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Therapeutic management of appendicular osteoarthritis in dogs using platelet rich plasma (PRP) and pentosan polysulphate sodium

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The present study was conducted on 12 dogs suffering from appendicular joint osteoarthritis (OA) presented at Veterinary Clinical Complex, Jabalpur during the study period of six months. The dogs were equally distributed into three treatment groups. In group I, the dogs were treated with inj. carprofen 4 mg/kg body wt, s.c. for 5 days and tab. glucosamine hydrochloride 500 mg, chondroitin sulphate 400 mg, manganese sulphate 5 mg, Boswellia serrata 50 mg, Withania somnifera 50 mg, Vitamin C 12.5 mg orally for 30 days. The dogs of group II were treated as in group I with addition of an intra-articular injection of 1 mL platelet-rich plasma (PRP) on days 0, 7, 14, 28 and 45. The dogs of group III were treated as in group I with addition of an intra-articular injection of 1 mL PRP and pentosan polysulfate sodium 3 mg/kg body wt, s.c. on days 0, 7, 14, 28 and 45. The results indicated that both the treatment regimens (group II and III) were equally effective in reducing osteoarthritic pain; however, significant (P<0.05) decrease in lameness and pain bearing scores were earlier in group III as compared to group II.

Keywords: *Dog, Osteoarthritis, Pentosan polysulfate sodium, Platelet rich plasma*

Osteoarthritis (OA) is a chronic inflammatory joint disease characterized by decreased cushioning in the joints and altered synovial fluid, causing pain, discomfort, stiffness and lameness (Vaughn-Scott and Taylor, 1997). Both local (joint trauma, overload, muscle weakness and developmental abnormalities) and systemic factors (genetic factors, high age, nutrition, gender and hormonal status) may significantly impact on development of OA (Garstang and Stitik, 2006).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used medication for the management of osteoarthritis (Henrotin *et al*., 2005). Platelet-rich plasma (PRP) has a high concentration of platelets, which contains a variety of growth factors that help to decrease the progression of OA by promoting cartilage anabolism when administered intra-articularly (Stief *et al*., 2011). Pentosan polysulfate sodium (PPSNa) is a polysulfated polysaccharide heparin analogue used in the management of canine osteoarthritis for its potential chondroprotective functions (Dart *et al*., 2001). In the present study, platelet rich plasma (PRP) and pentosan polysulphate sodium were evaluated for the therapeutic management of appendicular osteoarthritis in dogs.

Materials and Methods

The study was conducted during the period of six months from August 2023 to January 2024. Clinical and radiographic examinations were conducted in dogs with history of joint pain, lameness or abnormal gait. Twelve dogs, irrespective of sex, breed and age, presented for the treatment of osteoarthritis were used in the present study in three groups, I, II and III (Table 1).

From each dog, 5 mL of blood was collected in vials containing Acid Citrate Dextrose (3.2%) as anticoagulant. Centrifugation of blood was done at Relative Centrifuge Force (RCF) of 200 gravities (g) for 10 min and upper layer composed of plasma platelets and WBCs (buffy coat) was collected and transferred to a glass tube to be homogenized. Homogenized sample was then again centrifuged at RCF of 400 g for initial 5 min and subsequently at RCF of 600 g for another 5 min. The supernatant 2/3rd was discarded as platelet poor plasma and remaining 1/3rd called as platelet rich plasma was used for intra-articular injection (Kececi *et al*., 2014).

The dogs were kept off feed for 8 hr and off water for 4 hr prior to anaesthesia. General anaesthesia was induced using atropine sulphate (0.04 mg/kg body wt, i.m.), xylazine (0.8 mg/kg body wt, i.m.) and ketamine (5.0 mg/kg body wt, i.m.). The joint was aseptically prepared. For elbow joint, the animal was placed in lateral recumbency with the affected side up and the elbow joint was hyperextended to allow the joint to distend caudally. A needle (20-24 G) was then introduced lateral to and alongside the olecranon and inserted cranially toward the middle of the joint until contact was made with the humeral condyle. Other joints were also approached with standard technique for arthrocentesis in dogs (Fossum, 2007) (Fig. 1).

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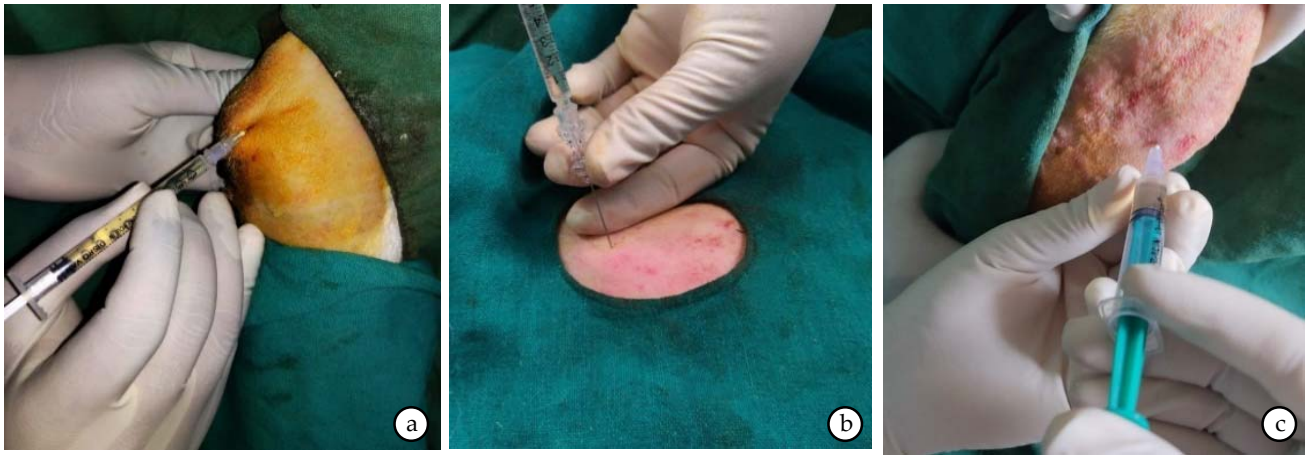

Fig. 1: Site for intra-articular injection (a) elbow, (b) hip and (c) stifle joint.

Clinical parameters such as degree of lameness, pain, degree of weight bearing and joint mobility were recorded on days 0, 7, 14, 28 and 45 of the treatment and were graded as per McCarthy *et al*. (2007). The degree of lameness was measured on a scale of 1 to 5; as walking normally (1), slightly lame when walking (2), moderately lame when walking (3), severely lame when walking (4), reluctant to rise and did not walk more than five paces (5). The degree of pain was measured on a scale of 1 to 5; as no clinical sign (1), mild signs, the dog turned its head in recognition (2), moderate signs, the dog pulled limb away (3), severe signs, dog vocalized or became aggressive (4), and the dog did not allow palpation (5). The degree of weight bearing was measured on a scale of 1 to 5; as equal on all limbs standing and walking (1), normal standing; favoured the affected limb when walking (2), partial weight-bearing while standing and walking (3), partial weight-bearing while standing; non-weight bearing while walking (4), and non-weight bearing while standing and walking (5). The grading for measuring the joint mobility was assigned as full range of motion (1), 10-20% range of motion (2), 10-20% range of motion with mild crepitus (3), 20-50% limitation range of motion with more crepitus (4) and >50% limitation in range of motion with high crepitus sounds (5). Radiographic examination was done to observe the signs of increased joint fluid, sub-chondral bone sclerosis and osteophytes formation on days 0, 7, 14, 28 and 45. Based on the radiographic signs, osteoarthritic dogs were graded (Nganvongpanit *et al*., 2013). The grading of observations after radiographic examination were described as radiographically normal joint (0), doubtful narrowing of joint space and possible osteophyte lipping (1), definite osteophytes, possible narrowing of joint space (2), moderate multiple osteophytes, definite narrowing of joint space, some sclerosis and possible deformity of bone contour (3), and large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour (4).

Computed tomography (CT) was performed at 180 mA and 120 kVp to observe any osteophytes, altered bone opacity and articular soft tissue mineralization (Ballegeer, 2016).

The synovial fluid samples were aseptically collected on day 0 by arthrocentesis and then transferred in sterile vials. Colour, turbidity, viscosity and total nucleated cell count of synovial fluid were estimated (Clements, 2006).

The data was analysed using two-way analysis of variance with the help of IBM SPSS version 27 and interpreted as per the method described by Snedecor and Cochran (1994).

Results and Discussion

A total of 6230 dogs were presented during the study period of six months, out of which 146 dogs had the history of joint pain, lameness or abnormal gait. Out of these 146 dogs, 26 dogs were diagnosed with appendicular osteoarthritis, with the occurrence of 0.41%.

The dogs were divided into three age groups as, < 5 yr, between 5 and 10 yr and > 10 yr. Maximum number of dogs (18, 69%) were recorded in age group of 5 to 10 yr, followed by >10 yr (7, 27%) and then <5 yr (1, 4%). Similar findings were also recorded by Areshkumar *et al*. (2018) and Lamani *et al*. (2019). Higher occurrence of OA in middle aged dogs might be due to age-related changes in their joints. As they get older, the cartilage in their joints might wear down, leading to development of osteoarthritis. Maximum number of cases were recorded in Labrador Retriever (11, 42%), followed by German shepherd (5, 19%), Non-descript (5, 19%), Golden Retriever (2, 8%), Boxer (1, 4%), Rottweiler (1, 4%) and Spitz (1, 4%). These findings were in agreement with the findings of Smith *et al*. (2006) and Bano *et al*. (2022). Higher occurrence of OA in Labrador Retriever, German shepherd and non-descript dogs might be due to preference of pet owners for these dog breeds in the population studied. The dogs were divided into three groups as, < 30 kg, between 30 and 40 kg, and > 40 kg body weight. Highest

occurrence of OA was recorded in dogs weighing >40 kg (11, 42%), followed by dogs weighing between 30-40 kg (8, 31%) and dogs weighing < 30 kg (7, 27%). Excess weight puts additional strain on the joints, accelerating the degenerative process. Marshall *et al.* (2010) and Anderson *et al*. (2020) reported that both obesity and increased body weight leads to increased pressure on the weight bearing joints and predisposes for the development of OA. Occurrence of OA according to joint was higher in hip joint (16, 62%), followed by stifle joint (6, 23%) and elbow joint (4, 15%). Similar findings were also reported by Stabile *et al*. (2019) and Bano *et al*. (2022). This might be due to repeated stress and loading on hip joint. Over the time, this mechanical stress leads to wear and tear of the joint cartilage, contributing to development of osteoarthritis.

Mean values of lameness score decreased significantly (P≤0.05) at days 45 and 28 in group II and III, respectively, indicating clinical improvement on day 45 of treatment as compared to day 0 (Table 2). A significant (P≤0.05) difference in mean lameness score was noticed between group III and group I on day 45. This might be due to decreased pain, increased viscosity of synovial fluid and decreased articular cartilage fibrillation due to intra-articular injections of PRP and Pentosan polysulfate sodium injections. Smith *et al*. (2002) reported that four weekly subcutaneous injections of pentosan by the s.c. route and four week daily oral administration of carprofen were effective in treating OA with significant improvements in lameness. Mean values of pain score decreased significantly (P≤0.05) at day 14 in group III, while in group II significant decrease in the mean pain score was recorded at day 28 (Table 3). Significant (P≤0.05) decrease in mean pain assessment score was noticed in groups II and III on day 45, as compared to group I. This might be attributed to anti-inflammatory action of PRP and pentosan polysulfate sodium resulting in decreased inflammation and joint pain. Innes (2012) stated that pentosan polysulfate can be used for management of canine osteoarthritis for its potential chondroprotective functions. Aminkov *et al*. (2021) observed that PRP exhibits a significant therapeutic potential to alleviate pain. Mean values of weight bearing score decreased significantly (P≤0.05) in groups II and III at days 45 and 28, respectively, indicating clinical improvement on day 45 of treatment as compared to day 0 (Table 4). Significant (P≤0.05) difference in mean weight bearing score was noticed between groups III and I on day 45. This can be due to injections of PRP and pentosan polysulfate sodium, which decreased pain and stimulated formation of hyaluronic acid helping lubrication of joint. Suheb *et al*. (2021) observed a significant improvement in weight bearing between days 14 and 45 in all the six dogs after receiving subcutaneous injection of pentosan polysulfate sodium (PPSNa). A non-significant decrease in the mean joint mobility score was observed between and

Table 1: Experimental design.

Groups	Therapeutic regimen
I	Inj. Carprofen @ 4 mg/kg body wt, s.c. for 5 days and tab. Glucosamine hydrochloride 500 mg, Chondroitin sulphate 400 mg, Manganese sulphate 5 mg, *Boswellia serrata* 50 mg, *Withania somnifera* 50 mg, Vitamin C 12.5 mg orally for 30 days
II	Medication in group I along with an intra-articular injection of 1 mL platelet-rich plasma (PRP) on days 0, 7, 14, 28 and 45
III	Medication in group I along with an intra-articular injection of 1 mL PRP and pentosan polysulfate sodium @ 3 mg/kg body wt, s.c. on days 0, 7, 14, 28 and 45

Table 2: Mean±SE values of lameness score at different time intervals.

Groups	Day 0	Day 7	Day 14	Day 28	Day 45
I	3.50±0.28	2.75±0.47	3.00±0.40	3.00±0.18	2.75^{A}±0.25
II	3.25^{a}±0.47	2.75^{ab}±0.25	2.50^{ab}±0.28	2.50^{ab}±0.28	1.75^{ABb}±0.25
III	3.50^{a}±0.28	2.75^{a}±0.25	2.50^{ab}±0.50	1.50^{b}±0.40	1.50^{Bb}±0.25

Superscripts (a, b) represent significant difference within the groups (P≤0.05)
Superscripts (A, B) represent significant difference between the groups (P≤0.0)

Table 3: Mean±SE values of pain assessment score at different time intervals.

Groups	Day 0	Day 7	Day 14	Day 28	Day 45
I	3.75±0.25	3.50±0.28	3.00±0.40	3.00±0.40	2.75^{A}±0.25
II	3.50^{a}±0.29	3.00^{ab}±0.40	2.75^{ab}±0.24	2.00^{b}±0.40	1.50^{Bb}±0.24
III	3.50^{a}±0.28	2.75^{ab}±0.25	2.25^{b}±0.25	1.75^{b}±0.25	1.50^{Bc}±0.28

Superscripts (a, b, c) represent significant difference within the groups (P≤0.05)
Superscripts (A, B) represent significant difference between the groups (P≤0.05)

Table 4: Mean±SE values of weight bearing score at different time intervals.

Groups	Day 0	Day 7	Day 14	Day 28	Day 45
I	3.50±0.28	3.25±0.25	3.25±0.47	2.75±0.25	2.75^{A}±0.25
II	3.75^{a}±0.25	3.50^{ab}±0.50	3.25^{ab}±0.25	2.50^{ab}±0.28	1.75^{ABb}±0.25
III	3.75^{a}±0.25	3.50^{a}±0.28	2.75^{ab}±0.25	2.00^{b}±0.40	1.50^{Bb}±0.28

Superscripts (a, b) represent significant difference within the groups (P≤0.05)
Superscripts (A, B) represent significant difference b/w the groups (P≤0.05)

Table 5: Mean±SE values of joint mobility score at different time intervals.

Groups	Day 0	Day 7	Day 14	Day 28	Day 45
I	3.25±0.47	3.25±0.47	3.00±0.40	2.75±0.25	2.75±0.62
II	3.25±0.47	2.75±0.47	2.50±0.28	2.25±0.62	2.00±0.40
III	3.25±0.47	3.00±0.41	2.50±0.28	2.00±0.40	1.75±0.25

Table 6: Mean±SE values of radiographic score at different time intervals.

Groups	Day 0	Day 7	Day 14	Day 28	Day 45
I	2.75±0.25	2.75±0.25	2.75±0.25	2.75±0.25	2.75±0.25
II	3.00±0.40	3.00±0.40	3.00±0.40	3.00±0.40	3.00±0.40
III	2.75±0.47	2.75±0.47	2.75±0.47	2.75±0.47	2.75±0.47

within the groups at different time intervals (Table 5). Cook *et al*. (2016) reported that PRP-treated knees showed improved range of motion and improved limb function. Elmesiry (2016) reported that PPSNa resulted in improvement in clinical signs (lameness and range of motion) and articular cartilage healing.

There was no significant difference in the mean radiographic score of the dogs within or between the groups (Table 6). This suggests that the treatment protocol had no impact on the bony changes in the joints affected by osteoarthritis. MacPhail (2000) reported that the osteoarthritic changes within the joint were irreversible. Further, Fahie *et al*. (2013) also observed no difference in radiographic appearance between day 0 and 90 following treatment with PRP in dogs diagnosed with osteoarthritis. CT scanning of osteoarthritic dogs revealed the presence of osteophytes, periostitis, loss of joint space and articular soft tissue mineralization (Fig. 2). Similar findings were noticed by Wenham *et al*. (2014) and Rhee *et al*. (2023). CT was found more useful than plain radiography in diagnosis of OA as it provided more accuracy and 3-dimensional view of the affected joint (Figs. 2 and 3). Rhee *et al*. (2023) also found that CT was helpful to provide most delicate bony structure information with a moderate examination. The synovial fluid was collected in eight dogs. The colour of synovial fluid ranged from colourless, slightly yellow to red-tinged. Similar findings were made by Anirudh and Ranganath (2015). Turbidity of synovial fluid ranged from clear, slightly turbid to opaque. Clements (2006) stated that turbid appearance of synovial fluid indicated a raised joint fluid cell count, which might be due to infection or immune-mediated disease. Viscosity of synovial fluid ranged from normal, slightly reduced to reduced and are in accordance with the findings of Fernandes *et al*. (2002) and MacWilliams and Friedrich (2003). Clements (2006) reported that reduced viscosity of synovial

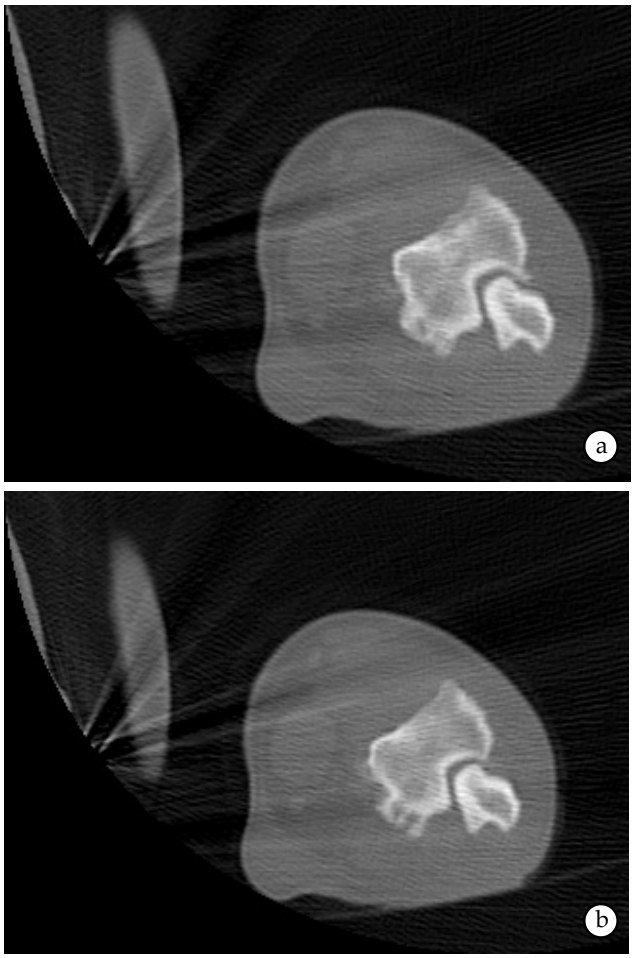

Fig. 2: CT images of osteoarthritic elbow joint (axial view, 5 mm); showing osteophytes at humeral condyle and olecranon process of ulna.

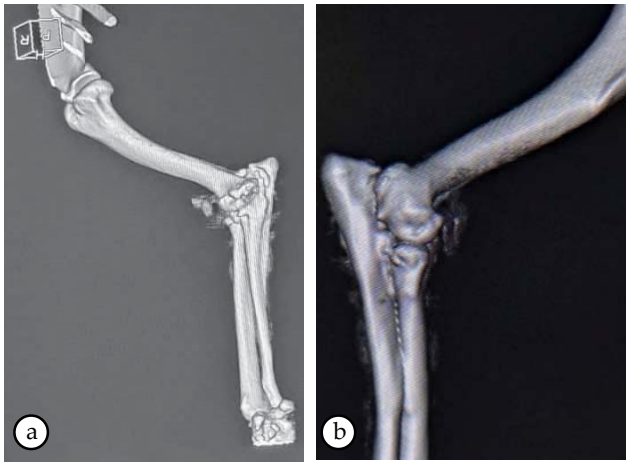

Fig. 3: D reconstructed CT images of osteoarthritic elbow joint; showing osteophytes at elbow joint along with periostitis of radius and ulna.

fluid was indicative of joint disease although, it did not define the pathology present.

Cytological examination of synovial fluid revealed the presence of mononuclear cells and neutrophils which was similar to the findings of MacWilliams and Friedrichs (2003). Total nucleated cell count (TNCC) ranged from 2892 cells/µL to 8122 cells/µL. Jacques *et al*. (2002) reported that increased TNCC might be due to non-inflammatory arthropathies, which have a normal to mildly increased total nucleated cell count.

On the basis of the present study, it was concluded that the occurrence of osteoarthritis was higher in older dogs with higher body weight. PRP and pentosan polysulfate sodium treatment showed better clinical outcome in dogs suffering with OA than analgesics and nutraceuticals.

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