanced level of active dipeptides like Proline- Hydroxyproline (PO) and Hydroxyproline- Glycine (OG). The product has been tested by LC/MS for the active dipeptide content in comparison with Conventional CP. Being the key regulatory factor in functionality, the 200 fold increase in the active molecule content in newly developed BACP shall exhibit high functional property in joint health compared to existing conventional collagen peptides. The average molecular weight of the new bioactive collagen peptides is ≤1000 Da. The objective of this study was to evaluate the potential anti-osteoarthritis effects of bioactive collagen peptides, in comparison with conventional collagen peptide against MIA (Monoiodoacetic acid) induced osteoarthritis in Wistar rats following consecutive oral dose for 21 days. In the present paper, we discuss the evidence on the efficacy on bioactive low dosage collagen peptides in management of OA.

Materials and Methods

BACP and Conventional Collagen peptides are products of Nitta Gelatin India Ltd. Etoricoxib tablet, manufactured by Sun Pharma Laboratories Ltd was procured as Reference drug for the study. Male Wistar Rats were sourced from CPCSEA approved vendor. Animal experiments were conducted with due clearance from the Institutional Animal Ethics committee at Centre for Toxicology and Developmental Research (CEFTE), GLP certified Facility. Approval was obtained from the institutional review board and animal care complied with the guidelines of the institution on the care and use of laboratory animals. Sixty four male Wistar rats of 6-8 weeks age were selected for the study. Animals were acclimatized in the experimental room for a period of 5 days. Young healthy rats were selected and grouped based on the stratified body weight method. After acclimatization, animals were randomized based on stratified body weight and assigned to eight groups (each group comprises of 8 animals) viz., Sham Control (GI), vehicle control (GII), Reference Drug (Etoricoxib 10mg/kg) (GIII), Conventional Collagen Peptide (GIV - 1033 mg/kg), Test item BACP (GV -1033mg/kg), Test item BACP (GVI-517mg/kg), Test item BACP (GVII -258 mg/kg), Test item BACP (GVIII-103mg/kg). The weight variation of animals was minimal and did not exceed ±20% of the mean body weight. Temperature, humidity and air exchange was maintained in the range of 19-23°C, 30- 70% and 12-15 air changes per hour respectively. The animals were provided with photo period of 12 h artificial light and 12 h dark. They were provided with standard rodent pelleted feed.

On day 0, rats were anaesthetized with ketamine hydrochloride (50 mg/kg body weight) and xylazine (10 mg/kg body weight) and the right knee was shaved and disinfected with 70% ethanol followed by povidone iodine. A single dose of 50µl sterile normal saline containing 3 mg monosodium iodoacetate was injected into right knee joint through infrapatellar ligament using a 300µl syringe fitted with a 29 G needle in two phases. Induction of Osteoarthritis in animals of all groups was confirmed by comparing the parameters of behavioral assessment of nociception, heat hyperalgesia and cold hypersensitivity before injection of MIA (0 Day) and on 7th day of injection. The test item was formulated using sterile water. On day 8 of post MIA induction, respective test items were orally treated to respective test groups till 28th day. The vehicle control animals received the same dose volume of vehicle (10 ml/kg/day weight) of sterile water during the treatment period. The dose volume of individual animal was calculated and adjusted based on their respective week body weight recorded during the treatment period. The parameters in the study were Rotarod fall latency monitoring, heat hyperalgesia, cold hypersensitivity, knee thickness, IL6 estimation and histopathology.

Rotarod Fall Latency Monitoring: Each animal was trained for 4 minutes (240 sec) at a constant speed of 40 rpm on the Rotarod. Every day, each animal was received a number of trials, separated by 30 minutes, at speeds accelerating from 4 to 40 rpm (with a 4 rpm increase every 30 seconds). Each animal was tested for a number of consecutive days. The latency to falling off the Rotarod was recorded on days 0 (before drug and MIA injection), 7, 14, 21 and 28 day during the period of the treatment.

Heat Hyperalgesia: Sensitivity to heat was determined by measuring paw flick latency to heat source. Plantar side of paw of animal was placed above the hot plate at 50°C and paw withdrawal latency were noted in seconds. A maximum cut-off time of 20 sec was used to minimize paw damage. Measurements were taken 2-3 times with at least 5 min gaps between the tests. Sensitivity to heat was assessed for each group before and after the induction of arthritis on days 0 (before drug and MIA injection), 7, 14, 21 and 28 day during the period of the treatment.

Cold Hypersensitivity: Paw withdrawal latency was recorded in seconds by submerging ipsilateral paw in cold water (4±1°C). Measurements were taken 2-3 times with at least 5 min gaps between the tests. Paw withdrawal latency was assessed for each group before and after the induction of arthritis on days 0 (before drug and MIA injection), 7, 14, 21 and 28 day during the period of the treatment.

Knee Thickness: Knee thickness was assessed with Vernier caliper of each group before and after the induction of arthritis on days 0 (before drug and MIA injection), 7, 14, 21 and 28 day during the period of the treatment.

IL6 Estimation: Blood was collected from retro-orbital plexus under anesthetic condition on day 28 of post MIA induction. The collected blood was centrifuged and the serum was separated and stored in a freezer at −80˚C and subjected to IL-6 estimation. The Rat IL-6 (Interleukin 6) ELISA Kit (E-EL-R0015) of Elabscience was used for analysis. This ELISA kit works on Sandwich-ELISA principle.

Histopathology: Necropsy: On 28th day of post MIA induction, animals of all groups were sacrificed by CO_2 inhalation and subjected to gross pathological examination. Right knee joints were collected in 10% neutral buffered formalin solution from all the rats after CO_2 euthanasia. The collected joints were decalcified using 10% formic acid and routinely processed. The sections were stained with hematoxylin and eosin stains and evaluated into 5 severity grades: minimal, mild, moderate, marked and severe.

Statistical Analysis

Statistical Analysis was performed using One Way Analysis of Variance, Tukey Multiple comparison, post hoc test with Sigma Plot 12.3 software. P value <0.05 is considered as significant.

Results and Discussion

Mortality was not observed in animals of different groups during the study. All the animals treated at the intended dose levels were observed to be normal throughout the experimental period. There was no significant change observed in the body weight between the groups on day 0, 7, 14, 21 and 28 day between the groups during inter group analysis. However, significant increase in body weight was noted from day 0 day 28 in all groups.

Behavioral Assessment of Nociception

Rotarod Fall Latency

Significant ($p<0.05$) improvement in the latency of fall was observed in all test groups and reference drug group when compared to Vehicle Control on days 14, 21 and 28 (Figure 1). When latency of fall on day 7 was compared with the same on days 14, 21 and 28; however, Etoricoxib group showed significant ($p<0.05$) improvement in all the days 14, 21 and 28. Conventional collagen peptide and BACP 1033 mg/kg groups showed a significant ($p<0.05$) improvement on day 28 when compared to day 7. BACP 517 mg/kg group showed significant ($p<0.05$) improvement on day 21 and 28. BACP 258 mg/kg and 103 mg/kg groups showed no significant ($p>0.05$) improvement in latency of fall on days 14, 21 and 28 when compared to day 7.

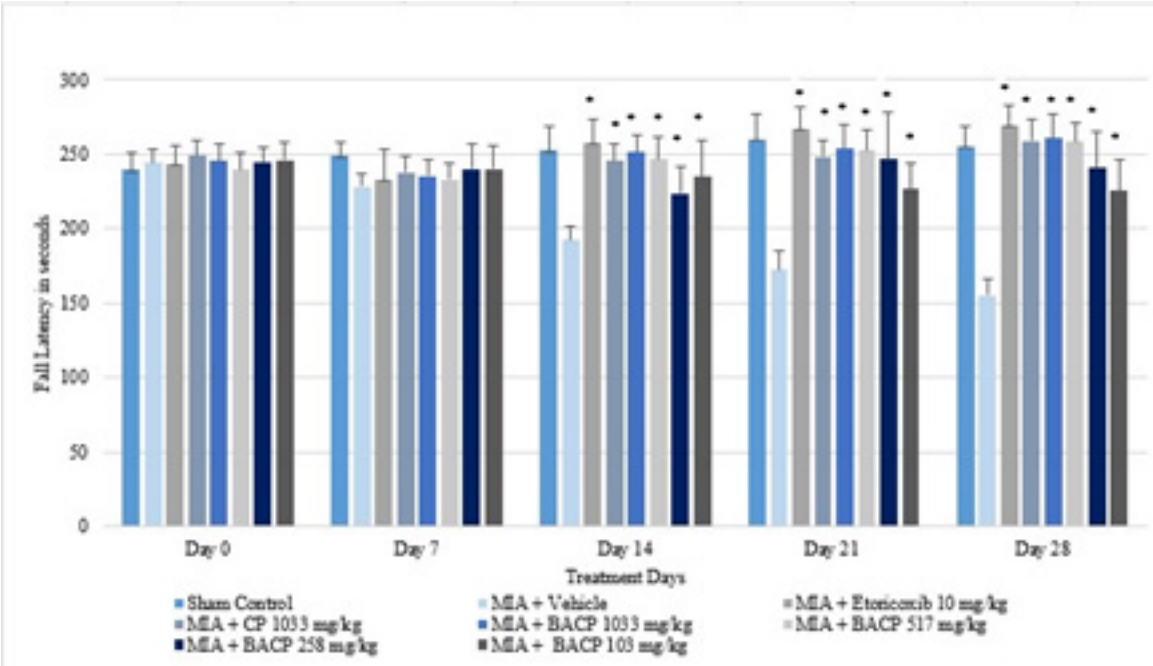

Figure:1 Rotarod Fall Latency

Heat Hyperalgesia

Significant ($p<0.05$) improvement in the paw withdrawal latency was observed in all test and reference drug groups when compared to Vehicle Control on days 14, 21 and 28 (Figure 2). When paw withdrawal latency on day 7 was compared with the same on days 14, 21 and 28, Etoricoxib, conventional collagen peptide, BACP 1033 mg/kg and 513 mg/kg groups showed significant ($p<0.05$) improvement on days 14, 21 and 28. BACP 258 mg/kg group showed a significant ($p<0.05$) improvement on day 21 when compared to day 7. BACP 103 mg/kg group has not shown any significant ($p>0.05$) improvement in latency of fall on days 14, 21 and 28 when compared to day 7.

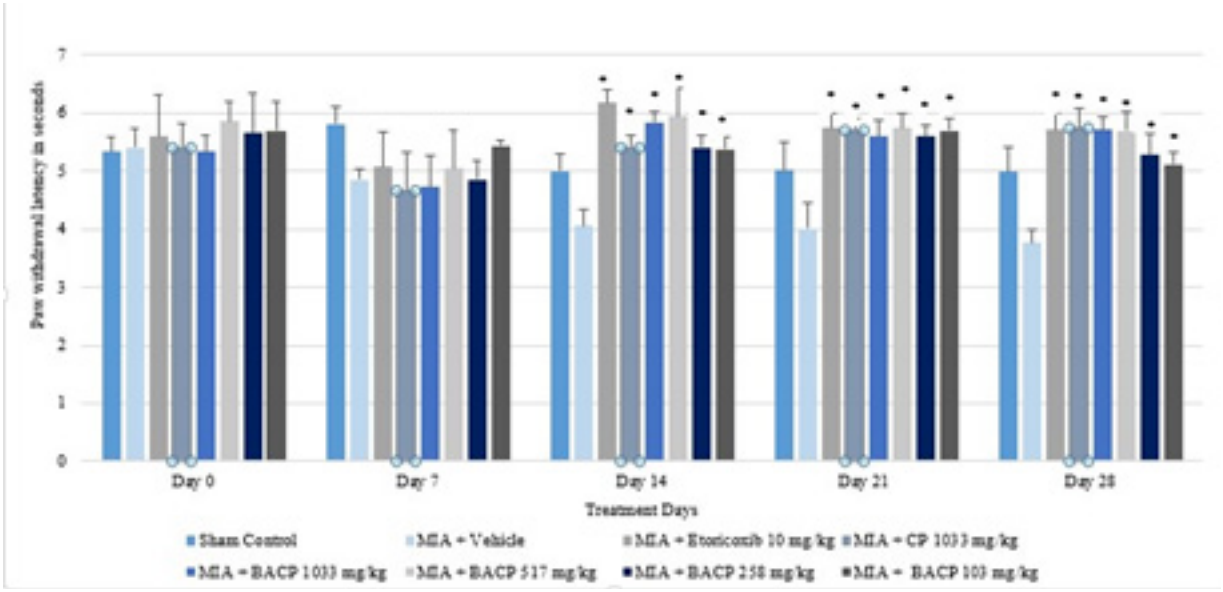

Figure:2 Paw Withdrawal Latency-Heat Hyperalgesia

Cold Hypersensitivity

Significant ($p<0.05$) improvement in the paw withdrawal latency was observed in all test groups and reference drug group when compared to vehicle control on days 14, 21 and 28 (Figure 3). When paw withdrawal latency on day 7 was compared with the same on days 14, 21 and 28; Etoricoxib group showed significant ($p<0.05$) improvement on days 14, 21 and 28. Conventional collagen peptide showed significant ($p<0.05$) improvement on day 28 when compared to day 7. BACP 1033 mg/kg group showed significant ($p<0.05$) improvement on days 21 and 28 when compared to day 7. BACP 517 mg/kg and 258 mg/kg showed significant ($p<0.05$) improvement on days 14, 21 and 28 when compared to day 7. BACP 103 mg/kg group showed no significant improvement ($p>0.05$) in latency of fall on days 14, 21 and 28.

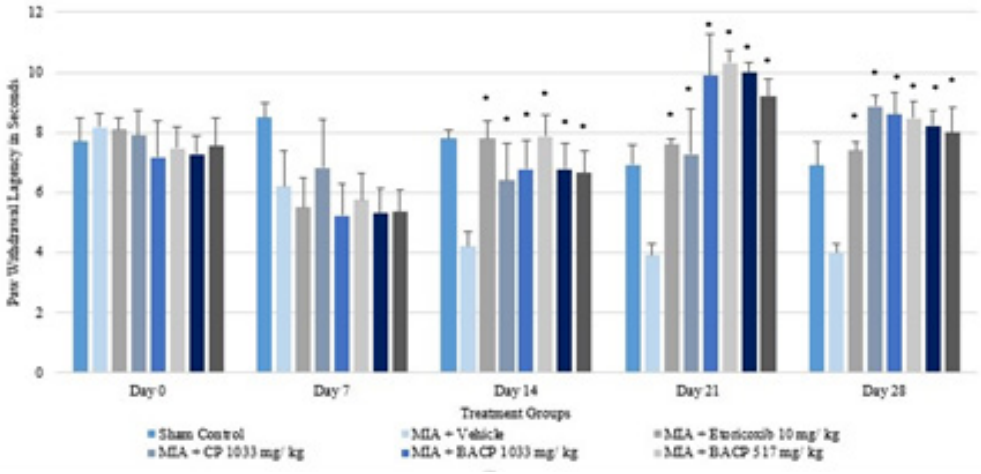

Figure: 3 Paw Withdrawal Latency-Cold Hypersensitivity

Knee Thickness

Significant ($p<0.05$) decrease in the knee thickness was observed in all test groups and reference drug group when compared to Vehicle Control on days 14, 21 and 28 (Figure 4).

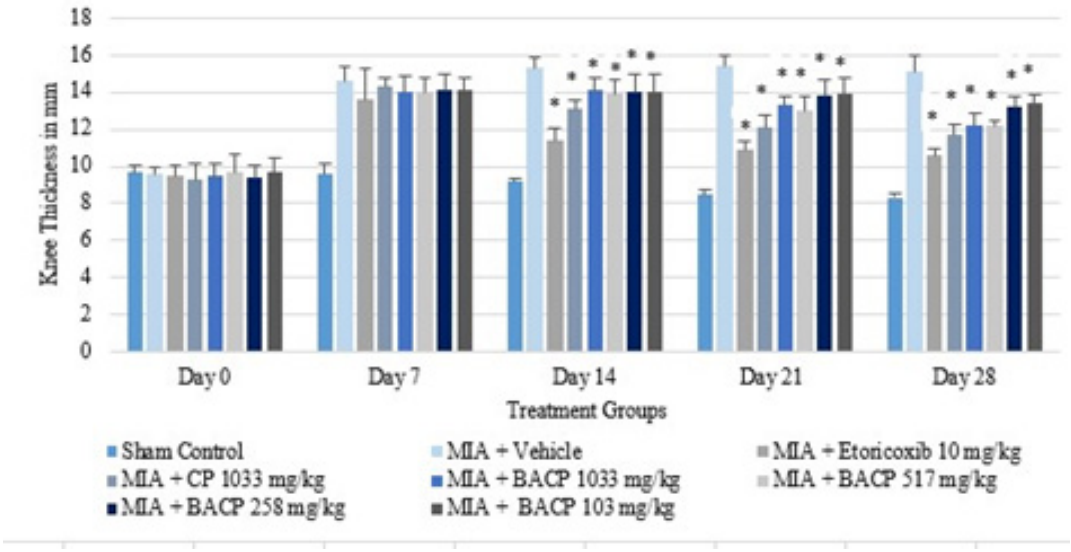

Figure: 4 Knee Thickness

When knee thickness on day 7 was compared with the same on days 14, 21 and 28, Etoricoxib and conventional collagen peptide groups showed significant ($p<0.05$) improvement on days 14, 21 and 28. BACP 1033 mg/kg and 517 mg/kg groups showed significant ($p<0.05$) improvement on day 28 when compared to day 7. BACP 258 mg/kg and 103 mg/kg groups showed no significant ($p>0.05$) improvement in knee thickness on days 14, 21 and 28.

Rotarod fall latency, heat hyperalgesia, cold hypersensitivity and knee thickness data of days 14, 21, 28 were compared with that of day 7 because arthritis induction in all groups was significantly evidenced on day 7 post MIA induction. After arthritis confirmation (day 7), supplementataion was initiated and continued till day 28. Hence comparison of data with day 7 provides the protective effect of supplements against arthritis progression

IL6

Significant ($p<0.05$) decrease in interleukin (IL6) level was observed in Etoricoxib, Conventional collagen peptide, BACP 1033 mg/kg, 517 mg/kg and 258 mg/kg groups on day 28 when compared to Vehicle Control (Figure 5). BACP 103 mg/kg group did not show any significant change in IL-6 levels on day 28 when compared with the vehicle control.

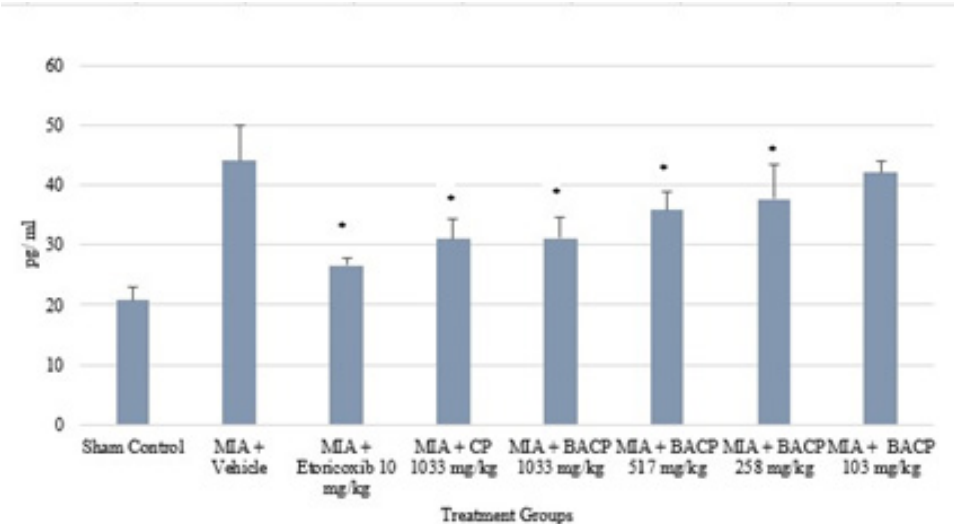

Figure: 5 Serum IL6 levels on Day 28

Histopathology Results

Histopathological evaluation was made for synovial hyperplasia, inflammatory cell infiltration, cartilage degeneration, cartilage necrosis, subchondralosteolysis/ trabaculae fragmentation. Scoring was done as per Janusz, et al. [11] using hematoxylin and eosin (H & E) stain. As scoring severity was not same in all the animals in all groups, statistical analysis between the group was not performed and the results are semi quantitative. Severity differences are also evidenced in vehicle control group. Cartilage necrosis and subchondralosteolysis/ trabeculae fragmentation occurrences (number of joints) at the end of treatment are given below in the Table 1.

	Sham Control	MIA + Vehicle	MIA + Etoricoxib (10mg/kg)	MIA + CP 1033 mg/ kg	MIA + BACP 1033 mg/ kg	MIA + BACP 517 mg/kg	MIA + BACP 258 mg/kg	MIA + BACP 103 mg/kg
Synovial hyperplasia	0	4	5	8	3	6	7	7
Inflammatory cell infiltration	2	1	1	4	2	6	7	7
Cartilage degeneration	0	0	4	2	1	2	1	0
Cartilage Necrosis	0	8	4	6	6	6	7	8
Sub chondral osteolysis/ trabeculae fragmentation	0	5	3	3	3	4	6	6

Table 1: Summary of Histopathology Study.

CP, BACP 1033 mg/kg and BACP 517 mg/kg treated groups had cartilage necrosis occurrences in 6 joints out of 8 joints examined. Vehicle control showed all 8 joints showing cartilage necrosis. CP and BACP 1033 mg/kg had sub chondral osteolysis/ trabeculae fragmentation occurrences in 3 joints, BACP 517 mg/kg showed 4; BACP 258 mg/kg and BACP 103 mg/kg showed sub chondral osteolysis/ trabeculae fragmentation occurrences in 6 joints. The representative micrographs of each group are shown below (Figure 6).

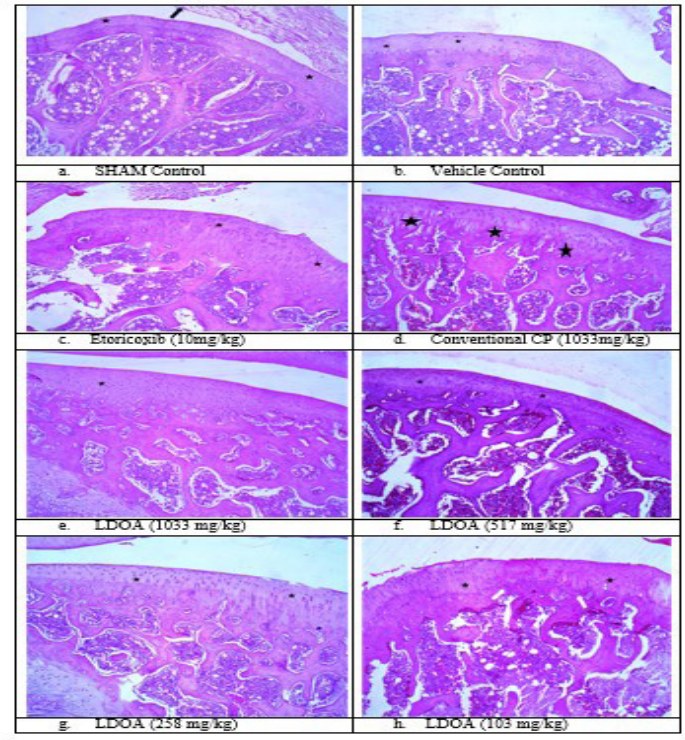

Figure:6 Histopathology Images of Knee Joint of Treated and Sham Control Animals

a. Sham Control: Knee joint showing normal articular cartilage (black star) and synovial membrane (black arrow). H&E, 100x magnification .b. Vehicle control: Knee joint showing marked cartilage necrosis (black stars) and minimal subchondral osteolysis (white arrow), H&E, 100x magnification .c. Etoricoxib (10mg/kg)- Knee joint showing minimal cartilage degeneration (black stars), H&E, 100x magnification. d. Conventional CP (1033mg/kg) - Knee joint showing mild cartilage degeneration (black stars), H&E, 100x magnification e. LDOA 1033mg/kg- Knee joint showing mild cartilage necrosis (black stars), H&E, 100x magnification. f. LDOA 517mg/kg- Knee joint showing minimal cartilage degeneration (black stars), H&E, 100x magnification. g. LDOA 258mg/kg- Knee joint showing minimal cartilage degeneration (black stars), H&E, 100x magnification. h. LDOA 103mg/kg- Knee joint showing moderate cartilage necrosis (black stars) and presence of minimal subchondral osteolysis (white arrows), H&E, 100x magnification

Discussion

The mechanism of action of collagen peptide has been extensively studied [12-16]. Experimental investigations have demonstrated that the degradation products of the collagen are principally able to influence cell metabolism [17].BACP is a mixture of Type I collagen peptides of low molecular weights that are generated via enzymatic digestion of Type I collagen extracted from animal bones. The peptide mixture, contains an abundance of proline- hydroxyproline and hydroxyproline-glycine dipeptides, which is absorbed to body following the oral delivery. Thus the potential role of collagen peptide in repair of damaged cartilage could be associated with the accumulation of orally administered collagen peptides. Cell culture experiments investigating the efficacy of collagen peptide on the biosynthesis of articular chondrocytes revealed that the treatment of cartilage cells with collagen peptide induced a statistically significant dose dependent increase in type II collagen synthesis of chondrocytes compared to the untreated controls [17]. The major component of collagen peptide that remained in the blood was identified as Pro-Hyp dipeptides [12]. Nakatani et al. concluded that Pro-Hyp, a dipeptide in Porcine Collagen Peptide (PCP), is an important factor that regulates chondrocyte differentiation and plays a key role in the maintenance of mature chondrocytes in cartilage [18]. They hypothesised that Pro-Hyp in collagen peptide and its regulatory mechanism seem to explain the therapeutic effect of collagen peptide in improving joint conditions. The real working mechanism of collagen derivatives in OA may be by the stimulation of collagen biosynthesis by chondrocytes [17], the use of collagen-specific peptides as building block for articular cartilage [6], or the inhibition of apoptosis and hypertrophy of chondrocytes [10]. Being one of the most important symptoms, pain reduction indirectly indicates the mark of improvement in joint conditions in patients with OA. Thus the administration of collagen peptide has much relevance with regard to reduction of pain in a patient with OA. The accumulated collagen peptide helps to maintain structure and function of cartilage, which in turn results in joint comfort and subsequent improvements in pain. The results clearly indicate that the new bioactive collagen peptides slow down or even halt the progression of OA.

Conclusion

Based on the above results, BACP at 517mg/kg and 1033 mg /kg showed potent anti-osteoarthritis effects along with Etoricoxib and conventional collagen peptide against MIA induced osteoarthritis in following consecutive oral dose for 21 days. Hence BACP 517 mg/kg can be considered as a low dosage supplementation for the management of osteoarthritis in the tested conditions. Clarifying the mechanism of action of collagen derivatives in OA and conducting randomized placebo-controlled trials will help in bringing robust evidence on the usefulness of this low dosage bioactive collagen derivative as a relevant prevention option for the management of osteoarthritis.

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