The Pharma Innovation Journal 2022; SP-11(8): 726-730

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(8): 726-730
© 2022 TPI
www.thepharmajournal.com
Received: 01-05-2022
Accepted: 04-06-2022

Sajida Bano
(1) Faculty of Veterinary and Animal Sciences, Rajeev Gandhi south Campus, Banaras Hindu University, Mirzapur, Uttar Pradesh, India
(2) Department of Veterinary Medicine, Ethics and Jurisprudence, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Samar Sarkar
Department of Veterinary Medicine, Ethics and Jurisprudence, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Chandan Lodh
Department of Veterinary Medicine, Ethics and Jurisprudence, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Subhasis Batabyal
Department of Veterinary Biochemistry, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Corresponding Author
Sajida Bano
(1) Faculty of Veterinary and Animal Sciences, Rajeev Gandhi south Campus, Banaras Hindu University, Mirzapur, Uttar Pradesh, India
(2) Department of Veterinary Medicine, Ethics and Jurisprudence, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Studies on bone related disorders in dog with special reference to osteoarthritis and its therapy with herbal components

Sajida Bano, Samar Sarkar, Chandan Lodh and Subhasis Batabyal

Abstract
The study was undertaken in 30 clinical cases of dogs diagnosed with osteoarthritis of various joints, and free of other concurrent neurological, metabolic and infectious diseases along with six healthy control dogs. Of these dogs, 36 dogs were divided into six groups of 06 dogs each. Animals of Group I were treated with collagen peptide orally at the dose rate of 1.0-2.0 mg/kg bwt and along with a combination of Sunthi (0.08gm/kg bwt), calcium (25mg/kg bwt) and Vitamin D (0.02-0.03mg/kg bwt) once daily for 60 days. The second groups were treated with meloxicam orally at the dose rate of 0.2 mg/kg body weight, once daily for 7 days along with a combination of collagen peptide (1.0-2.0 mg/kg bwt) for 60 days. The third group was treated with Herbal component like Sunthi at a dose rate of 0.08 gm/kg body weight orally, daily for 60 days. The fourth group was treated with Glucosamine sulphate (25mg/kg bwt) and Chondroitin Sulphate (20mg/kg b wt) orally, daily for 60 days. The fifth group was treated with calcium (25mg/bwt) and Vitamin D (0.02-0.03mg/kg bwt) orally, daily for 60 days. The sixth group was kept as healthy control. The parameters studied included incidence of Bone Related Disorders in dogs. In osteoarthritis sex wise incidence, breed wise incidence, age wise incidence, Joint wise incidence, radiographical examination, haematological parameters, serum biochemical parameters were seen. Group I animals showed significant reduction in lameness on day 60 which could be attributed to the effect of collagen peptide, sunthi, calcium and vitamin D in providing relief to osteoarthritic pain as compared to other four groups which showed very mild improvement and also there was significant decrease (P<0.01) in uric acid and C-RP. Radiography and clinical sign was the used in diagnosis of OA. Hence, Group I is the better as compared to other groups.

Keywords: Calcium, chondroitin sulphate, glucosamine sulphate, osteoarthritis, sunthi, vitamin D

Introduction
Dogs provide companionship for humans and the effects of such relationships are often associated with physical and psychological health benefits in humans (Barker & Dawson, 1998; Barker & Wolen, 2008; Dembicki & Anderson, 1996) [1, 2, 4]. Health issues for aging dogs and risk of osteoarthritis increases steadily from youth and older dogs are also at risk. Obesity can start as a problem in young dogs and continue into later years or it can develop in an arthritic dog that can no longer exercise normally. The cycle of inflammation, degradation, and chondrocyte damage in osteoarthritis can be promoted by joint stress because of excess body weight. Obesity also increases the likelihood of other diseases in addition to osteoarthritis. Overfeeding or an inappropriate diet often leads to a degenerative osteoarthritic joint disease with symptoms of chronic pain and increasing lameness. Restricted vitality and disability is a direct consequence of the osteoarthritic defect in the dogs. Bone related disorders constitute an important cause for lameness in dogs. Bone disorders may be congenital or may be the result of injury to the joint, abnormal development, immune-related conditions, or infections and nutritional deficiency. Articular cartilage undergo age related changes that increased the risk of joint degeneration leading to the development of osteoarthritis and that the ability of chondrocytes to maintain metabolic homeostasis was shown to decline with age leading to alterations in the proteoglycans and collagen composition and organization.(Nesic *et al.* (2012) [15]. Osteoarthritis also known as degenerative joint disease (DJD) is a chronic inflammatory joint disease followed by progressive degenerative changes of synovial joints characterized by pain, disability, destruction of articular cartilage, and bony remodeling. It is the most common, most frequent and one of the oldest known diseases in both man and animals encountered joint disease in dogs (May, 1994) [13]. Canine hip dysplasia is an inherent, developmental orthopedic disease of the hip joint in dogs (Lust

1980) [12]. Now a days canine hip dysplasia is the most common inherited orthopaedic disease in dogs. The cause of canine hip dysplasia is due to a genetic basis with heritability estimates in the range of 0.2 to 0.6. (Leighton 1997 and Hedhammar *et al*., 1974) [7]. The non-genetic causes include body size, growth rate, nutrition, dietary anion gap, in utero endocrine influences and muscle mass. The diagnosis of canine hip dysplasia has been based on a combination of clinical examination and pelvic radiography. Rickets is an important metabolic disease commonly seen in young growing dogs due to their rapid skeletal growth (Dittmer and Thompson, 2011) [5]. Bowed legs, enlarged joints, and lameness in young dogs is a diagnosis of rickets. Fracture is dissolution of bony continuity with or without displacement of the fragments. It is always accompanied by soft tissue damage of varying degrees; there are torn vessels, bruised muscles, lacerated periosteum, and contused nerves. Sometimes there are injured internal organs and lacerated skin. Fracture of the long bone is a commonly encountered orthopaedic problem in canine practice. There are many methods of treating osteoarthritis by conventional method in which Non-steroidal anti-inflammatory drugs (NSAIDs) are used in dogs because of their ability to reduce joint pain, decrease synovitis, and reduce lameness. The main adverse effects of NSAIDs are associated with the gastrointestinal tract, the kidneys and the impairment of platelet activity (Innes 2012). As dogs are more susceptible to the side effects of NSAIDs than people and most of the drugs in this class have narrow safe margins, accurate dosing is vital (Lamont & Mathews 2007). NSAIDs can cause direct irritation of the mucosa after oral administration or following secretion in bile after hepatic elimination and increased production of leukotrienes and inhibition of aspirin triggered lipoxin (KuKanich *et al*. 2012). NSAIDs may also be harmful for reproductive function and should not be used during pregnancy (Lamont & Mathews 2007). Ayurveda is the oldest system of medicine in the world and by far the most commonly practiced form of non-allopathic medicine in India. At present, it is one of the fastest-growing Complementary and Alternative Medicine (CAM) therapies worldwide (Gogtay NJ *et al*., 2002) [6]. Ayurveda medicine and Panchakarma (detoxification technique) has promising relief effect in osteoarthritis. Several controlled drug trials were conducted to demonstrate efficacy and safety of standardized Ayurvedic drugs containing several plants mentioned in Ayurveda classics, for treatment of osteoarthritis (OA). The primary outcome of Ayurvedic medicinal plants have analgesic, anti-inflammatory, chondroprotection, soft tissue healing, antiosteoporosis, immune-modulation, anti-lipogenesis, anabolic effect, and anti-oxidative stress (Subramoniam A., 2013) [19]. The goal of OA treatment for medical profession is not only control symptoms but also prevent disease progression, minimize disability, and improve quality of life. Ayurveda is one of the fastest growing traditional medicines. Ayurveda treatment outcome is better than conventional standard care in the treatment of OA. The efficacy of some herbal products is beyond doubt. The most recent example for the treatment of osteoarthritis is the use of ginger (*Zingiber officinale*), commonly known as Sunthi in Ayurveda. It is used externally as a paste to subside the inflammations, osteoarthritis. All these above reasons indicate that conventional method of treatment has many side effects, and cannot be used for a longer duration and unsafe for osteoarthritis (OA) patients. Hence in the present study an effort was made to treat the osteoarthritis by Ayurvedic remedy i.e. Sunthi. In this context, the present research work had been carried out in studies on bone related disorders in dog with special reference to osteoarthritis and its therapy with herbal components in TVCC

Materials and Methods

The entire work involved in the study was carried out in the Laboratory of the Department of Veterinary Medicine Ethics and Jurisprudence, Laboratory of the Department of Veterinary Surgery and Radiology, Laboratory of the Department Veterinary Biochemistry, West Bengal University of Animal and Fishery Sciences. The study was conducted for a continuous period of one year i.e. from October 2017 to September 2018.The study was conducted in the Teaching Veterinary Clinical Complex, Clinical cases presented with history and clinical symptoms suggestive of OA formed subject of the study.

A total number of 1952 suspected cases were screened for the presence of bone related disorders, in dogs from Teaching Veterinary Clinical Complex (TVCC) in the month from October 2017 to September 2018. The samples were collected from client owned dogs presented with history and clinical signs suggestive of OA like lameness, unwilling to jumpy run or climb stairs, preference to sit or lie down rather than standing and difficulty in rising and stiffness after resting were common complaints. These were subjected to detailed physical, orthopedic and radiographic examinations to confirm the diagnosis of OA. Out of them 173 animal were positive for osteoarthritis and 36 dogs (30 diseased and free of other concurrent neurologic, metabolic or infectious diseases and 6 healthy) were selected for this study. For their treatment the following studies were undertaken.

Collection of Blood and serum Samples

In this research work, the aim was to see the effects of osteoarthritis in dogs on certain haematological and biochemical profiles. Eight (8) ml blood was withdrawn aseptically from the radial vein or recurrent tarsal vein of the dogs. The blood samples were considered for examination from the enlisted suspected cases of osteoarthritis as per their clinical signs and symptoms, some cases were also considered where there were previous history of such illness and compared with some healthy dogs for assessing the critical condition. Out of which three (3) ml of whole blood were collected in 5ml sterilized plastic vial containing the requisite quantity of ethylene-di-amine tetra acetate (EDTA) @ 1mg/ml of blood for the estimation of haematological parameters. The serum also extracted from collected bloods.

Haematological studies

The haemoglobin (Hb) concentration of the blood was estimated by Cyanmethaemoglobin method as described by Brar *et al.,* (2000) [3] and the values were expressed as gm/dl. Packed Cell Volume (PCV) were measured by Wintrobes Haematocrite Method as described by Brar *et al.,* (2000) [3] and the values were expressed as percentage (%) of the total volume. TEC values were estimated by the Hemocytometer as described by Brar *et al.,* (2000) [3] and was values were expressed as million per microliter ($x10^6$/µl). TLC was estimated by Hemocytometer as described by Brar *et al.* (2000) [3] and was expressed as thousands per microliter ($x10^6$/µl). The DLC was counted by standard Leishman's staining method as described by Schalm *et al.* (1975) [17].

Biochemical studies

The biochemical parameters viz. serum SA, SC, BUN, ALT, AST, ALP, Ca, Uric acid, P, C-RP, Vitamin D were also studied. Serum albumin (SA) was estimated spectrophotometrically by Modified Biuret and Dumas method (1971) as per the protocol provided with the kit and the values were expressed as gm/dl or gm%. Serum creatinine level was estimated spectrophotometrically by Alkaline Picrate Method of Toro *et al.*, (1975) [20] by using serum creatinine kit and expressed as mg/dl. BUN was estimated by DAM method (urea kit) from blood serum and expressed as mg/dl. Serum alanine amino-transferase (ALT) was estimated spectrophotometrically by2,4- dinitrophenylhydrazine (2,4-DNPH) method (Reitman and Frankel, 1957) [16] and the value was expressed as IU/L. Serum aspartate amino-transferase (AST) was estimated spectrophotometrically by2,4- dinitrophenylhydrazine (2,4-DNPH) method (Reitman and Frankel, 1997) [16] and the value was expressed as IU/L. Serum alkaline phosphatase (ALP) was estimated spectrophotometrically by2,4- dinitrophenylhydrazine (2,4-DNPH) method (Reitman and Frankel, 1987) and the value was expressed as IU/L. OCPC method by bagainski (1973) is used for the determination of Calcium concentration in the plasma. Calcium in an alkaline methods combines with o-cresolphthalein complexone to form a purple colored complex. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample and measured in UV- VIS spectrophotometer at 570 nm and expressed as mg/dl. Plasma Phosphorus concentration is measured by Molybdate U.V method described by Fiske and Subbarow (1985). Phosphate ions in acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex has an absorbance in the ultraviolet range and measured of 340 nm. Intensity of the complex is directly proportional to the amount of inorganic phosphorus present in the sample and express as mg/dl. C-reactive protein was estimated by by high sensitive c-reactive protein (hs CRP) assay in an automated immunoturbidimetric method and expressed as mg/dl. (Freedman DM *et al*, 1999). Vitamin D test was done by liquid chromatography/tandem mass spectrometry method for determination of 25-hydroxy vitamin D3. (Newman MS *et al*, 2009).

Radiographical examinations

The affected joints were radiographed in ventro-dorsal views before and after treatment. The extended ventral-dorsal view was used to examine the hip joints.

Therapeutic trial

For this study a total of 36 dogs were selected randomly and then divided into six (6) groups comprising of six dogs in each group. Group VI was kept as control healthy to which no treatment was given. Group I was treated with collagen peptide (Tendocare Forte, a product of pharmed Pvt. Ltd) @1.0-2.0 mg/kg bwt, Sunthi (GMP certified company) @0.08gm/kg bwt and calcium and vitamin D (Petcal, Pfizer Animal Health Limited)@ 25mg/kg bwtand 0.02-0.03mg/kg bwt orally respectively, once daily for 60 days. Group II was treated with meloxicam (Melobest, Intas Pharmaceuticals Pvt. Ltd) orally at the dose rate of 0.2 mg/kg body weight, once daily for 7 days along with a combination of collagen peptide (Tendocare Forte, a product of pharmed Pvt. Ltd) @1.0-2.0 mg/kg bwt, orally, daily for 60 days. Group III was treated with Herbal component like Sunthi (GMP certified company) dose rate 0.08gm/kg body weight orally, daily for 60 days. Group IV was treated with Glucosamine sulphate and Chondroitin Sulphate (Glycoflex, Vetriscience Pharmaceuticals Pvt. Ltd) @ 25mg/kg bwt and 20mg/kg bwtorally respectively, once daily for 60 days. Group V was treated with calcium vitamin D at the dose rate of 25mg/kg bwtand 0.02-0.03mg/kg bwt orally respectively, once daily for 60 days.

The efficacy was judged noticing on the basis of development of clinical signs, blood parameters and radiographical examinations.

Statistical Analysis

All the data which were obtained during the present investigation were analysed in IBM, statistically to draw valid conclusion in SPSS (Version 21.0) software, using general linear model for univariate data. Data related to the study on bone related disorders in dog special reference to osteoarthritis and its therapy with herbal components, the efficacy was judged noticing on the basis of development of clinical signs, blood parameters and radiographical examinations and were analysed by ANOVA according to Tukey'sHSD (Honest Significant Difference) Test. The results were expressed in terms of mean and standard error (SE). A probability value of (p<0.05) was described as significant and (p<0.01) was noted as highly significant.

Results and Discussions

The present study was carried out to investigate about osteoarthritis (OA) in dogs in regards to its clinical, haematological, blood serum biochemical changes and radiographical examinations in different days of treatment. Therapeutics aspects were also highlighted in this study which has a definite role in minimizing OA changes in dogs. The efficacies of drugs against OA were observed based on observation of clinical signs, estimation of hemato-biochemical profiles and radiographical examinations.

A total of 1952 suspected dogs were screened for the presence of bone related disorders in TVCC, WBUAFS, Kolkata, West Bengal. Out of which 173 dogs were positive for OA. In the present study 30 clinical cases of OA in dogs were selected showing the most common clinical signs such as slowly progressive lameness or gait change and exercise intolerance were frequently reported. Unwilling to jumpy run or climb stairs and that they preferred to sit or lie down rather than stand. Difficulty in rising and stiffness after resting and difficulty in jumping over obstacles are the typical clinical signs were common complaints. Many OA patients had a history of earlier acute painful, non-weight bearing lameness, reduced height of step, and shortened stride length, bunny hopping.

Among the incidence of bone related disorders in dog a total number of positive cases were 1952 out of which fracture was 741 (38.00 per cent), hip dysplasia 689 (35.33 per cent), osteoarthritis 173 (8.86 per cent), rickets 86 (4.00 per cent), others 263 (14.20 per cent).Among the dogs with osteoarthritis 84 were females (49.46 per cent) and 89 were males (50.53 per cent). The results of the present study indicated higher incidence of joint disorders in male dogs than female dogs which could be attributed to the aggressive behavior of males compared to females and also due to their faster growth rate or a sex-linked factor. The increased incidence of osteoarthritis in male dogs could be due to the effect of testosterone which influenced the roaming behavior

of intact males which concurred with the findings of Jayaprakash *et al.* (2007) [9] and Simon *et al.* (2010) [18].

In the present study, Out of the 173 dogs affected with osteoarthritis, the breeds are Labrador 52 (29.66 per cent), Spitz 30 (17.35 per cent), German shepherd 34 (19.48 per cent), Doberman 22 (12.77 per cent), Golden Retriever 06 (3.72 per cent), Great Dane 8 (4.79 per cent) and Non-descript dogs 21 (12.23 per cent). The possible reasons in the variation in reported incidence of osteoarthritis recorded among different breeds may possibly be due to the different types of joint disorders or a close association of a certain type of joint affection in a particular breed, rate of body weight gain or due to nutritional, heredity and environmental factors.

Out of the 173 dogs with various joint wise disorders, the joints affected were hip joint 121 (70 per cent), Stifle 35 (20 per cent), hock 06 (3.69 per cent), shoulder 5 (3.12 per cent), elbow 06 (3.19 per cent). The higher occurrence of hip joint disorders recorded in the present study could possibly be attributed to the complexity of the motion of the joint which renders the joint to trauma and it can also be attributed to the differences in genetic line, mutation and environmental factors which influence the growth of the dogs directly.

The animals with OA were found to be distributed in various age groups as, less than one year 29 (17.02 per cent), 1-3 years 35 (20.44 per cent), 4-6 years 30 (17.04 per cent), 7-9 years 42 (24 per cent) and above 10 years 37 (21.50 per cent). High incidence of joint disorders in dogs older than 6 years can be attributed to a large number of dogs that undergo articular cartilage related changes with age that increased the risk of joint degeneration and that the ability of chondrocytes to maintain metabolic homeostasis was shown to decline with age leading to alterations in the proteoglycans and collagen composition and organization. Similar findings were reported by Mele (2007) [14] and Nesic *et al.* (2012) [15].

Apart from estimation of incidence (sex-wise, breed-wise, joint-wise, age-wise) of bone rated disorders, the therapeutic aspect was also covered in this study. These cases were divided into five groups (Gr I, II, III, IV and V) randomly, having six dogs in each groups, and provided different combinations of treatments in groups I, II, III, IV and V. The animals of group I were treated with collagen peptide, sunthi, in addition with the supportive treatment of calcium and vitamin D. The animals of group II were treated with meloxicam and collagen peptide. The animals of group III were treated with a herbal component, sunthi (*Zingiber officinale*). Group IV animals were treated with a combination of glucosamine and chondroitin sulphate. Group V animals were treated with calcium and vitamin D. The groups VI dogs were kept as healthy control group. The six healthy control group dogs were without any characteristic signs and symptoms of osteoarthritis and which were found to be free from any other concurrent diseases were considered for healthy control group. Periodical examinations were done on clinical and hemato-biochemical parameters before and after therapy.

In the present study, the clinical manifestations of the dogs of group I was found to improve gradually at 30 days to 60 days of treatment. The haematological changes of Haemoglobin concentration (gm/dl), Packed Cell Volume(%), Total Erythrocyte Count (X 10^6/cumm), Total Leucocyte Count(/ cumm),Differential Leucocyte Count(%) in osteoarthritis were within normal limits and were not significant in groups I, II, III, IV and V as compared to healthy control group on 0, 30 and 60 days of study. Serum Albumin (gm/dl), Serum Creatinine(mg/dl), BUN(mg/dl),ALT(IU/L), AST(IU/L), ALP(IU/L), Total protein (g/dl) in osteoarthritis were within normal limits and were not significant in groups I, II, III, IV and V as compared to healthy control group on 0, 30 and 60 days of study.

Ca (table 1), Phosphorus (Table 2), Vitamin D(mg/dl) (table 3) in osteoarthritis increased significantly (p<0.01) in Gr.I, II, III, IV, V and VI. The values of uric acid (g/dl) (table 4) and C-RP (mg/dl) (table 5) in osteoarthritis decreased significantly (p<0.01) in Gr. I, II, III, IV, V and VI respectively on 0 day observation compared to healthy control group. But following treatment animals showed gradual improvements in the treated groups on 30 and 60 days of treatment.

Osteoarthritis is considered to be a problem of geriatric dog population. Hematological parameters showed no significant changes in dogs with osteoarthritis. Biochemical changes in all the treatment groups, mean value of Uric acid was decreased. However, the relative decrease on day 60 was more for group I (0.41±.01) (0.018*)* as compared with group II (1.06±0.34), group III (1.13±0.45), group IV (1.08±0.46) and group V (0.98±0.37). In all the treatment groups, mean value of CR-P was decreased. However, the relative decrease on day 60 was more for group I (3.13±0.32) (p<.001) as compared with group II (3.43±0.40), group III (4.6±0.65), group IV (5.18±0.81) and group V (6.5±1.26). In Radiograph osteophyte formation, increased bone opacity, development of sclerosis, loss of the articular cartilage and narrowing of joint spaces were reduced.

Ginger seems to have potent ant inflammatory and analgesic property in conjunction with other conventional drugs that have evidence in the clinical, biochemical and radiological studies of osteoarthritic dogs in Group I. It was found that herbal drugs are economic, has low risk of side effects, can be used for a long duration and complementary and alternative medicine. Group I showed marked improvement by the end of day 60 while Group II, III , IV and V showed mild improvement by day 60 which was evident from parameters such as clinical signs, blood parameters and radiography. Treatment with collagen peptide along with combination of Sunthi, calcium and Vitamin D in oral form showed good therapeutic response and is better than conventional treatment alone.

Table 1: Calcium alteration (gm/dl) in non-infected and infected groups of dogs (Mean±S.E)*

Group	Days		
	0 days	30 days	60 days
Group I	7.05 ± 0.28^{bz}	9.95 ± 0.43^{ay}	10.45 ± 0.30^{ax}
Group II	7.15 ± 0.26^{bx}	7.15 ± 0.26^{cd}x	6.93 ± 0.22^{dy}
Group III	6.85 ± 0.31^{c}	7.05 ± 0.22^{d}	7.05 ± 0.22^{d}
Group IV	6.98 ± 0.30^{cy}	8.08 ± 0.10^{bx}	8.11 ± 0.17^{bx}
Group V	7.35 ± 0.16^{cy}	7.36 ± 0.08^{cy}	7.41 ± 0.15^{bx}
Group VI	8.86 ± 0.13^{a}	9.23 ± 0.16^{a}	9.31 ± 0.18^{a}

Table 2: Phosphorus alteration (mg/dl) in non-infected and infected groups of dogs (Mean±S.E)*

Group	Days		
	0 days	30 days	60 days
Group I	3.35 ± 0.03^{bz}	5.65 ± 0.16^{ay}	6.38 ± 0.17^{ax}
Group II	3.78 ± 0.21^{by}	4.05 ± 0.24^{bx}	3.96 ± 0.20^{cx}
Group III	2.93 ± 0.21^{c}	3.10 ± 0.14^{c}	3.10 ± 0.14^{d}
Group IV	3.76 ± 0.13^{bx}	3.33 ± 0.12^{cy}	3.30 ± 0.13^{cdy}
Group V	3.43 ± 0.10^{cy}	3.46 ± 0.10^{bcy}	3.58 ± 0.12^{bx}
Group VI	4.3 ± 0.08^{a}	4.41 ± 0.12^{a}	4.45 ± 0.09^{b}

Table 3: Vit D alteration (gm/dl) in non-infected and infected groups of dogs (Mean±S.E)*

Group	Days		
	0 days	30 days	60 days
Group I	71.85±7.89^{by}	79.58±10.49^{by}	97.73±1.15^{ax}
Group II	59.28±4.55^{cx}	56.16±4.15^{cdy}	56.68±4.52c^{y}
Group III	63.08±4.95^{bc}	61.05±4.81^{c}	61.73±4.86^{b}
Group IV	57.75±0.69^{cy}	57.51±0.72^{cy}	56.66±0.14^{cx}
Group V	49.15±*1*.67^{dy}	49.35±*1*.60^{dy}	50.41±3.76^{dx}
Group VI	100.28±1.14^{a}	100.28±0.98^{a}	100.25±1.13^{a}

Table 4: Uric Acid alteration (gm/dl) in non-infected and infected groups of dogs (Mean±S.E)*

Group	Days		
	0 days	30 days	60 days
Group I	1.20±0.45^{bx}	0.58±0.21^{cy}	0.41±0.01^{cy}
Group II	1.33±0.39^{ax}	1.08±0.35^{ay}	1.06±0.34^{by}
Group III	1.23±0.46^{bx}	1.18±0 .48^{ay}	1.13±0.45^{ay}
Group IV	1.13±0.46^{c}	1.13±0.45^{a}	1.08±0.46^{b}
Group V	1.00±0.38^{c}	1.00±0.38^{b}	0.98±0.37^{b}
Group VI	0.13±0.01^{d}	0.13±0.02^{d}	0.13±0.01^{d}

Table 5: CR-P protein alteration (gm/dl) in non-infected and infected groups of dogs (Mean±S.E)*

Group	Days		
	0 days	30 days	60 days
Group I	6.51±1.36b^{x}	4.58±0.61b^{cy}	3.13±0.32^{cz}
Group II	6.7±1.78^{bx}	3.6±0.48^{cy}	3.43±0.40^{cy}
Group III	7.91±1.66^{abx}	5.05±.78^{by}	4.6±0.65^{by}
Group IV	8.61±1.78^{ax}	7.61±1.43^{ay}	5.18±0.81^{bz}
Group V	6.2±1.12^{bx}	4.43±1.02^{cy}	6.5±1.26^{ax}
Group VI	2.08±0.01^{c}	2.08±0.01^{d}	2.08±0.02^{d}

*Different superscripts row wise (a, b, c and d) and column wise (x and y) differ significantly. Significance level (p<0.05) is significant whereas (p<0.01) is highly significant according to Tukey's HSD test.

Acknowledgment

The authors are very much thankful to the Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, for providing necessary facilities for this study. This work was a part of Ph.D. thesis of first author.

References

1. Barker SB, Dawson SKS. The effects of animal-assisted therapy on anxiety ratings of hospitalized psychiatric patients. Psychiatric Services. 1998;49:797-801.
2. Barker SB, Wolen AR. The benefits of human-companion animal interaction: A review. Journal of Veterinary Medical Education. 2008;35:487-495.
3. Brar RS, Sandhu HS, Singh A. Veterinary clinical diagnosis by laboratory methods. Kalyani publishers., Kolkata, 2000.
4. Dembicki D, Anderson J. Pet ownership may be a factor in improved health of the elderly. Journal of Nutrition for the Elderly. 1996;15:15-31.
5. Dittmer KE, Thompson KG. Vitamin D metabolism and rickets in domestic Animals: A review. Veterinary Pathology. 2011;48:389-407.
6. Gogtay NJ, Bhatt HA, Dalvi SS, Kshirsagar NA. The use and safety of non-allopathic Indian medicines. Drug Saf. 2002;25:1005-1019.
7. Hedhammar A, Wu FM, Krook L. Overnutrition and skeletal disease. Cornell Veterinarian. 1974;64:83-114.
8. Innes JF, Clayton J, Lascelles BD. Review of the safety and efficacy of long-term NSAID use in the treatment of canine osteoarthritis. Vet Rec. 2010;166:226-230.
9. Jayaprakash R, Dhanalakshmi N, Suresh RK, Ganesh TN. A retrospective study on incidence of canine hip dysplasia. Ind. Vet. J. 2007;84:519-520.
10. Kukanich B, Papich MG. Pharmacokinetics and antinociceptive effects of oral tramadol hydrochloride administration in Greyhounds. Am J Vet Res. 2011;72:256-262.
11. Leighton RL. Surgical treatment of osteochondritis dissecans of the canine stifle. J Vet. Med. Small Anim. Clin. 1981;76:1733.
12. Lust G, Beilman WT, Dueland DJ. Intra-articular volume and hip joint instability in dogs with hip dysplasia. J Bone joint Surg. 1980;62(A):576,
13. May SA. Degenerative Joint disease (Oseoarthritis, Osteoarthrosis, Secondary Joint disease). In : Houlton, J.E.F. and R.W. Collinson (Eds.), Manual of Small Animal Arthrology, British Small Animal Veterinary Association, Lowa State University Press, Ames, 1994, 62-74.
14. Mele E. Epidemology of osteoarthritis. Vet. foc. 2007;17(3):4-10
15. Nesic D, Whiteside R, Brittberg M, Wendt D, Martin I, Mainil-Varlet P. Cartilage tissue engineering for degenerative joint disease. Adv. drug delivery rev. 2012;58:300-322.
16. Reitman S, Frankel S. Colorimetric method for the determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. Amer. Journal. Clin. Paathol. 1957;28:56-63
17. Schalam OW, Jain NC, Caroll EJ. Veterinary haematology. 3rd edn. Lea and Febiger, Philadelphia, 1975, 410-461.
18. Simon MS, Ganesh R, Ayyapan S, Rao GD, Suresh Kumar R, Kundave VR, Das BC. Incidences of pelvic limb fractures in dogs:A survey of 478 cases. Vet. World. 2010;3(3):120-121.
19. Subramoniam A, Madhavachandran V, Gangaprasad A. Medicinal plants in the treatment of arthritis. Annals of Phytomedicine. 2013;2:3-36.
20. Toro G. Practical Clinical Chem, 1975, 154.