Experimental study on synovial fluid matrix metalloproteinases in dogs affected with osteoarthritis

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1. Abstract

Arthritis is one of the most important diseases causing lameness in dogs as well as other species of animals. Osteoarthritis (OA)cause considerable deterioration of the cartilage and marked enzymatic changes in the synovial fluid, and the joint tissue. The present study was conducted on 25 clinically healthy Mongrel male dogs, with body weight of 15.0 ±1.7kg. Arthritis was induced in 20 dogs and 5 dogs were left as a negative control (normal) group. Osteoarthritis was induced by performing arthrotomy and curettage of the cartilage by creation of two 8.0 mm diameter full thickness chondral defect till exposing the subchondral bone. The dogs were left for 10 days till complete healing of the surgical wound. Amphotericin-B was injected intra-articularly at a dose of 10 mg twice weekly for 2 successive weeks. The dogs were left one week till complete typical symptoms of arthritis were observed. The experimental groups were classified into five groups as control negative (normal), dexamethasone treated, sodium hyaluronate treated, poly sulfated glycosaminoglycan treated and control positive (sham operated). Sensory characteristics of the treated groups were evaluated. Besides, synovial analysis was performed for each animal at the end of the 1st, 2nd, 3rd, and 4th weeks. Matrix metalloproteinase (MMPs) 3 and 13 and tissue inhibitor metalloproteinase (TIMP-1) were detected by ELISA assay. The obtained results revealed that osteoarthritis OA was associated with significant increases in the enzymatic activities of MMPs 3 and 13 and decrease of TIMP-1 as biomarkers of OA. Among the used treatments, polysulfated glucose aminoglycan is the highly recommended therapeutic approach as it caused the highest improvement compared with other treatments.

Keywords: *Osteoarthritis; matrix metalloproteinases; dexamethasone; sodium hyaluronate; polysulfated glucose aminoglycan*

2. Introduction

Osteoarthritis is a complicated condition involving several biomechanical and metabolic disturbances. It is characterized by articular cartilage degradation, osteophyte development, and bone remodeling; periarticular tissue growth; and a low-grade, non-purulent inflammation of varying severity [1]. The underlying molecular pathways are complex and have not been fully understood, despite the obvious macroscopic changes [2].

Osteoarthritis causes considerable cartilage remodeling and deterioration. An enzymatic pathway appears to be involved in the terminal breakdown of articular cartilage and periarticular tissues [3]. Matrix metalloproteinases (MMPs), enzymes that degrade all components of the extracellular matrix, are stimulated by the previous inflammatory mediators. MMPs regulate cell proliferation, differentiation, apoptosis, and host defense. Furthermore, MMPs are involved in both physiological and pathological tissue regeneration processes, such as wound healing,

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inflammation, and cancer [4]**.** In addition, arthritis increases the expression of additional MMPs, such as MMP-2, MMP-3, and MMP-9, which destroy non-collagen matrix components of joints [5]. MMP inhibitors can be used to treat cancer by inhibiting the enzymes that aid tumor spread [6]. MMP-13 inhibitors have been employed to treat osteoarthritis OA in recent research, with an emphasis on their enzyme inhibitory characteristics [7]**.** In orthopedics, experimental *in vivo* models of OA are commonly employed to confirm a working theory [8,9]**.** In particular, models that demonstrate the most relevant symptoms of natural disease are highly recommended [10]. Injection of chemical reagents and biological mediators into the joint of small animals leads rapidly to macroscopic and/or histopathologic lesions simulating naturally occurring OA. Physical, chemical, and surgical methods have been used to develop *in vivo* OA models. several chemical models of OA have been utilized, each causing a rapid inflammatory response (within hours) followed by cytotoxic and structural damage to joint cartilage [8,10]. Amphotericin B is an antifungal polyene antibiotic produced by a *Streptomyces nodosus* strain that can be used for induction of OA. The medicine works by attaching to sterols in susceptible fungi's cell membranes, causing a shift in membrane permeability that allows intracellular components to seep out. Sterols are also found in mammalian cell membranes, and it has been postulated that human and fungal cell damage may be caused by the same mechanisms [11,12,13]**.** The goal of OA treatment is primarily to alleviate the unpleasant symptoms associated with the disease. The degree of pathologic change, including bone and soft tissue abnormalities, is widely acknowledged factors that affect the prognosis of the disease. Therefore, it is difficult to provide a treatment protocol that fits well for all OA cases. Therefore, multimodal therapy is highly recommended to deal with difficult cases [14].

Hyaluronic acid (HA) and glycosaminoglycan are found in synovial fluid and the matrix of cartilage. Hyaluronic acid is needed for the proper function of the joint via promotion of cartilage viscosity and lubrication [15].In degenerative joint disease, the concentration of HA drops dramatically, reducing the viscosity of the synovial fluid [16]. Thus, exogenous supply of HA is among the suggested treatment protocols of OA.

Dexamethasone medications are among the most prescribed treatments for OA via alleviating the painful symptoms of OA [17]. The current study aimed at induction of OA model in healthy Mongrel dogs, recording of the clinical symptoms before and after treatment with different protocols including dexamethasone, sodium hyaluronate, and polysulfated glycosaminoglycan. Determination of matrix metalloproteinases activities and their inhibitor TIMP-1 was done using ELISA in synovial fluid.

3. Materials and Methods

Ethical approval

All the study procedures were done in accordance with and approved by the Institutional Animal Care and Use Committee (IACUC) of Faculty of Veterinary Medicine- Cairo University (Approval # Vet CU 12/10/2021/355)

3.1. Experimental animals and study design

Twenty-five clinically healthy Mongrel dogs (males) were used (body weight 15.0 ± 1.7 kg) and age ranged from 20-25 months. Before enrollment in the study, each dog was given a complete clinical, physical, and radiographic examination to exclude the evidence of any systemic, orthopedical and neurological disease. Animals were housed indoor in

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individual cages with free access to drinking water and dry canine food for maintenance. The dogs were randomly assigned to 5 groups 5 dogs per each, control negative group, dexamethasone treated group, Sodium hyaluronate treated group, Polysulfated glycosaminoglycan treated group and 5th group' control positive group. The dogs were fastened 12 hours prior to surgery and allowed free access to drinking water 2 hours before anesthesia. The dogs were prepared in lateral recumbency with the operated side upper most. The cephalic vein was cannulated using 20 G I.V. cannula. The skin over the right pelvic limb from the dorsal midline to the tarsal joint was circumferentially clipped and prepared with routine aseptic preoperative preparation. Dogs were premedicated with atropine sulphate (Atropine®, ADWIA Co. 10th of Ramadan City, Egypt) subcutaneously 10 minutes before induction of anesthesia at a dose rate of 0.05 mg/kg body weight and tranquilized with xylazine HCL (Xylaject®, ADWIA Co. 10th of Ramadan City, Egypt)1mg/kg body weight I.M. Ketamine HCL (Ketamax®, Gujarat, India) was used for anesthetic induction at a dose rate of 5 mg/kg body weight I.V. via cephalic catheter. Anesthesia was maintained during the operative time by venous drip (0.5 g) thiopental/500 ml dextrose 5%) with a drip rate of 28-40 drops /minute (Thipen®, Sigma, Egypt) [18]. Dogs were intubated to keep the respiratory airway patent by the use of endo-tracheal tube. Osteoarthritis was induced by performing arthrotomy of the right stifle joint and curettage of the cartilage by creation of two 8.0 mm diameter full thickness chondral defect till exposure of subchondral bone. The right stifle joint was approached through a 4 cm parapatellar incision as was previously described for dogs [19]. Then the incision was sutured in layers and the dogs were left for 10 days. Two successive doses of (10 mg amphotericin-B® Sigma-Aldrich, USA) were injected intraarticularly at 10 and 17 days post-operative according to [20]. All dogs were left for 10 days post injection of amphotericin before treatment.

3.2. Treatment protocol

The five groups presented as 1st control negative group (normal**),** the 2nd group treated with 8mg/joint dexamethasone (Dexamethasone sodium phosphate® EPICO Co. 10th of Ramadan City, Egypt) through intra articular injection , the 3rd group was treated by intrarticular injection of sodium hyaluronate 10 mg/ml (Hyalgan® TRB Chemedica, Switzerland), the 4th group treated with polysulfated glycosaminoglycan 100 mg/ml (Adequan® Canine, American Regent, Inc). all treated groups treated by intraarticular injection with 0.2-0.8 ml according to aspirated synovial volume every other day for 3 weeks. The 5th group control positive (sham operated) **after** induction of osteoarthritis no treatment applied.

3.3. Clinical and physical examination before and after treatment

A clinical scoring system adopted from Nganvongpanit et al. [21] was used to record the severity of clinical signs at each visit (weekly). The treatments efficacy was determined using a clinical grading system after Kuroki K. et. al. [22] that looked at the animal's lameness, pain on palpation, and weight-bearing ability. Authors have evaluated the dogs' lameness by walking and trotting 6 meters three times. Palpation of the stifle joint was then performed to determine signs of joint pain and range of motion.

3.4. Synovial analysis

Synovial analysis was performed for every dog at the end of 1st, 2nd,3rd, and 4th weeks after treatment to estimate the MMPs in addition to determine the TIMP1. Dogs were placed in dorsal recumbency with both rear legs dangling over the end of the

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table immediately after anesthesia. The area around the stifle was aseptically prepared. Direct arthrocentesis was used to get synovial fluid. Aliquots were inserted in polypropylene screw cap micro tubes for the study (72.694, Sarstedt AG & Co., Newton, NC). The samples were frozen at -70 °C for assaying within 15 minutes of harvest. For the experiment, at least 0.4 mL of synovial fluid was kept. If there was any remaining material, it was placed in a vacuum glass tube coated with potassium EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and sent for fluid analysis. The Determination of matrix metalloproteinases was performed according to Burkert et. al. [23]. The MMP-3 and MMP-13 concentration was determined using a Human MMP-3 double-antibody ELISA kit (RPN2613; Amersham Biosciences, Piscataway, NJ). An automatic plate reader was used to read the optical density of each well in under 10 minutes at 450 nm (MRX TC Revelation, Dynex Technologies, Inc., Chantilly, VA). The plate reader software (Dynex Revelation 4.02, Dynex Technologies, Inc.) determined MMP-3 concentrations from optical densities using the standard curve. The detection of TIMP-1 concentrations was determined using a Human TIMP-1 double-antibody ELISA kit (RPN2611; Amersham Biosciences). The ELISA detects both free TIMP-1 and TIMP-1 bound to MMP-1, MMP-2, MMP-3, and MMP-9 using two monoclonal antibodies. The procedures followed the same pattern as those described earlier [23].

3.5. Statistical analysis

One way analysis of variance (ANOVA) followed by a post-hoc, Tukey's-Kramer HSD, tests were used to examine the data in this study, and a $P< 0.05$ was considered to be statistically significant. All data are expressed as means ± standard deviation (SD).

4. Results

The obtained results revealed induction of osteoarthritis could be achieved by both intra articular injection of amphotericin-B in combination with articular curettage. The negative control (normal) group mean score of lameness was around 1.0±0.0 till the fourth week of treatment of treated groups.

The positive control (sham operated) group with no therapeutic interference had significantly ($p< 0.05$) the highest lameness score during the experimental duration. Groups that received medical interference had lower lameness scores. The experimental group received polysulfated glycosaminoglycan had significantly the lowest lameness scores during study duration returning to the normal score at the fourth week of treatment **(Fig. 1).**

Similar to lameness evaluation, The negative control (normal) group mean score of pain palpation was around 1.0±0.0 till the fourth week of treatment of treated groups. The recorded results revealed that the positive control group had significantly ($p< 0.05$) the highest score for pain on palpation during the whole experimental duration. Groups that received medical interference had lower scores compared to the positive control group. The experimental group received polysulfated glucose aminoglycan had significantly the lowest scores for pain on palpation during the experimental duration returning to the normal score at the fourth week of treatment **(Fig. 2)**.

The negative control (normal) group mean score of weight bearing was around 1.0±0.0 till the fourth week of treatment of treated groups.The obtained results of pain on palpation declared that the positive control group had significantly ($p< 0.05$) the highest score for weight bearing (non-weight-bearing on standing and walking) during the whole experimental duration. Groups that received medical interference had lower scores compared to the positive control group. The experimental group received

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polysulfated glycosaminoglycan had significantly the lowest scores for weight bearing during the experimental duration returning to the normal score at the fourth week of treatment **(Fig. 3).**

The obtained results in **Fig. 4** showed that the negative control (normal) group mean score of activity of MMP-3 was around 2.10 to 3.26 till the fourth week of treatment of treated groups. The positive control group had significantly ($p< 0.05$) the highest MMP-3 compared with other experimental groups during the whole experimental duration. Unlikely, groups that received medical treatment had significantly lower MMP-3 activities compared to the positive control group. The experimental group received polysulfated glycosaminoglycan had significantly the lowest MMP-3 activities during the experimental duration. The negative control (normal) group mean score of activity of MMP-13 was around 4.46 to 4.56 till the fourth week of treatment of treated groups. The recorded findings in **Fig. 5** showed that the positive control group had significantly ($p< 0.05$) the highest MMP-13 compared with other experimental groups during the whole experimental duration. Interestingly, groups that received medical treatments including dexamethasone, sodium hyaluronate, and polysulfated glucose aminoglycan had significantly lower MMP-13 activities compared to the positive control group. The experimental group treated with polysulfated glucose aminoglycan had significantly the lowest MMP-13 activities during the experimental duration approaching to the normal levels at the third week.

The obtained results in **Fig. 6** showed that the negative control (normal) group mean score of activity of TIMP-1 was around 3.86 to 4.20 till the fourth week of treatment of treated groups. The positive control group had significantly ($p< 0.05$) the lowest TIMP-1 activity compared with other experimental groups during the whole experimental duration. However, groups that received medical treatments including dexamethasone, sodium hyaluronate, and polysulfated glucose aminoglycan had significantly higher TIMP-1 activities compared to the positive control group returning to the normal levels at the third week.

5. Discussion

Osteoarthritis is a common progressive disease in senior dogs, particularly of large breeds. Osteoarthritis is also described as a degenerative joint disease caused by the deterioration of the cartilage. In osteoarthritis, cartilage cushion begins to break down because of factors such as age, injury, repetitive stress, or disease. The damage of the cartilage cushion results in pain, inflammation, decreased range of motion, and the development of bone spurs. Osteoarthritis can affect any joint in the body; however, such conditions affect mainly limbs [1]. Osteoarthritis might lead to massive economic losses because of the failure of the treatment of such cases [24] In this direction, this study was undertaken to investigate firstly the ideal treatment of OA in experimentally induced arthritis in dogs. The obtained results revealed a successful induction of OA in the experimentally treated dogs with amphotericin-B. The development of OA was apparent in terms of the highest significant ($p < 0.05$) score of lameness in untreated dogs during the whole experimental duration. The high score of lameness was demonstrated hence the animals were unable to raise their legs and to move few steps. Unlikely, groups that received medical treatment had lower lameness scores. The used treatments were dexamethasone, sodium hyaluronate, and polysulfated glucose aminoglycan. The experimental group received polysulfated glucose aminoglycan had significantly the lowest lameness scores during the experimental duration returning to the normal score at the fourth week of treatment [25]. Another major clinical sign

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for OA is the pain on palpation. Similarly, the recorded results revealed that the positive control group with no medical intervention had significantly ($p < 0.05$) the highest score for pain on palpation during the whole experimental duration. However, groups that received medical intervention had lower scores compared to the positive control group. The experimental group received polysulfated glycosaminoglycan had significantly the lowest scores for pain on palpation during the experimental duration returning to the normal score at the fourth week of treatment [26]. The score of the weight bearing abilities is regarded as one of the major sensory evaluation parameters for the development of OA [27]. In agreement with the lameness score, and pain on palpation, the positive control group with no treatment had significantly ($p < 0.05$) the highest score for weight bearing (non-weight-bearing on standing and walking) during the whole experimental duration. Unlikely, groups that received medical treatments had lower scores compared to the positive control group. The experimental group received polysulfated glycosaminoglycan had significantly the lowest scores for weight bearing during the experimental duration returning to the normal score at the fourth week of treatment. Lameness, pain on palpation, and difficulty on bearing weight are suggested to be gold standards for sensory evaluation of OA in dogs [28,29]. Severe pain on palpation, lameness, and difficulty in weight bearing were similarly observed upon induction of OA in dogs in several reports [30,31].

The mechanism of OA is a progressive degenerative disease that leads to disability to move and pain syndrome. Generally, the exudative and proliferative phenomenon in the capsular and ligamentous apparatus and osteocartilagenous elements causes functional disturbances which resulting in impairment of the weight-bearing and the motor functions disturbances [32].

The wrinkling of the fibrous capsule, changes in the synovial membrane, the dystrophic process in the ligaments, the connective tissue formation, the development of the osteocartilagenous erosions, the area of osteonecrosis plays an important role in shape, deformity, and the mechanism of locomotion [33].

From the obtained results, it was found that proteinases enzymes were the most prominent feature of arthritis in the early stages. This observation was agreed with other investigators [34,35]. For this reason, synovial analysis was considered a very useful step for diagnosis.

Chemoattractant substance may be a main cause for the destruction of the osteocartilagenous tissues and the other structure of the joint [36]. The end result of this destruction is the liberation of more metalloproteases enzymes and all the cytokines. This result coincides with the observation of [37]. It is known that metalloproteases enzymes are produced normally in tissues in inactive forms but after liberation from the cells, they become active but on the other side the tissues of the body produce inhibitors to all the proteases to inactivate it to protect the body and the healthy tissues from destruction. From the obtained results after the induction of OA, the MMPs become more active and increased in its concentration. The end result is degradation of the cartilage and denaturation of the collagen and the appearance of all the symptoms of arthritis. This observation agreed with that occurred in human [38].

Corticosteroids have been used for treatment of OA in dogs since decades for the purpose of reducing the pain and the structural changes in the joints. In this regard, dexamethasone used in the current study could slightly improve the sensory evaluation of OA in dogs. Likely, injections of 4 mg dexamethasone every other day interval were reported to reduce the development of osteophytes and the severity of structural changes.

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Hyaluronic acid is a glycosaminoglycan that consists of alternating units of glucuronic acid and N-acetylglucosamine. It plays an important role in the structure and organization of the extracellular matrix. HA is found at high concentration in the tissues of the body which have a rapid regeneration [39].

HA promotes the detachment process that allows cells to migrate to the tissue of regeneration or formation. This effect is due to the ability of HA to take a large quantity of water of hydration which opens up tissue spaces for cellular migration [40].

Differences between adult and fetal tissues with respect to hyaluronic acid and its receptor CD44 are well documented in both adult and fetal wounds. The early matrix is rich in hyaluronic acid but in adult HA is degraded by hyaluronidase 41].

Intra-articular injections of HA are regarded as the first-line treatment for OA. This lies on the elastic properties of HA, increasing the lubricating properties similar to that of the synovial fluid in OA joints. Furthermore, HA can directly induce endogenous HA synthesis and has endogenous anti-inflammatory and antinociceptive properties [42,43]. The used sodium hyaluronate could significantly improve the sensory evaluation of OA in the current study. Similarly, [44] Lee. et al. (2019) reported that intra-articular injections of hyaluronic acid and a novel hyaluronic acid-platelet-rich plasma conjugate could significantly improve OA in in a canine model of osteoarthritis. However, in the present study the improvement in the structure of the joint was not as expected that may explain the hyaluronate can degrade the injected material. For this fact other researchers suggested that HA can be mixed with another material or in complex with collagen which may enhance the healing and reduce the scar tissue formation [45].

The proteoglycans are composed of glucose aminoglycan (GAGs) covalently linked to the core proteins. It consists of repeating carbohydrate units that are sulfated. This material might be involved with growth factor regulation such as osteonectin, fibronectin, fibrilin, osteopontin, and bone sialoprotein [46]. These non-collagenous proteins may modulate the cellular attachment and at the same time mediate and assist the calcification of the organic matrix and facilitate the rapid healing the OA [47]. These findings may explain and clear up the question, why polysulfated glycosaminoglycan showed good results in the reading of the enzymes and the newly formed structures of the joint after the treatment. Interestingly, the obtained results in the current investigation revealed that the used polysulfated glucoseaminoglycan had significantly the lowest scores for lameness, pain on palpation, and weight bearing, and the continuation of the treatment for four weeks led to recovery of OA and returning to the normal condition. These results correspond well with Salazar A. et. al. (2019) [31] who recorded that the use of a single oral dose of either firocoxib (Previcox®; BoehringerIngelheim), or grapiprant (Galliprant®, Elanco Animal Health), two new introduced NSAIDs, in an induced synovitis model of acute arthritis and pain in dogs could significantly improve OA and pain records on the examined dogs. Similar findings were reported before [48,49]. The anti-inflammatory effects of NSAIDs are attributed mainly to the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2) that are produced by the breakdown of arachidonic acid resulting from cell-wall damage [46].

Osteoarthritis is leading to significant destruction in the extracellular matrix (ECM) within synovial joints, particularly those in the knee, hands, and hips. Matrix metalloproteinases, enzymes that may degrade all components of the ECM, are stimulated by inflammatory mediators such as tumor necrosis factor

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alpha (TNF-) and the interleukins including IL-1 and IL-7 that produced by both joint cells and immune cells [50]. Matrix metalloproteinase-3 (MMP-3), and MMP-13 critical enzymes involved in cartilage degeneration, are assumed to have substantial contributions to the degenerative process that occurs during OA pathogenesis [5]. The obtained results in the present study showed that MMP-3-related activity was significantly increased in the positive control group with no medical treatment compared with other experimental groups during the whole experimental duration. Unlikely, groups that received medical treatment had significantly lower MMP-3 activities compared to the positive control group. The experimental group received polysulfated glucose aminoglycan had significantly the lowest MMP-3 activities during the experimental duration. Similarly, MMP-13 related activity was significantly increased in the positive control group compared with other treated groups during the whole experimental duration. Interestingly, groups that received medical treatments including dexamethasone, sodium hyaluronate, and polysulfated glucose aminoglycan had significantly lower MMP-13 activities compared to the positive control group. Same to MMP-3, the experimental group treated with polysulfated glucose aminoglycan had significantly the lowest MMP-13 activities during the experimental duration approaching to the normal levels at the third week. These results strongly suggest that MMP-3, and MMP-13 could be used as ideal markers for canine OA. In agreement with this assumption, MMPs-1, -2, -3, -9, -13 have been associated with OA and have been reported as the most influential MMPs in OA disease [51]. In addition, Williams et. al. [52] reported that MMP-3, and MMP-13 are studied as surrogate *in vitro* biomarkers for OA. Besides, Mehana et. al. [5] reported that MMP-13 acts as a key enzyme responsible for the degenerative changes in cartilage during OA, and that is a major contribution occurring during OA pathogenesis.

Tissue inhibitor of metalloproteinases-1 (TIMP-1) is acting as a natural inhibitor and regulator for several MMPs including MMP-1, MMP3, MMP-9, and MMP-13 [4]**.** In this direction, TIMP-1 related activities were assayed in the present study. Unlike MMP-3 and MMP-13, TIMP-1 was significantly ($p< 0.05$) high in the negative control group indicating that arthritis cause reduction TIMP-1 related activity [53]. The obtained results showed that the positive control group with no medical treatment had significantly ($p< 0.05$) the lowest TIMP-1 activity compared with other experimental groups during the whole experimental duration. However, groups that received medical treatments including dexamethasone, sodium hyaluronate, and polysulfated glucose aminoglycan had significantly higher TIMP-1 activities compared to the positive control group returning to the normal levels at the third week. These results suggest that the used treatments were successful in retaining the normal functions of the joint.

6. Conclusion

This study demonstrated that Experimental *in vivo* models of OA are highly suggested to establish an efficient treatment protocol. Lameness, difficulty in bearing weight, and pain on palpation are among the most apparent clinical signs of OA in dogs. Osteoarthritis was associated with a significant increase in the enzymatic activities of MMPs, particularly MMP-3, and MMP-13. Therefore, it is highly suggested to use MMP-3, and MMP-13 as biomarkers for OA in dogs. TIMP-1 is regarded as an inhibitor and regulator for MMPs, such as MMP-3, and MMP-13, and it had negative correlation with the clinical signs of OA, and the examined MMPs, therefore, it is highly suggested to consider TIMP-1 inducers as targets for treatment protocols of OA in dogs. Among the used treatments, polysulfated

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glycosaminoglycan is the highly recommended therapeutic approach as it caused the highest improvement compared with other treatments.

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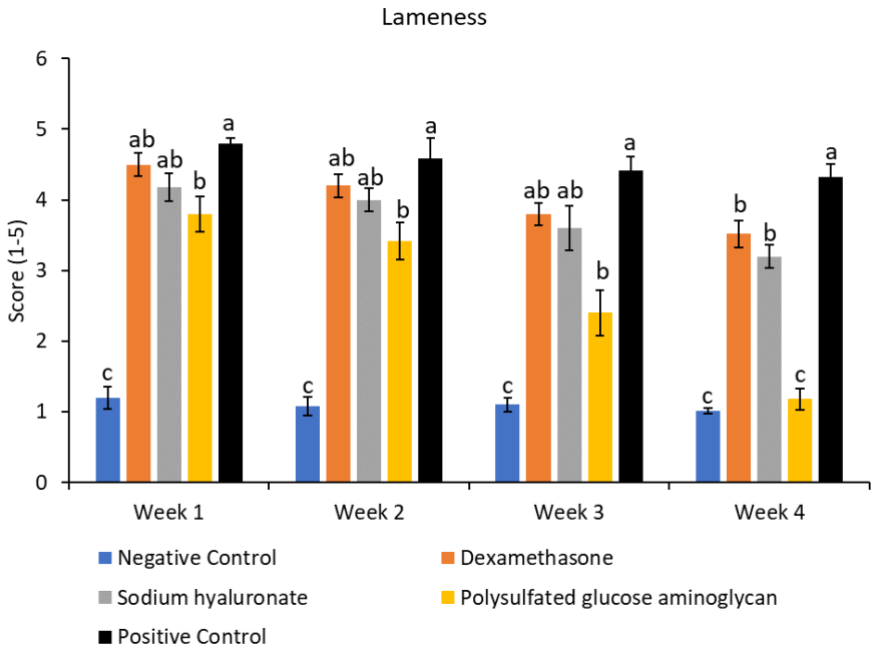

Fig. 1: The score of lameness among dogs with induced-arthritis in comparison with treatment groups

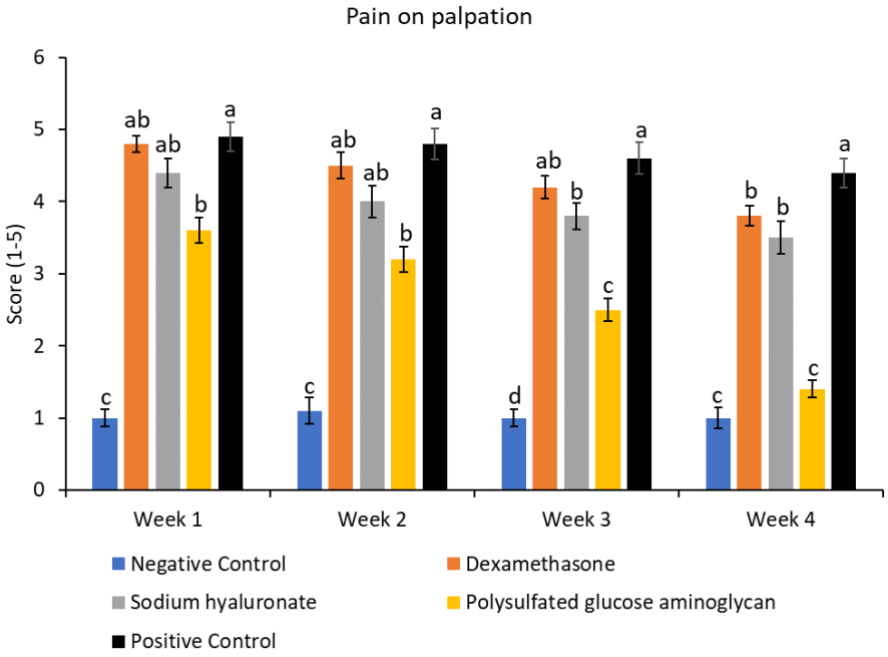

Fig. 2: The score of pain on palpation among dogs with induced-arthritis in comparison with treatment groups

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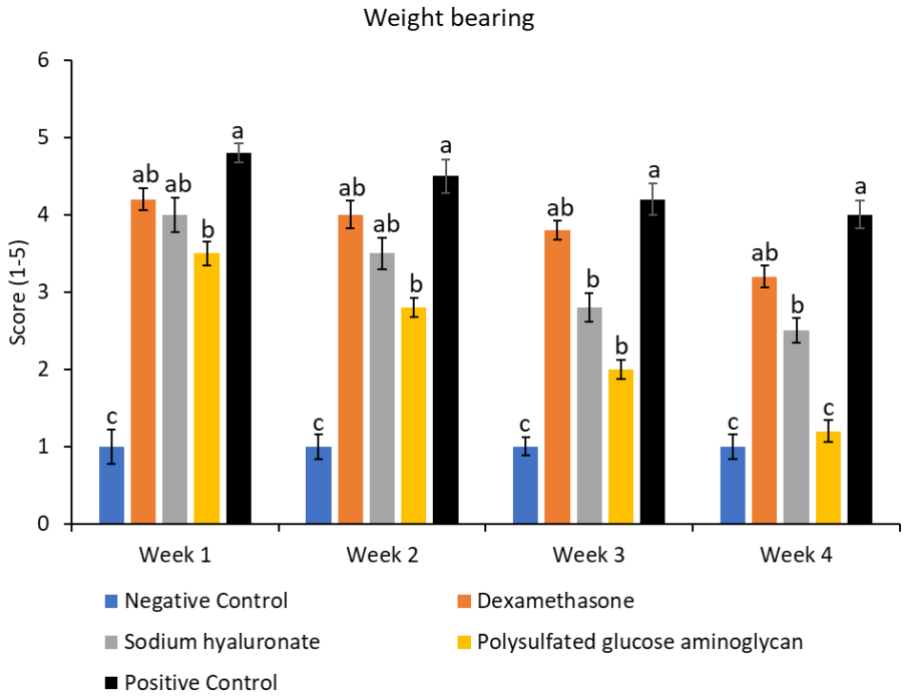

Fig. 3: The score of the weight bearing abilities among dogs with induced-arthritis in comparison with treatment groups

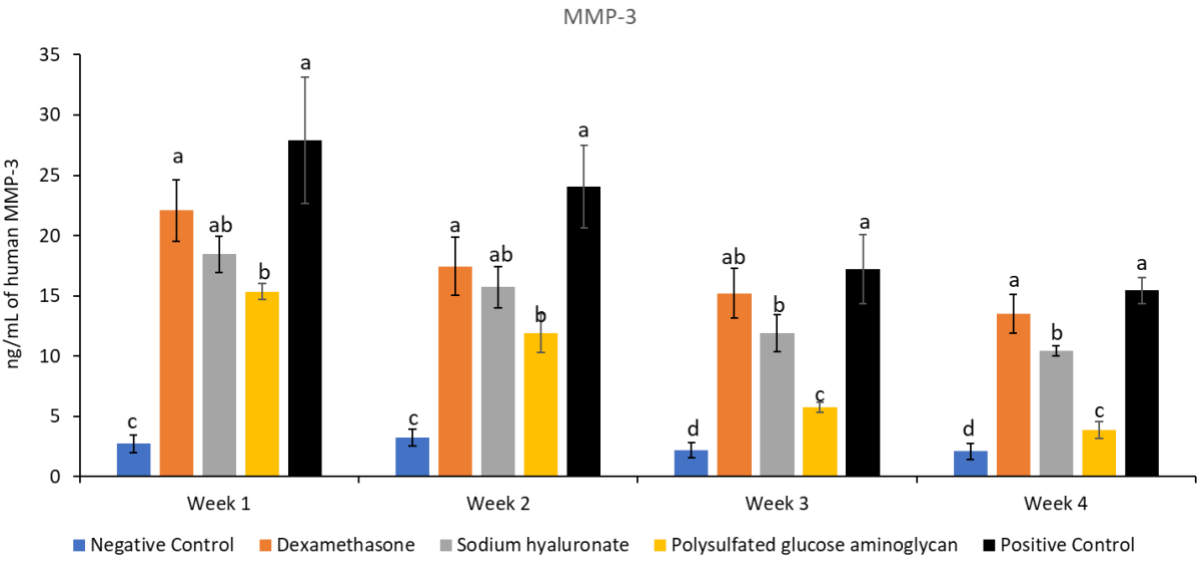

Fig. 4: Activity of MMP-3 among dogs with induced-arthritis in comparison with treatment groups

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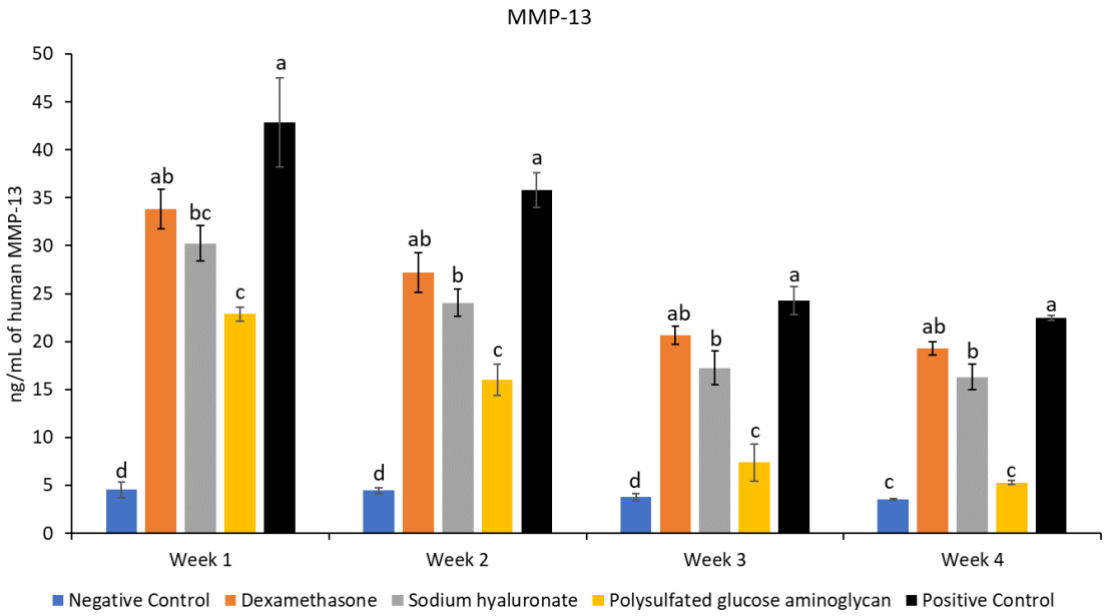

Fig. 5: Activity of MMP-13 among dogs with induced-arthritis in comparison with treatment groups

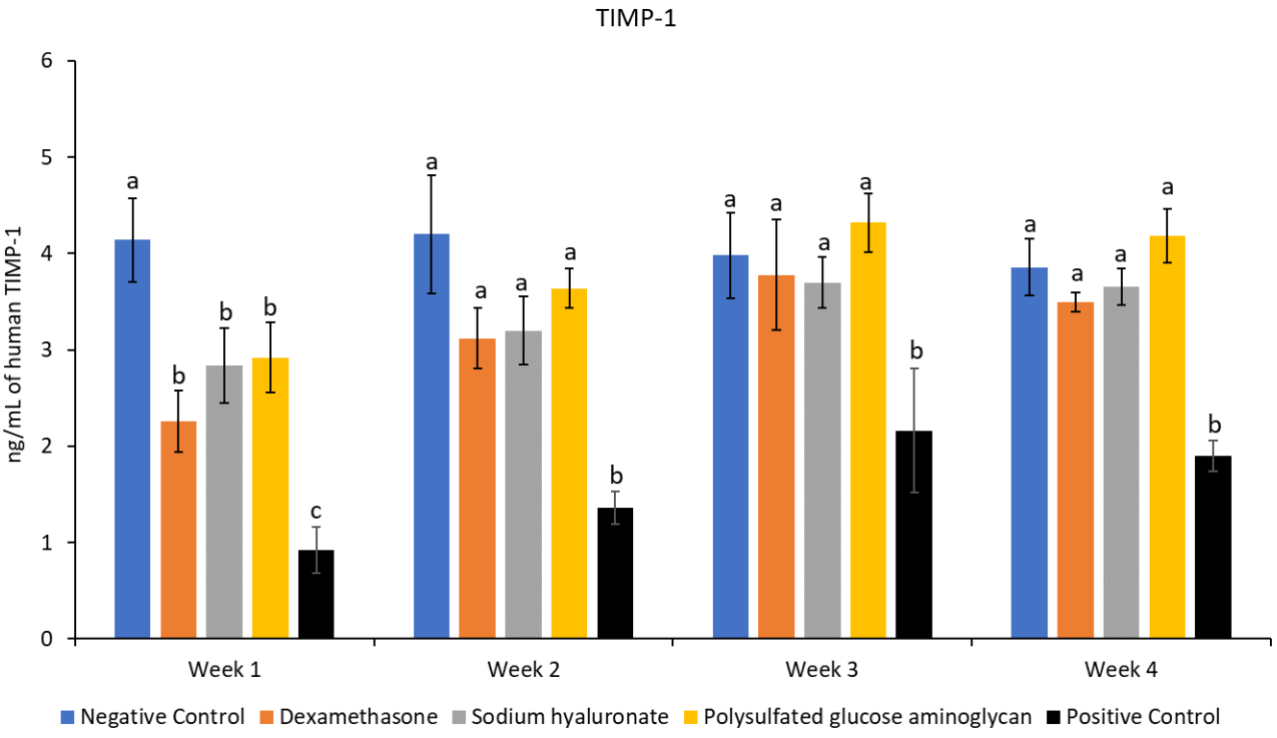

Fig. 6: Activity of TIMP-1 among dogs with induced-arthritis in comparison with treatment groups

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