CLINICAL STUDIES ON NUTRACEUTICALS VERSUS AUTOLOGOUS UNCULTURED BONE MARROW MONO-NUCLEATED STEM CELLS (BMNSc) FOR TREATMENT OF HIP DYSPLASIA IN CANINE

THESIS

BY

Dr. SUKHJINDER SINGH

(BVC/M/VSR/005/2017-18)

Submitted to

BIHAR ANIMAL SCIENCES UNIVERSITY

PATNA, BIHAR

In partial fulfillment of the requirements

FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

IN

VETERINARY SURGERY AND RADIOLOGY

2019

DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY
Bihar Veterinary College, Patna-800014
(Bihar Animal Sciences University, Patna, Bihar)

CERTIFICATE-I

This is to certify that the thesis entitled "***Clinical Studies on Nutraceuticals versus Autologous Uncultured Bone Marrow Derived Mono-Nucleated Cells (BMNCs) for treatment of Hip Dysplasia in Canine***" submitted in partial fulfillment of requirement for the award of the degree of **Master of Veterinary Science in the discipline of Veterinary Surgery and Radiology** of faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is the bonafide research carried out by **Dr. Sukhjinder Singh** son/~~daughter~~ of Sh. Satwant Singh under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Endorsed

Place: Patna

Date:

(Dr. Ramesh Tiwary)
Major Advisor
Assistant Professor cum Junior Scientist
Department of Veterinary Surgery and Radiology,
Bihar Veterinary College, Patna-14

DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY
Bihar Veterinary College, Patna-800014
(Bihar Animal Sciences University, Patna, BIHAR)

CERTIFICATE- II

This is to certify that the thesis entitled "***Clinical Studies on Nutraceuticals versus Autologous Uncultured Bone Marrow Derived Mono-Nucleated Cells (BMNCs) for treatment of Hip Dysplasia in Canine***" submitted by **Dr. Sukhjinder Singh,** Registration No.– **BVC/M/VSR/005//2017-18,** son/~~daughter~~ of Sh. Satwant Singh to Bihar Animal Sciences University, Patna, Bihar in partial fulfillment of the requirement for the degree of **Master of Veterinary Science in the discipline of Veterinary Surgery and Radiology** has been approved by the advisory Committee after an oral examination of the student in collaboration with an External examiner.

Dr. Ramesh Tiwary
Chairman, Advisory Committee

Dr. A. K. Gangwar
(External Examiner)

Members of the advisory Committee:

1. **Dr. Archana Kumari**
   Asstt. Prof.-cum-Jr. Scientist
   ……
   Dept. of Veterinary Surgery & Radiology
2. **Dr. Pallav Shekhar**
   Asstt. Prof.-cum-Jr. Scientist
   ……
   Dept. of Veterinary Medicine
3. **Dr. Ajeet Kumar**
   Asstt. Prof.-cum-Jr. Scientist
   ……
   Dept. of Veterinary Biochemistry
4. **Dr. G.D. Singh**
   Asstt. Prof.-cum-Jr. Scientist
   ……
   Dept. of Veterinary Surgery & Radiology
   (**Nominee of Dean, PGS)**

Dean, Postgraduate studies

(Dr. Mithilesh Kumar)
Head of Department

ACKNOWLEDGEMENT

I would like to express my deep sense of gratitude and indebtedness to my guide and major advisor, ***Dr. Ramesh Tiwary*** *Asstt. Prof. cum Jr. Scientist Department of Veterinary Surgery and Radiology, Bihar Veterinary College, Patna, for valuable guidance, keen interest, close supervision, constant encouragement and healthy criticisms during the course of investigation. His painstaking supervision of the manuscript warrants special mention, without which this research undertaking would not have completed.*

I am highly obliged to ***Dr. Mithilesh Kumar,*** *Head, Dept. of Veterinary Surgery and Radiology, for his useful suggestions and needful facilitation of contrivance during the course of investigation.*

I am grateful to the other members of my advisory committee, ***Dr. Archana Kumari,*** *Assistant Professor-cum Jr. Scientist Department of Veterinary Surgery and Radiology,* ***Dr. Pallav Shekhar****, Assistant Professor-cum Jr. Scientist Department of Veterinary Medicine,* ***Dr. Ajeet Kumar****, Assistant Professor-cum Jr. Scientist Dept. of Veterinary biochemistry., and* ***Dr. G. D. Singh,*** *Assistant Professor-cum Jr. Scientist Dept. of Veterinary Surgery and Radiology, Bihar Veterinary College, Patna, for their valuable guidance, constructive suggestions and timely help during the entire period of investigation.*

My sincere thanks are also to all Assistant Professor-cum Jr. Scientist of Bihar Veterinary College, Patna for his co-operative behavior, valuable suggestions and moral support during the research work.

I, with great pleasure, acknowledge my thanks to ***Dr. J. K, Prasad,*** *Dean, Bihar Veterinary college, Patna-14, for providing the necessary facilities during the tenure of this investigation.*

A deep sense of gratitude is expressed to Bihar Animal Sciences University (BASU), Patna, Bihar, for providing facilities to conduct this investigation.

A deep sense of gratitude is expressed to ***Honorable Vice Chancellor Sir****,* ***Registrar Sir , DRI cum Dean PGS Sir, Director Research Sir, D.S.W. Sir; Hostel Warden Sir, Hostel Superintendent Sir and All University Officer*** *of Bihar Animal Sciences University, Patna, Bihar, for providing facilities to conduct this investigation.*

My thanks are also extended to all the respected seniors ***Dr. Awadhesh Kumar, Dr. Babul Kumar, Dr. Ravi Ranjan Kumar, Dr. Harmanpreet Singh Sodhi, Dr. Shyamdeo Kumar, Dr. Armannullah ,*** *many colleagues like* ***Dr. Kanchan Rawal, Dr. Rajapartap Singh Sandhu, Dr. Kirpal Singh Uppal, Dr. Harmandeep Singh Josan, Dr. Kabal Singh Brar, Dr. Chandan Kumar, Dr. Ritesh Kumar, Dr. Hitesh Purohit, Dr. Sudhanshupartap Singh, Dr. Soni Kumari*** *most loving junior,* ***Dr. Manish Kumar Mukherjee, Dr. Komal , Dr. Agyey pusp, Dr. Ayush*** *and* ***all other friends*** *who helped me directly or indirectly during my research work with a company of whom helped me to overcome the stressful moment of investigation and physically help from time to time during the course of study.*

I am also thankful to the Librarian and the staff-members of the library of the Bihar Veterinary College, Patna-14 for rendering their cooperation.

Thanks are also to the non-teaching staff members ***Mr. Jagat Ram, Mr. Jai Ram Shah, Mr. Neeraj Kumar and Mr. Prem Kumar*** *department of Veterinary Surgery and Radiology for their kind help during the research work.*

Gratitude alone fails to convey my feelings which cannot be expressed in words for the affectionate care, thought fullness, moral support and encouragement constantly received from all members of my family specially my father ***Dr. Satwant Singh****, my elder brother* ***Dr. Khushvir Singh*** *and my younger sister* ***Dr. Mamta Heer*** *and my lovely mother* ***Smt. Sukhwinder Kaur*** *for their divine support and source of inspiration during the study.*

I thank God for giving me patience and strength to overcome the difficulties which crossed my way in accomplishment of this endeavor.

Last but not the least I would thank every person connected to my life here in Patna or anywhere else who couldn't find a separate mention in this acknowledgement.

Place: - Bihar Veterinary College, Patna

Date: - 27/09/2019 (*Dr. Sukhjinder Singh)*

DEDICATED

TO MY

GRAND PARENTS

CONTENTS

Abbreviations

%	Per cent
/	Per
@	at the rate
<	Less than
=	Equal to
>	Greater than
±	Plus or minus
°F	Fahrenheit
µL	Microliter
µM	micromole
AC	Acetabular rim
ANOVA	Analysis of variance
ASCs	Adipose derived stem cells
b.wt.	Body weight
BMNCs	Bone marrow mononuclear stem cells
BMSCs	Bone marrow mesenchymal stem cells
BVA	British veterinary association

CAT	Catalase
CBC	Complete blood count
CCO	Caudo curvilinear osteophyte
CFHO	Circumferential femoral head osteophyte
CHD	Canine hip dysplasia
CRP	C- reactive protein
CS	Chondroitin sulfate
CT	Computed tomography
CVDs	Cardiovascular diseases
d	Distance
D	Diameter
DAR	Dorsal acetabular rim
DAS	Dorsal acetabular slope
DI	Distraction Index
DJD	Degenerative joint disease
DLC	Differential leukocyte count
DLS	Dorsolateral subluxation
DMSO	Dimethyl sulfoxide

DTNB	5, 5'-dithiobis (2-nitrobenzoic acid)
EBVs	Estimated breeding values
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
et al.	And others
F	Female
FCI	Federation Cynologique Internationale
FHC	Femoral head coverage
FHO	Femoral head ostectomy
Fig.	Figure
g/dl	Gram per deciliter
GF	Growth factors
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Glutathione disulfide
H_2O_2	Hydrogen peroxide
HA	Hyaluronan
Hb	Hemoglobin

HD	Hip dysplasia
HJL	Hip joint laxity
HR	Heart rate
i.e.	That is
IGF	Insulin like growth factor
IL-1β	Interleukin-1- beta
IM	Intra muscular
IU	International Unit
KC	Kennel Club
Kg	Kilograms
LCP	Legg-Calvé-Perthés disease
LFO	Linear femoral overlap
LPO	Lipid peroxidation
lt.	Left
M	Month
M	Male
MDA	Malondialdehyde
mg	Milligram

min	Minute
ml	Milliliter
ML	Morgan Line
mM	millimole
mm^2	Square millimeters
mm^3	Cubic millimeters
MTT	3-(4-5 dimethyl thiazol 2-xl) 2, 5 diphenyl tetrazolium bromide
n	Number
NA	Norberg angle
NADPH	Nicotinamide adenine dinucleotide phosphate
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteo-arthritis
OD	Optical density
OFA	Orthopedic Foundation for Animals
PBS	Phosphate buffer solution
PCV	Packed cell volume
PDGF	Platelets derived growth factors

PFHC	Percent femoral head coverage
pH	Potential of hydrogen
P-PRP	Pure Platelets rich plasma
PRP	Platelets rich plasma
r	Radius
RA	Reduction angle
RBC	Red blood cells
rpm	Revolutions per minute
RPMI-1640	Roswell park memorial institute
RR	Respiratory rate
RT	Rectal temperature
rt.	Right
SE	Standard error
SFO	Surface femoral overlap
SOD	Superoxide dismutase
SVF	Stromal vascular fraction
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid

TEC	Total erythrocyte count
TGF-β	Transforming growth factor
TLC	Total leukocyte count
TNB	5-thio (2-nitrobenzoic acid)
TNF-α	Tumor necrosis factor
TPO	Triple pelvic osteotomy
V/D	Ventrodorsal
VEGF	Vascular endothelial growth factor
WBC	White blood cells
Y	Year

List of tables

	various time intervals.
Table no. 13	Mean ± S.E. values of scores of animals for Ability to climb stairs in different groups at various time intervals.
Table no. 14	Mean ± S.E. values of norberg angle scores of animals in different groups at various time intervals.
Table no. 15	Mean ± S.E. values of percent femoral head coverage of animals in different groups at various time intervals.
Table no. 16	Mean ± S.E. values of distraction index of animals in different groups at various time intervals.
Table no. 17	Mean ± S.E. values of Haemoglobin (Hb) (g/dl) of animals in different groups at various time intervals.
Table no. 18	Mean ± S.E. values of packed cell volume (PCV) (%) of animals in different groups at various time intervals.
Table no. 19	Mean ± S.E. values of total erythrocyte count (TEC) (10^6/µL) of animals in different groups at various time intervals.
Table no. 20	Mean ± S.E. values of total leukocyte count (TLC) (10^3/µL) of animals in different groups at various time intervals.
Table no. 21	Mean ± S.E. values of Neutrophils (%) of animals in different groups at various time intervals.
Table no. 22	Mean ± S.E. values of Eosinophils (%) of animals in different groups at various time intervals.
Table no. 23	Mean ± S.E. values of Basophils (%) of animals in different groups at various time intervals.

List of Figures

Figure no. 7	Breed wise distribution of animals included for treatment of hip dysplasia.
Figure no. 8	Age (month) wise distribution of animals suffering from hip dysplasia.
Figure no. 9	Sex wise distribution of animals suffering from hip dysplasia.
Figure no. 10	Body weight (kg) wise distribution of dogs suffering from hip dysplasia.
Figure no. 11	Percent distribution of duration of lameness (days) in dogs suffering from hip dysplasia.
Figure no. 12	Histogram showing Mean ± S.E. of rectal temperature (°F) of animals not significant in different groups at various time intervals
Figure no. 13	Histogram showing Mean ± S.E. of heart rate (beats/min) of animals not significant in different groups at various time intervals
Figure no. 14	Histogram showing Mean ± S.E. of respiration rate (breaths/min) of animals not significant in different groups at various time intervals.
Figure no. 15	Figure Showing (a) mild (b) moderate and (c) severe thigh muscle atrophy in dogs suffering from canine hip dysplasia.
Figure no. 16	Histogram showing Mean ± S.E. of scores of animals for pain in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).
Figure no. 17	Histogram showing Mean ± S.E. of scores of animals for Lameness in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).
Figure no. 18	Histogram showing Mean ± S.E. of scores of animals for ability to jump in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).

Figure no. 19	Histogram showing Mean ± S.E. of scores of animals for ability to climb stairs in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).
Figure no. 20	Histogram showing Mean ± S.E. of Norberg angle (NA) of animals in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).
Figure no. 21	Histogram showing Mean ± S.E. of Percent femoral head coverage (PFHC) of animals in different groups at various time intervals. The highest value for PFHC showed least luxation of hip joint in group C followed by groups B and A.
Figure no. 22	Histogram showing Mean ± S.E. of Distraction Index (DI) of animals in different groups at various time interval. The lowest mean value for DI showed least luxation of hip joint in group C followed by groups B and A.
Figure no. 23	Histogram showing Mean ± S.E. of Haemoglobin (Hb) (g/dl) of animals not significant in different groups at various time intervals
Figure no. 24	Histogram showing Mean ± S.E. values of packed cell volume (PCV) (%) of animals not significant in different groups at various time intervals.
Figure no. 25	Histogram showing Mean ± S.E. values of total erythrocyte count (TEC) (106/µL) of animals not significant in different groups at various time intervals
Figure no. 26	Histogram showing Mean ± S.E. values of total leukocyte count (TLC) (10^3/µL) of animals not significant in different groups at various time intervals
Figure no. 27	Histogram showing Mean ± S.E. values of Neutrophils (%) of animals not significant in different groups at various time intervals
Figure no. 28	Histogram showing Mean ± S.E. values of Eosinophils (%) of animals not significant in different groups at various time intervals
Figure no. 29	Histogram showing Mean ± S.E. values of Lymphocytes (%) of animals not

	significant in different groups at various time intervals
Figure no. 30	Histogram showing Mean ± S.E. values of Monocytes (%) of animals not significant in different groups at various time intervals.
Figure no. 31	Histogram showing Mean ± S.E. of C - reactive protein (CRP) (mg/dl) of animals in different groups at various time intervals. The lowest value of CRP suggested least inflammatory condition in group C followed by groups B and A.
Figure no. 32	Histogram showing Mean ± S.E. of Malondialdehyde (MDA) (n mol/ml of blood serum) of animals in different groups at various time intervals. The lowest value of MDA suggested least oxidative stress in group C followed by groups B and A.
Figure no. 33	Histogram showing mean ± S.E. of Superoxide dismutase (SOD) (K/gram protein) of animals in different groups at various time intervals. The highest value of SOD suggested least oxidative stress in group C followed by groups B and A.
Figure no. 34	Histogram showing Mean ± S.E. of Catalase (CAT) (k/gram of protein) of animals in different groups at various time intervals. The highest value of Catalase suggested least oxidative stress in group C followed by groups B and A.
Figure no. 35	Histogram showing mean ± S.E. of Glutathione peroxidase (U/ml) of animals in different groups at various time intervals. The highest value of GPx suggested least oxidative stress in group C followed by groups B and A.
Figure no. 36	Histogram showing the response of treatment in animals suffering from hip dysplasia on the basis of observation on days 90. Group A (Control), Group B (Bone marrow mono-nuclear cells-BMNCs), Group C (BMNCs + Activated platelets).
Figure no. 37	Figure showing ventro-dorsal view radiographs of pelvic bone in dogs at different time intervals. The radiographs showed better response in group C followed by groups B and A in terms of disappearance of osteophytes, clearly visibility of joint space, norberg angle, percent femora head coverage and

	distraction index. Group A (Control), Group B (Bone marrow mono-nuclear cells-BMNCs), Group C (BMNCs + Activated platelets).

DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY
BIHAR ANIMAL SCIENCES UNIVERSITY,
PATNA, BIHAR

Title of thesis:	Clinical Studies on Nutraceuticals versus Autologous Uncultured Bone Marrow Derived Mono-Nucleated Cells (BMNCs) for treatment of Hip Dysplasia in Canine
Name of student:	Sukhjinder Singh
Major discipline:	Veterinary Surgery and Radiology
Minor discipline:	Veterinary Medicine
Date of thesis submission:	27/09/2019
Total pages of thesis:	122
Major advisor:	Dr. Ramesh Tiwary

Abstract:

Eighteen dogs suffering from either sex were used in this study. The animal were randomly divided into three groups A, B, & C consisting of six animals each to study the effect of biomaterials i.e. once in affected hip joint with uncultured autologous bone marrow derived mono-nuclear cells (BMNCs) (4.35 ($\pm 0.07 \times 10^6$) (group B), BMNCs (4.35 ($\pm 0.07 \times 10^6$) and activated platelets (2.54 ($\pm 0.12 \times 10^8$) (group C) and nutraceuticals orally for three months and 1 ml RPMI intra- articularly once (group A-control). Evaluation of treatment protocols were done at four weeks intervals for twelve weeks on the basis of clinical examination like Ortolani test, scores for pain, lameness, ability to jump and ability to climb stairs. Radiographic evaluation based on Norberg Angle (NA), Percent Femoral Head Coverage (PFHC) and Distraction Index (DI), haematobiochemical test for CBC and C reactive protein (CRP). The oxidative stress parameters were also observed for malondialdehyde (MDA), superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx). No significant result were observed in physiological as well as Haematobiochemical parameters whereas, significant improvement in the scores for pain, lameness, ability to jump, ability to climb stairs and norberg angles were observed with better response in group C followed by group B and A at various interval of time. The mean value of C reactive protein on days 90 were found significantly different among the groups that indicates minimal inflammatory reaction in hip joint with lowest value in the animals of groups C (4.02 ± 0.14) followed by groups B (4.60 ± 0.13) and A (8.50 ± 0.20). There were significant differences in the mean value of Percent Femoral Head Coverage (PFHC) and Distraction Index (DI) and oxidative stress parameters observed with better response group C followed by group B and A. in osteoarthritis (OA) glutathione provides resistance and resilience to damage of cartilage from oxidative stress. On day 90^{th} the mean value of glutathione peroxidase was also significant and highest in group C (0.148 ± 0.001) followed by group B (0.124 ± 0.000) and A (0.0850 ± 0.001) that further support better improvement in clinical signs of animals suffering from hip dysplasia in group C followed by group B and A. At the end of study it was concluded that the Nutraceuticals can ameliorate the clinical signs associated with canine hip dysplasia whereas, the implantation of uncultured autologous BMNCs in hip joint can improve the clinical signs and augment the healing of cartilage combination of BMNCs and activated platelets further improve the clinical signs and healing of cartilage in canine hip dysplasia.

Student
(Sukhjinder Singh)

Major Advisor
(Dr. Ramesh Tiwary)

HOD
(Dr. Mithilesh Kumar)

1 INTRODUCTION

Canine hip dysplasia (CHD) is a heritable polygenic and painful disorder affecting all breed types and sizes, with a higher pervasiveness in purebreds (Sanchez-Molano, 2014). In the first month of ordinary growth, the teres ligament is responsible for keeping the femoral head in location. The teres ligament is too short in canines with hip dysplasia, so the femoral head is not correctly attached and luxation develops after about 2 months. At age of 2-3 months, canines suffering with hip dysplasia, when viewed radiographically shows dramatic changes in the development of hips as compared to ordinary hip development. (Wilson *et al.,* 2012). Canines without hip dysplasia have healthy articular cartilage covering the femoral head whereas in canines suffering with Hip Dysplasia, have worn articular cartilage in contact with acetabulum. The acetabulum and femoral head recedes from each other resulting in loosening and unstable hip joints. New bony growth is formed in the acetabulum to compensate the loss of cartilage, causing further luxation (Newton and Nunamaker, 1985)

Hip dysplasia (HD) is an inherited, non-congenital orthopaedic disease with highest incidence and heritability of up to 95% in the canine species. It is more prevalent in large and giant breeds of dog (Mäki *et al.,* 2004; Janutta *et al.,* 2005; Ginja *et al.,* 2008; Guo *et al.,* 2011; Sanchez-Molono *et al.,* 2015). All canines are usually born with normal hip conformation but when reaches the age around three weeks changes starts due to both environmental and genetic predisposition leads to the development of hip dysplasia (Janutta and Distl, 2006). It is characterized by degenerative joint disease that can progressively trigger to the development of osteoarthritis (OA) of the affected joints (Smith *et al.,* 2001), characterized by articular cartilage lesions, bone remodelling with the presence of osteophytes and inflammation in hip joint (Johnston *et al.,* 2008).

Dogs suffering from hip dysplasia shows considerable lameness and painful arthritis in coxo-femoral joints. The most common clinical signs of OA are joint pain, gait abnormalities such as stiffness, reduced height of step, shortened stride length, bunny hopping, difficulty in rising, climbing stairs or in jumping over obstacles. (Fry and Clark., 1992; Ginja *et al.,* 2008). These clinical symptoms may alter the behaviour of dog making them uncomfortable, irritable

and potentially harmful. This condition of dog(s) make emotionally stressful to the pet owners. The surgical treatment to get rid of this condition may cost owners greater and it will only help to alleviate the pain despite of curing the actual disease (Zhu *et al.,* 2012)

The actual confirmatory diagnosis of canine hip dysplasia is a major challenge now a days. Various popular frequently used methods for diagnosing CHD are based on phenotypic observations such as using British veterinary association (BVA) or Orthopaedic Foundation for Animals (OFA) Scores and Estimated Breeding Value (EBVs). The biasness of these methods produces false positive and negative results. (Janutta and Distl, 2006). In subjective hip scoring systems, CHD is easily diagnosed if there is radiographic evidence of hip subluxation (joint laxity), DJD or both. Radiographic evidence of DJD includes one or more of the following femoral periarticular osteophyte formation or subchondral sclerosis of the craniodorsal acetabulum or osteophytes on the cranial or caudal acetabular margin and joint remodelling from chronic wear (Owens and Biery, 1999)

The aim of the treatment is to reduce or eliminate pain thereby improving or restoring limb function to normal. Two approaches of canine HD management have been described, which include conservative management and surgery (Anderson, 2011). In dogs, one of the principal conservative therapeutic approaches involves oral administration of nutraceuticals, whose formulation is primarily composed of glucosamine and chondroitin sulfate together with the use of non-steroids anti-inflammatory drugs (NSAIDs) (McCarthy *et al.,* 2007; Sauve *et al.,* 2003). However, prolonged use of NSAIDs can be associated with side effects, especially in the digestive system and kidneys (Luna *et al.,* 2007).

In past few years stem cell-based therapies has widened leading to progress in stem cell therapy as a clinical standard for certain circumstances, such as hematopoietic stem cell therapy for leukaemia and epithelial stem cell-based therapy for burns and various corneal diseases (Kalra and Tomar, 2014). Stem cells are unspecialized and undifferentiated cells having clonogenic and self-renewing capabilities through mitotic cell division and differentiate into multiple cell lineages (Kalra and Tomar, 2014). Bone marrow mononuclear cells and cultivated bone marrow stromal cells represent a phenotypically and functionally heterogeneous population of mesenchymal stem cells cells. Cultured BMSCs were used in small breeds of dog suffered from aseptic necrosis of the femoral head (Legg-Calvé-Perthés disease- LCP) and found good

recovery after 4–5 weeks without signs of pain and lameness (Crovace *et al.,* 2008). The effect of autologous stromal vascular fraction (SVF) or allogeneic cultured adipose-derived stem cells (ASCs) injected into acupuncture points with hip dysplasia in dogs having weak response to drug therapy showed an important therapeutic for this type of pathology (Marx *et al.,* 2014). Cartilage has limited capacity for regeneration, and when lesion is limited to the articular cartilage only and does not extend to the underlying bone, it fails to heal spontaneously (Kim *et al.,* 1995; Hunziker and Rosenberg, 1996; Hunziker, 1999) leading to the osteoarthritis, lameness, and permanent disability. Intra-articular implantation of uncultured bone marrow derived Mono-nucleated cells in osteochondral defects of stifle joint facilitate healing of defects, however the combination of BMNCs and IGF-1 induces faster and biologically better healing than BMNCs alone in Rabbits (Tiwary *et al*., 2014). The mesenchymal cells from bone marrow isolates found to be a valid alternative to the more invasive traditional techniques to correct large bone defects and cartilage repair. (Crovace *et al.,* 2004; Ouyang *et al.,* 2004; Mastrogiacomo *et al.,* 2006; Marcacci *et al.,* 2007). Adult stem cells have high potential to form a wide range of cell types, thus various sources of stem cells such umbilical cord blood and bone marrow are regularly used in medical therapies. Through cell culture technique, stem cells can now be cultivated and converted into specialized cells with features consistent with cells of different tissues such as nerves or muscles (Chapman *et al.,* 1999)

Various mesenchymal stromal cell therapies are being utilized for successful treatment of variety of diseases like equine tendinopathies (Smith *et al.,* 2003; Smith, 2008) or cartilage degeneration in dogs (Black *et al.,* 2007; 2008)

Platelet-rich plasma (PRP) is an autologous product of blood having concentration higher than blood (Everts *et al.,* 2006). It is a rich source of autologous growth factors, such as platelets derived growth factors (PDGF), Transforming growth factor β (TGF- β), Insulin like growth factor (IGF) and vascular endothelial growth factor (VEGF) (Ham *et al.,* 2012). The active secretion of these growth factors begins within minutes of the start of activation and more than 90% are secreted during the first hour (Smith *et al.,* 2007). PRP counteract cartilage erosion by inhibiting the catabolic cytokines of IL-1β and TNF-α (Daheshia *et al.,* 2008 and Chen *et al.,* 2008) and by promoting factors associated with cartilage matrix synthesis including fibroblast

growth factor, transforming growth factor-β (TGF-β), and others (Park and Na, 2008, Solorio *et al.,* 2012 and Boswell *et al.,* 2012)

The growth factors derived by platelets stimulate chondrogenic differentiation of bone marrow derived mesenchymal stem cells, enhance chondrocyte proliferation and extracellular matrix biosynthesis in porcine (Akeda *et al*., 2006; Jung *et al*., 2009). Uncultured BMNCs are autologous source without a risk of disease transmission or immunogenic reaction. It may be an alternative choice for clinical treatment of cartilage repair as compared to culture BMNCs that requires costly lab setup. The use of autologous activated platelets may also be an alternative option to synthetic recombinant growth factors. The proposed work planned with hypothesis that use of uncultured bone marrow mononucleated cells and its combination with activated platelets may be useful for therapy in canine hip dysplasia to overcome the complications related with conventional surgical as well as medicinal treatment with following objectives.

Objectives

- ➢ To evaluate efficacy of Nutraceuticals in treatment of hip dysplasia in canine.
- ➢ To evaluate efficacy of autologous uncultured BMNCs in treatment of hip dysplasia in canine.
- ➢ To study the effect of activated platelets on BMNCs for treatment of hip dysplasia in canine.

2 REVIEW OF LITERATURE

Functional anatomy of canine hip joint

The coxofemoral joint or hip joint is a diarthrodial joint, formed by an articulation between the pelvic acetabulum and the head of the femur.it is a ball and socket synovial joint in which the ball is the femoral head and the socket is the acetabulum. It connects the axial skeleton with the lower extremity. The joint capsule helps in maintaining the stability during the flexion and extension of the joint. The most important stabilizers of the hip joint are ligamentum teres, the joint capsule, and the transverse ligament, which completes the acetabular fossa ventrally. The teres or round ligament emerges from the acetabular fossa and inserts on the fovea capitis of the femur head. The joint capsule arises from the acetabulum, dorsal acetabular rim and labrum periphery. The labrum is composed of fibro cartilaginous band between the acetabular rim and the joint capsule. The joint stability depends upon the concavity of acetabulum and convexity of femoral head, which fits into the acetabulum completely (Evans and Lahunta, 1993).

Femur

The hemispherical head of femur is covered with articular cartilage (hyaline cartilage), which is thicker at the places of weight bearing. The femoral neck extended from the head to its insertion on the proximal aspect of femoral diaphysis (Evans and Lahunta, 1993). The angle formed by the axis of the shaft of the femur with the long axis of the femoral neck and head is within the range of 135° to 145° and is called as the angle of inclination. The angle of anteversion is another angle of femoral neck in relationship to the shaft of the femur. It normally ranges between 20° to 27° (Rischer, 1975 and Schulz and Dejardin, 2003).

Montavon *et al*. (1985) reported that 30 mongrel dogs were evaluated radiographically using biplanar technique for the determination of angles of anteversion, angle of inclination of the femoral head and neck. The angle of anteversion of the 30 samples of necropsy were evaluated directly and compared with the in vivo radiographic measurements. The obtained

average values of angles of anteversion were 31.3°, average values of inclination were 148.8°and corrected real angles of inclination were 144.7° respectively.

Acetabulum

The acetabulum is the point where the three components of the pelvis (ilium, ischium, and pubis) meets. At the distal part of the acetabulum of os coxae is the acetabular notch, which is continuous with the acetabular fossa (a circular depression), at the bottom of the cavity of the acetabulum. At around three months of age, acetabular notch fuses and forms a socket that faces laterally and slightly caudally and has a prominent edge across the cranial, dorsal and caudal areas. The cup shaped acetabulum is divided into acetabular fossa, the lunate surface, the labrum and the cranio dorsal buttress (Evans and Lahunta, 1993).

The femur head is attached to the acetabular fossa by ligamentum teres and the articular cartilage is absent. The lunate surface is articulated with femoral head by hyaline cartilage. The transverse acetabular ligament bridges the two ventral aspects of the lunate surface together (Evans and Lahunta, 1993).

Blood supply

The arterial supply to the hip joint is extensively via medial (dorsally, caudally and ventrally) and lateral (dorsally, cranially and ventrally) circumflex femoral arteries. These are the branches of the profunda femoris artery (deep femoral artery) and they all penetrate the femoral head at joint capsule. They anastomose at the base of the femoral head and neck to form a vascular ring, from which smaller arteries arise to supply the hip joint itself. From teres ligament no vital blood vessels penetrate the femoral head (Kaderly *et al.,* 1983).

Nerve supply

Kinzel *et al.* (1998) studied the coxofemoral joints of 16 dogs and reported that branches of cranial gluteal nerve innervates the cranio-lateral region of coxofemoral joint and the branches of femoral nerve innervates the medial region, also the branches of ischiatic nerve innervates the caudo-lateral region.

Thirty canine acetabular fragments were evaluated to compare the nerve fibre density of the periosteum. The results depicted a significant difference between mean densities of nerve fibres at the caudolateral region, approximately 60 fibres/mm^2, of the acetabulum. Periosteal fibres were arranged in a sagittal plane, directing towards the joint capsule, suggesting equivalent density in the latter region (Schmaedecke *et al.,* 2008).

Huang *et al*. (2013) observed that the canine hip joint capsule is innervated by four important nerves branches. It includes branches of cranial gluteal nerve, femoral nerve, ischiatic nerve and obturator nerve which innervates the coxofemoral joint capsule laterally, cranio-medially, caudo-laterally and caudo-medially respectively.

Hip dysplasia

Canine hip dysplasia is a common developmental orthopaedic disease affecting medium as well as large breed of dogs that is characterised by coxofemoral joint subluxation and incongruity. The critical period in the development of hip dysplasia in canine is the period of maximal growth and coxofemoral joint development occurring between 3 and 8 months of age (Schnelle, 1935; Todhunter *et al.,* 1999)

Canine hip dysplasia is a heritable polygenic and painful disorder affecting all breed types and sizes, with a higher pervasiveness in purebreds (Sanchez-Molano 2014). Hip dysplasia (HD) is an inherited, non-congenital Orthopaedic disease with highest incidence and heritability of up to 95% in the canine species. It is more prevalent in large and giant breeds of dog (Mäki *et al.,* 2004; Janutta and Distl, 2006; Ginja *et al.,* 2008; Guo *et al.,* 2011; Sanchez-Molono *et al.,* 2015). All canines are usually born with normal hip conformation but when reaches the age around three weeks development of hip dysplasia starts with both environmental and genetic predisposition to the disorder (Janutta and Distl, 2006). It is characterized by degenerative joint disease that can progressively trigger to the development of osteoarthritis (OA) of the affected joints (Smith *et al.,* 2001), characterized by articular cartilage lesions, bone remodeling with the presence of osteophytes and inflammation in hip joint (Johnston *et al.,* 2008).

Passive hip laxity is necessary for the development of coxofemoral osteoarthritis in dogs, but alone it is insufficient for osteoarthritis development. Most dogs developed hip dysplasia in milder forms of degenerative joint disease (DJD) until their geriatric age group without any

clinical signs. It has been hypothesized that DJD occurs in biphasic manners but new evidence suggests that the incidence of DJD occurs linearly as the aged progress. (Kapatkin *et al.,* 2002; Gatineau *et al*., 2012).

In 1937, Schnelle reported canine hip dysplasia as disease that was thought to be uncommon and published the first radiographic description of canine hip dysplasia (CHD) termed as *bilateral congenital subluxation*

In 1966, Henricson and colleagues defined CHD as a variable degree of laxity of the hip joint causing subluxation during young age, resulting in variable degrees of shallow acetabulum and flattening of the femoral head, certainly leading to osteoarthritis.

All dogs are born with ordinary hips, but the growth of hip dysplasia starts in the femoral head and pelvic socket areas around week 3 (Kealy *et al.,* 1992).

Incidence

Brinker *et al*. (1990) reported that the stability of hip is prevented by conditions associated with hip joint includes acetabular fracture, hip laxity, femoral head and neck fracture, capital femoral physeal fracture, Legg-Calve-Perth's disease and degenerative changes in the coxofemoral joint.

Dyce *et al*. (2000) evaluated 285 dogs for breed incidence of coxofemoral joint which were undergone for surgical total hip replacement. 20 cases (7.8%) were recorded for Postoperative complications and dorsal luxation was the most common complication in 12 dogs (4.7%). commonly affected breeds were German Shepherd Dogs (20%), Golden retriever (15%), Labrador Retriever (21%), Rottweiler (6%) and cross bred (16%).

Genevois *et al.* (2000) conducted epidemiologic study, analyzing 9738 standard radiographs for canine hip dysplasia and observed 42 per cent of unilateral hip dysplasia. Obvious degenerative changes were noticed in 31.4 % of the dysplastic canines ageing from 1 to 1.5 years, 55 % of the canines ageing from 2 to 4 years and 63 % of the canines over 5 years of age.

LaFond *et al*. (2002) conducted epidemiological study to determine breeds at risk for developmental orthopaedic diseases including canine hip dysplasia (CHD) and reported that breeds such as German Shepherd Dog, Mastiff, Rottweiler, Saint Bernard, Golden Retriever, Labrador retriever, Chow – Chow, Bull dog and Bull mastiff are at high risk for canine hip dysplasia. Legg Calve-Perth's disease has more prevalence in Lhasa apso, Dachshunds, Miniature poodle and Pug.

Todhunter and Lust (2003) reported that during the growth phase between 3 to 8 months of age dogs shows clinical signs like lameness, swaying of back, difficulty in rising, bunny hopping gait and other gait abnormalities due to hip dysplasia. It can be diagnosed by palpation of hip joint and from radiographic examination.

Van Hagen *et al*. (2005) reported that large and fast-growing breeds are more susceptible to canine hip dysplasia

Shiju Simon *et al*. (2010) reviewed a total of 272 cases of hip dysplasia in canine. A standard ventro-dorsal hip extended radiographic examination was done in young animals of over three months to one-year age group. The radiograph revealed highest incidence of disease (52.94 %). In Labrador retriever, the breed-wise prevalence was discovered to be greater (36.76%). It was noticed that male dogs were more vulnerable (59.55%) than female dogs and bilateral hip dysplasia was more common (88.60 %) as compare to unilateral hip dysplasia. It was observed that left coxofemoral joint was more affected than right coxofemoral joint in the unilateral hip dysplasia.

Etiopathology/ Causes

Various instability issues like proliferation of the dorsal acetabular rim, atrophy of local muscles, stretching of ligaments in the femoral head, cartilage degeneration, and thickening of the joint capsule and femoral neck occurs if the dog is obese or has extra weight (Fries and Remedios 1995).

As observed by Rischer (1975), joint laxity which initiated damage to the femoral head, erosion of acetabular cartilage, subchondral of the dorsal acetabular rim the load bearing surface and microfracture of the trabeculae of the limbs led to appearance of the first sign of lameness.

Evidence of Osteophytes on cranial acetabular rim leading to alterations in the shape of dorsal acetabular edge might be seen. Lust *et al*. (1973), Kasstrom (1975) and Kealy *et al*. (1992) studied rapid growth rate and high food consumption are the factors regarding development of CHD while most investigators conclude that overfed dogs grow faster than dogs fed a restricted diet and hence are more prone to the development of CHD although the etiology of this condition is not fully understood.

Kasstrom (1975) reported that young dogs with age less than one year higher had higher occurrence of hip dysplasia. He noticed that early rapid growth, disproportionate skeletal and muscular growth, overloading of articular areas and tearing or stretching of round ligament were prime reasons for this higher occurrence.

Riser (1975b) reported that there is reduction in joint's ability to lubricate due to changes in the composition of the synovial fluid within the dysplastic hip, leading to increased cartilage wear.

Fries and Remedios (1995) opined that excess dietary calcium and vitamin D could predispose the development of canine hip dysplasia in genetically predisposed animals and should be avoided in young, rapidly growing dogs.

Cardinet *et al*. (1997) opined that development of canine hip dysplasia was associated with deformities and abnormalities of pelvic musculature like weakened thigh and pelvis muscle mass and altered muscle fiber composition and size in dogs.

Leighton *et al*. (1977) Lust and Farrell (1977) and Hedhammar *et al*. (1979) reported that the most probable genetic basis for CHD was multifactorial mode of inheritance.

Lust and Summers (1981) reported that the cartilage damage can be quantified in canine hip dysplasia that could be histologically detected and included loss of Chondrocytes, reduction in proteoglycan content will lead to cartilage damage, fibrillation and fissure in the cartilage.

Carney and Muir (1988) reported aggrecans are the major proteoglycan of cartilage and they constitute a large core protein with chondroitin sulphate and some keratin sulphate chains and their loss will result in decreased ability to bear load, mechanical strength and cartilage deformation. Mackenzie *et al*. (1985) and Shepherd (1986) reported that hip dysplasia is

caused by the interaction of hundreds of genes, each contributing a small part to the disease and thus is a polygenic trait.

Frost (1989) and Alexander (1992) reported that the genetic blueprint for the hip's shape, size, anatomical relationships, musculature, and innervation, and a program for its growth and remodeling is determined by genotype.

In 1992, Kealy and his co-workers reported that in total Forty-eight Labrador Retrievers with age of 8-week-old were allotted to 2 groups of 24 dogs each to determine the Effects of limited food consumption on the incidence of hip dysplasia in growing dogs. The study reveals that out of 24 Labrador Retrievers only 7 with “restricted feeding” were diagnosed with hip dysplasia, while in other 24 canines fed with an “ad libitum” diet 16 dogs were diagnosed with hip dysplasia.

Fries and Remedios (1995) and Janutta and Distl (2006) reported that canine hip dysplasia being a genetic disease also appears to be affected by environmental factors for its occurrence in genetically predisposed puppies.

Leighton (1997) noticed that the Complex interactions within multiple genetic loci and environmental factors are the major etiological factors for development of canine hip dysplasia.

Lust (1997) reported that hip dysplasia is a highly acquired trait of medium- and large-breed dogs. Subluxation of the femoral heads and joint laxity are considered to be inherited developmental condition which lead to osteoarthritis of the hip joints. New study suggests that even the age of 2 is too young to accurately predict the hip phenotype (Smith *et al.,* 2001).

Morgan (1997) reported that shallow acetabulum, joint subluxation, erosion of articular cartilage and remodelling of the acetabular and femoral surface are some structural deformities of canine hip dysplasia. The gross pathological changes of hip joint included sclerosis of subchondral bone, osteophytes, surface irregularity, and joint capsule thickening. Fibroblastic proliferation, increased collagen production and fibro vascular stroma (Pannus) are common histological changes leading to pain. Hypertrophy, hyperplasia and necrosis are the possible reactions of synoviocytes to injury. Increase in Type A synoviocytes led to increase in the

appropriate ultrastructural features. Type B synoviocytes induced rough endoplasmic reticulum which would be associated with increased production of lysosomal hydrolase and thus, potentiated exacerbation of cartilage damage. Articular cartilage got degenerated due to impaired nutrition supply to cartilage leading to osteoarthritis.

Flecknell and Waterman-Pearson (2000) studied that the vigorous exercise especially during growth phase of dog act as a predisposing factor for osteoarthritis and joint surgery elevated the onset of arthritis in coxo-femoral joint.

Impellizeri *et al.* (2000) and Macphail (2000) studied that for development of osteoarthritis in dogs, obesity was considered to be an important risk factor and incidence of obesity and osteoarthritis was found in 25 % of dog population.

Rettenmaier *et al.* (2002) noticed no significant difference was observed in the prevalence of canine hip dysplasia between pure bred and mix bred dogs also between sexes.

Wood *et al.* (2002) observed that among Labrador Retrievers while screening males had higher scores for hip dysplasia than females.

Todhunter and Lust (2003) reported that canine hip dysplasia (CHD) is a developmental condition which is characterized by instability of the hip joint, leading to degenerative arthritis commonly affecting medium-sized and large-breed dogs.

Braden *et al.* (2004) reported that the condition was bilateral and was attributed to hip dysplasia (93%) as observed in 286 dogs with hip pain or rear leg lameness with spontaneous occurrence of degenerative joint disease of the coxofemoral joint. He also noted that unilateral degenerative joint disease which was attributed to trauma or primary osteoarthritis was also present in few dogs (7%).

Citi *et al.* (2005) studied that incidence of hip dysplasia was highest in dogs less than 12 months of age (37.6 %), followed by dogs between 25-72 months of age (25.5%), than between 12-24 months of age (22.8%), and in dogs older than 73 months (14.1%).

Hagen *et al*. (2005) mentioned that dogs with exposure to a slippery floor surface earlier to weaning had higher risk of developing clinical signs of hip dysplasia in a cohort of Boxers.

They reported that the proper role of housing conditions in development of canine hip dysplasia had not been extensively studied.

Berg-Foels *et al*. (2006) found in Labrador retriever that with increased birth weight and during postnatal body weight, the probability of degenerative changes in the hip joint increased in later life.

Kate and Keith (2006) opined that there was there was no significant linkage in 28 Boykin Spaniels that underwent genotyping but there was a positive correlation between the occurrences of hip dysplasia and increased joint laxity.

Corr (2007) reported that joint laxity which leads to subluxation of hip joint causing stretching of the joint capsule, ligament of femoral head and joint effusion which cause erosion of dorsal acetabulum and capsule followed by capsular fibrosis leading to development of osteoarthritis with bone remodeling were influenced mainly by the genetic and environmental factors.

Genevois *et al.* (2008) reported that dogs belonging to large and giant breeds had highest prevalence of hip dysplasia. He also reported that this prevalence varied from 2 per cent to 67 per cent albeit exceptions existed.

Benzioni *et al.* (2008) reported that in dogs, hip dysplasia more often lead to secondary osteoarthritis.

Coopman *et al.* (2008) studied that in Belgium 60 per cent of dogs affected with hip dysplasia were females.

Roberts and Paul (2009) reported that breeds of dog with long body easily predisposed to hip dysplasia.

Zhou *et al*. (2010) reported that genes associated with hip dysplasia were Four Single nucleotide polymorphisms (SNP) and gene loci associated with hip osteoarthritis were two Single nucleotide polymorphisms (SNP).

Krontveit *et al.* (2012a) opined that as compared to smaller breeds, occurrence of hip dysplasia with higher frequency were observed in giant breeds of dogs although there were

exceptions. Krontveit *et al.* (2012b) mentioned main risk factors for hip dysplasia that had been most commonly studied in dogs. The risk factors such as growth rate, body conformation and size, food consumption, composition of the diet and muscle disorder and weak muscle mass predisposed canine hip dysplasia. He also reported that puppies ≤ 3 months of age should be allowed outdoor exercise on soft ground but should not be allowed access to stairs and exercise should not be on irregular land. The movements of puppies should be restricted to decrease the risk for developing hip dysplasia.

Samir *et al.* (2014) noticed that the incidence of canine hip dysplasia in small-sized breeds, medium-sized breeds, giant size breeds and large-sized breeds was 4.29 per cent, 5.59 per cent, 19.29 per cent and 31.79 per cent, respectively.

Kimeli *et al*. (2015) reported that the key roles in developing canine hip dysplasia is played by environmental influences such as obesity, injury at a young age, overexertion on hip joint or round ligament tear at a young age, repetitive motion on forming coxofemoral joint, and excess dietary calcium/ vitamin D.

Dezateux and Rosendahl, (2007) and Noordin *et al.* (2010) reported that Development dysplasia of hip (DDH) in humans can be diagnosed clinically at birth as hip joint instability. It was reported that the condition was considered congenital although some children had normal femoro-acetabular relationship at birth and only later develop a dysplastic hip joint.

Riser (1975a) found no recognizable evidence of CHD before seven weeks of age in four German Shepherd dogs followed from birth to maturity but later in life all four dogs developed severe canine hip dysplasia.

Alexander, (1992) and Riser, (1975b) reported that the normal development of hip joint might be affected by changes in biomechanical balance, stress, compression, traction, muscle pull, lubrication, or congruity between the joint components. It was reported that by six months of age, under normal circumstances, function, tissue strength, and ossification have progressed sufficiently to prevent development of CHD. He noticed that during the first two to three months of life the joints are vulnerable to injury and abnormal development as the muscles and nerves are immature and the tissues are soft and plastic.

Lust *et al.* (1973) and Olsewski *et al.* (1983) opined that period between three and eight months of age in canines appeared important in the development of hip dysplasia.

Clinical signs

Brinker *et al.* (1990) reported that the clinical signs associated with any joint involvement included pain on palpation, stiffness of the joint with decreased range of motion, crepitation due to severity of osteoarthritis, obesity, bunny hopping gait and boxy hip. In case of hip dysplasia in young dogs 'Ortolani sign' was noticed due to increased hip laxity and in older animals degenerative joint changes with thigh and pelvic muscle atrophy and in cranio lateral luxation, short limb with adducted thigh and outwardly rotated stifle was noticed as the femur rested on the dorsal and cranial aspect of acetabulum.

Olmstead (1995) observed that the dogs with history of disabling disease exhibited one or more of the following clinical signs such as progressive hind limb(s) lameness, difficulty during rise or lay down, reluctant to walk or run, reluctance to climb stairs or get into car, pelvic muscle weakness, atrophied muscles, exercise intolerance and an aggressive nature when the hip was palpated.

Morgan (1997) reported that the animals suffering from pain associated with canine hip dysplasia might be an indication of physical changes such as distension of joint capsule, periosteal new bone formation, pressure and ischemia in subchondral bone as well as ancillary soft tissue pain.

Riser (1975a) and Manley *et al*. (2007) noticed two ages at which animals present with overt clinical signs associated with canine hip dysplasia (1) dogs younger than 1 year of age with hip instability and overloading of some articular areas and where pain is caused mainly due to tearing or stretching of the round ligament, synovitis and acetabular microfractures and (2) geriatric dogs with chronic pain from osteoarthritis.

The dogs with hip dysplasia shows variable clinical presentation and it does not correlate with the radiographic changes in joint morphology (Barr *et al*., 1987; Ginja *et al*., 2008a).

Fry and Clarke (1992) reported that due to coxofemoral joint discomfort older dogs exhibiting lameness frequently shift weight to their thoracic limbs thus have well-developed forelimb musculature. Depending upon the severity of the disease the pelvic limb muscles get atrophied from mild to severe and may be symmetric or asymmetric. These dogs often have a waddling gait, weak pelvic limbs, reluctant to exercise and generally prefer sitting to standing. "Bunny hopping" gait have also been reported.

Dassler (2003) opined that dogs suffering with hip dysplasia showed initial clinical signs between the age of three and twelve months. Albeit the degenerative joint disease was becoming established and the predominant clinical manifestation including joint laxity, pain due to synovitis, joint remodelling and established periarticular fibrosis disease might be abated. The dogs often entered a quiescent phase with a certain clinical signs. In mature dogs the onsets of clinical signs were from 2 to 12 years of age.

Todhunter and Lust (2003) reported that dogs between 3 and 8 months of age, on clinical examination, revealed signs of pain, increased joint laxity and subluxation of joint during palpation whereas clinical signs such as signs of joint pain, articular degeneration, bone remodeling and loss of function due to development of osteoarthritis were observed in older dogs.

Ginja *et al*. (2008a) observed some typical clinical signs of canine hip dysplasia such as stiffness, gait abnormalities, reduced height of step, shortened stride length, bunny hopping, difficulty in rising, climbing stairs or in jumping over obstacles.

Greene *et al.* (2013) conducted a study on sixty Labrador Retrievers suffering with hip dysplasia to evaluate various factors associated with severity of lameness and range of motion of coxofemoral joint. It was concluded that with hip dysplasia dogs have lower lameness scores when exercised daily for longer duration and dogs with hip joint luxation secondary to hip dysplasia had higher lameness scores than dogs without hip joint laxity.

Nouh *et al*. (2014) noticed gait abnormalities, difficulty in rising, climbing stairs or in jumping over obstacles as clinical signs of canine hip dysplasia.

Panigrahi (2014) recorded history of abnormal gait, difficulties in jumping, intermittent lameness in its hind legs, reluctant to rise upstairs and exercise intolerance in a two and half years old male Labrador retriever dog. On the basis of history, clinical signs, physical examination and palpation of hip joint the case was suspected for hip dysplasia. On Radiographic examination, there was clear subluxation of the femoral head and increased angle of femoral neck inclination. The case was successfully managed by Chondro-protective agent, non- steroidal anti-inflammatory drugs (NSAID) therapy, weight reduction, physiotherapy, diet and exercise restriction.

Souza *et al*. (2015) studied the correlation between kinetic parameters and radiographic hip grade in German shepherd dogs. Dogs were distributed into five groups each containing eight dogs with respect to hip grades (A, B, C, D or E). Clinical evaluation and kinetic analysis was done. Peak vertical force, vertical impulse and stance phase duration were evaluated at velocity of (1·2 to 1·4 m/s) ±0·1m/s acceleration. It was observed that mean peak vertical force decreased progressively from grade C to grade E. The vertical impulse was decreased in groups C and E compared to groups A, B and D. it was concluded that hip dysplasia degree can affect severity of lameness.

Diagnosis

Subluxation is not always entirely favored as the only diagnosis of CHD thus it is sometimes described as a "risk factor" rather than CHD (Ginja *et al.,* 2010).

D'Amico *et al.* (2011) reported that, while predisposed to find new methods of diagnosing CHD regardless of clinical symptoms, found that laxity often decreased with maturation of the dog.

Various diagnostic method including the use of history, clinical signs, palpation and radiography have also been reported but Ortolani maneuver is the most frequently used palpation technique for early detection of coxofemoral laxity in canine (Chalman and Butler, 1985). Hip laxity evaluation by palpation method i.e. Ortolani test was moderately co-related with hip laxity on distraction radiography but similar co-relation was not found in dogs with radiographic evidence of DJD (Puerto *et al*., 1999).

New diagnostic modalities such as ultrasonography, computed tomography, magnetic resonance imaging, force plate, kinematics, molecular techniques are still under investigation whereas, radiography has become the accepted convention for the diagnosis of canine hip dysplasia (Riley *et al.,* 1996).

Gait analysis

Gendreau and Cawley (1977) reported that after femoral head osteotomy dogs might exhibit an obvious permanent gait abnormality from leg shortening and chronic muscle atrophy. It might be more obvious in unilateral femoral head osteotomy because of difference between hind limb after surgery.

The dogs with hip disorder when made to walk showed the gait abnormality like boxy hip and bunny hopping gait which indicate hip luxation. In severe stage of degeneration, clunking sound in hip due to relocation of the luxated head of femur into hip joint. Stand test created extension of the hip and lordosis of the spine and in case of trauma the postural attitude altered depending on the severity of the trauma (Slocum and Slocum, 1998 and Hulse and Johnson, 2007).

Bockstahler *et al.* (2007) studied the kinematics gait analysis in 20 Belgium shepherd dogs by walking on the tread mill and found that dogs with borderline hip dysplasia diagnosed by radiographic examination had alerted kinematics.

Physical examination

Barlow (1962) described Barlow's sign for first time in the human medicalliterature.

Bardens and Hardwick (1968) reported that a positive Bardens sign consisted of a 2 mm or greater estimation of palpable hip laxity.

Chalman and Butler (1985) and Ginja *et al*. (2008b) reported that the Ortolani test is a common physical manipulation examination that is used in veterinary clinical medicine to diagnose hip joint laxity (HJL). Hip joints are considered to exhibit a positive Ortolani sign when a palpable or audible ‘clunk’ is present during hip joint reduction. If a ‘clunk’ cannot be elicited, the result of the Ortolani test is considered negative.

Fry and Clark (1992) reported that a complete clinical examination should include observation of the patient at rest, walking and trotting, and re-examination after vigorous exercise. However, they also reported that a number of clinical tests that can give information about the hip joint have long been advocated and these can be separated into two groups: (1) to provide information on HJL, recommended mainly for use on young animals (Ortolani, Bardens and Barlow tests) and (2) to detect signs of osteoarthritis (palpation and range of motion tests).

Adams *et al*. (2000) and Ginja *et al*. (2009) reported that the Ortolani test lacks sensitivity in puppies around 8 weeks of age but is most sensitive in young dogs older than 4 months.

Ginja *et al*. (2008b) studied on 104 Estrela Mountain dogs to determine the reliability of early passive hip laxity examination in predicting moderate and severe hip dysplasia by using the Ortolani method, conventional hip-extended radiography and the PennHIP method, when the animals were between four and twelve months of age. After the age of one, dogs were re-examined for hip dysplasia using the Federation Cynologique Internationale scoring system. The passive hip laxity results were subsequently compared with the Federation Cynologique Internationale scores for sensitivity, specificity, positive predictive value, negative predictive value and accuracy. The PennHIP method achieved the best results in specificity (93 %), positive predictive value (91 %) and accuracy (88 %), and the Ortolani method was the best in sensitivity (92 %) and negative predictive value (92 %).

Clinical Examination

Barden"s test has been recommended to evaluate hip joint laxity in puppies at six to eight weeks of age. The animal was kept in lateral recumbency, with the examiner standing behind the animal and holding the upper femur. Upward pressure was applied by this hand to elevate the femur horizontally. The index finger of the other hand was located on the greater trochanter and its mobility was used to estimate hip joint laxity (Bardens and Hardwick, 1968).

Fry and Clark (1992) categorized the clinical tests that could give information about the hip joint into two groups. The first group of signs provided information on hip joint laxity, recommended mainly for use in young animals (Ortolani, Barden’s and Barlow tests) and second

group of signs to detect osteoarthritic changes (palpation and range of motion tests**).** They also reported that crepitus may be detected during palpation of the hip joint with osteoarthritis and range of motion might be decreased due to capsular fibrosis, subluxation or fixed luxation and osteophytes.

Puerto *et al*. (1999) stated that a positive Ortolani test suggested excessive laxity, but its absence did not always indicate a tight hip. Fibrosis and thickening of the joint capsule and the acetabular rim, and femoral head destruction prevented the detectable "clunk" sound.

Quinn *et al*. (2007) evaluated three methods of lameness scoring and reported that numerical rating scales (NRS) and visual analogue scales (VAS) did not replace the force plate analysis. NRS and VAS scoring scales most precisely reflected force plate gait analysis when lameness was severe.

Ginja *et al.* (2008b) noted that Ortolani test was a common physical manipulation examination that was used in veterinary clinical medicine to diagnose hip joint laxity in dogs. The dog was positioned in lateral recumbency; the examiner stood behind the animal and held the upper stifle firmly putting the hip in a neutral position and the femur parallel to the surface of the examination table. A proximally directed force was applied to the shaft of the femur to provoke hip subluxation, while the pelvis was supported with the other hand. Then the stifle was slowly abducted to reduce the hip joint. Hip joints were considered to exhibit a positive Ortolani sign when a palpable or audible „clunk" was present.

Jaegger and Marcellin-Little (2002) and Goldring and Goldring (2006) reported that joint pain was one of the hallmarks of osteoarthritis and the major cause of lameness associated with the disease and the symptoms of osteoarthritis were often associated with significant functional impairment as well as signs and symptoms of inflammation including pain, stiffness and loss of mobility.

Manley *et al*. (2007) found that there were two ages at which dogs were presented with clinical signs of hip dysplasia; younger than one year of age with hip instability and overloading of some articular areas where in the pain was caused mainly by tearing or stretching of the round ligament, synovitis and acetabular micro fractures and in adult dogs with chronic pain due to osteoarthritis.

Macphail (2000) reported that obesity was a risk factor for osteoarthritis and increased loads placed on an arthritic joint contributed to cartilage deterioration and that weight control might alleviate the severity of clinical signs of osteoarthritis by decreasing the amount of abnormal force put on the joint leading to significant lameness ranging from mild to severe.

Nielson and Pluhar (2005) noted that the orthopedic examination consists of three major components, including observing the animal at rest, observing in motion, and physical examination of the animal both standing and in lateral recumbency. He also stated that Visual observation of lameness will include change in stride length, change in joint angles both in stride and stance phases of gait, weight shifting, motion of the head and trunk, and changes in gait symmetry from normal.

Thomas *et al*. (2006) reported that Lameness can be objectively quantified through biomechanics, which applies mechanical principles to the study of the animal in motion, either via kinematics or kinetics. They also stated that Kinematics is concerned with the study of the description of motion, while kinetics is the study of the action of forces. Kinematics describes motion, and kinetics explains motion during hip joint reduction. Ortolani test was considered to be negative when audible „clunk" sound was absent.

Farrell *et al.,* (2007) reported that in case of Osteoarthritis crepitus might be detected during palpation of hip joint and the range of motion might be decreased due to the presence of osteophytes, capsular fibrosis, subluxation or fixed luxation.

Physiological parameters

Ranganath and Subin (2006) reported that there was no significant difference observed in the rectal temperature, heart rate and respiratory rate in dogs with and without hip dysplasia. They reported that all the values were in reference range throughout the study.

Vishal (2011) studied that no significant variations in the physiological parameters like heart rate, respiration rate and temperature in both normal and joint affected dogs was observed and the respective values reported were within the reference or normal range throughout the study period.

Radiographic Examination

Gaspar *et al.* (2016) reported that norberg angle is not an accurate prediction of normal hip conformation with cut point of ≥105 degree based on distraction index and dorsolateral subluxation score.

Distraction index at 4 months of age was highly correlated with distraction index at 24 months and was associated with the resemblance that dogs would develop radiographic evidence of canine hip dysplasia (Vezzoni, 2004).

It has been shown that the conventional ventrodorsal radiographic projection has a decreased sensitivity when it comes to identifying hip joint laxity, as this position tightens the joint capsule, the femoral head ligaments and the related muscles (Smith *et al*., 1990 and Vezzoni *et al*., 2005). Smith *et al*. (1995) reported that Norberg angle measurement may not be a precise sign of CHD development.

Riser (1973) studied the hip joint instability radiographically and acetabular remodelling histologically in puppies as young as seven weeks.

Riser (1975a) reported femoral head subluxation radiographically as the first signs of HD and a lag in the development of the craniodorsal acetabular rim which was noted by seventh week of age.

Morgan (1987) conducted a radiographic study on 605 dogs of five breeds from pelvic radiograph. He observed that there was a greater frequency (54%) of bony change in cases diagnosed radiographically as dysplastic than in cases diagnosed as normal (15%). Thus, it was suggested that for early detection of canine hip dysplasia this minimal radiographic change can be used as an indicator of early canine hip dysplasia, especially in the absence of femoral head subluxation.

Brass (1989) reported that there is no direct correlation between the degree of pain and the severity of radiographic changes within the joints in hip dysplasia.

A longitudinal cohort study was conducted on forty-eight Labrador Retrievers to determine the relationship between a circumferential femoral head osteophyte (CFHO) and

osteoarthritis characteristic of canine hip dysplasia. The study was conducted to find whether CFHO varies between diet-restricted and control-fed dogs as it varies in osteoarthritis. It was concluded that there is relationship between CFHO and subsequent development of radiographic signs of osteoarthritis. The presence of CFHO in Labrador Retrievers might be considered as an early indicator of osteoarthritis (Szabo *et al*., 2007).

Ginja *et al.* (2008a) studied that for an accurate radiographic examination and interpretation the correct positioning of the dog is of utmost importance. He concluded that the pelvis should be positioned symmetrically with the femurs parallel to each other and the patellae superimposed over the center of the femoral condyles.

Rungea *et al*. (2010) studied prevalence in 4349 dogs by 3 radiographic projections i.e. the hip extended view for presence of osteoarthritis, the compression view and the distraction view to calculate the distraction index and reported that osteoarthritis increases with hip joint laxity as measured by the distraction index irrespective of breed.

Smith *et al*. (2012) reported that with increase in age of dog, radiographic examination becomes more accurate for predicting the status of the hip joint morphology.

Ventrodorsal hip extended view

Corley (1992) and Ginja *et al*. (2010) conducted a study for the ventro-dorsal projection by placing the dogs in dorsal recumbency on the X-ray table with the rear limbs extended parallel to each other and to the table top and the stifles internally rotated.

Corley *et al*. (1997) conducted preliminary hip evaluations using the Ventro Dorsal view on young dogs less than 2 years of age. He concluded that OFA scoring system in young dogs is reliable to predict hip dysplasia (CHD) at later ages.

Ginja *et al*. (2010) opined that for diagnosis of canine hip dysplasia in dogs older than 1 or 2 years of age, characteristic radiographic signs were observed on the standard ventrodorsal (VD) hip-extended projection.

Norberg angle and femoral head coverage

Henrigson *et al*. (1966) and Comhaire and Schoonjans (2011) suggested that for measurement of norberg angle, a line was drawn connecting the center of both femoral heads and an another line was drawn connecting the center of the femoral head and the craniolateral aspect of the ipsilateral acetabular rim the angle formed between two lines was considered as norberg angle.

Adams *et al*. (2000) observed that dogs with distracted hips showing norberg angle 85° or greater did not develop degenerative joint disease and 69% of dogs with hips distracted showing norberg angles less than 85° did develop degenerative joint disease.

Tomlinson and Johnson (2000) conducted a study on 1841 dogs between age of 24 to 48 months which including 545 Rottweilers, 455 Labrador Retrievers, 423 Golden Retrievers, and 418 German Shepherd dogs. Percentage femoral head coverage and Norberg angle (NA) were measured in 4 common breeds of dogs and to distinguish between normal and dysplastic hip on the basis of OFA hip evaluation. They concluded that the cut off points of NA of 105° and PC of 50% do not differentiate normal versus dysplastic hip status also found that each of 4 breeds had different values for norberg angle and percent femoral head coverage that distinguished normal from dysplastic hip status.

Ohlerth *et al*. (2001) conducted a study on 664 full and half-siblings from a colony of Labrador Retrievers and estimated genetic population variables for 6 radiographic criteria of canine hip dysplasia and concluded that Canine hip dysplasia is heritable to a moderate degree. Signs of subluxation revealed the highest heritability estimates. The criteria craniodorsal acetabular rim (ACR), subchondral bone sclerosis (SUBCH), femoral head and neck (FHN), and joint capsule (JC) were strongly influenced by Norberg angel (NA) and coverage of femoral head (COV).

Culp *et al*. (2006) did a retrospective study on 350 clinically normal dogs in 7 breeds of dogs (American Bulldog, Australian Shepherd, Borzoi, German Shepherd, Golden Retriever, Labrador Retriever, and Rottweiler) and evaluated the thresholds of 2 radiographic methods to determine coxofemoral joint laxity and they reported that by using the Norberg angle threshold of 105 degrees there was a high percentage of false- negative and false-positive diagnoses. Breeds like the Labrador retriever and Rottweiler would have

large numbers of hip dysplasia susceptible dogs remain in the breeding population based on this Norberg angel threshold. False-positive diagnoses were common in breeds like the Australian Shepherd, Borzoi, and German shepherd effectively eliminating hip dysplasia non-susceptible dogs from the breeding population. They found that Norberg angle was not an accurate predictor of Degenerative Joint Disease susceptibility in these 7 breeds of dogs when using a Norberg angel threshold of 105 degrees.

Janssens *et al*. (2014) conducted a radiographic examination of hips on hundred dogs to compare Norberg angle, linear femoral overlap and surface femoral overlap. They concluded that Norberg angle (NA) and Linear femoral overlap (LFO) or Surface femoral overlap (SFO) cannot be used as comparable methods.

A study was conducted on 437 dogs to determine whether Norberg angle (NA) ≥105°) accurately predicts a non-dysplastic hip based on a distraction index (DI) cut-off of ≤0.3 or a dorsolateral subluxation (DLS) score cut-off of ≥55%. It was concluded that the commonly used cut- point for NA i.e. ≥105° is not an accurate measurement of normal hip conformation (Gaspar *et al*., 2016).

Morgan line

Whittington *et al*. (1961) observed the radiographic identification of a caudolateral curvilinear osteophyte (CCO) at the insertion of the joint capsule on the femoral neck.

Morgan (1987) stated that remodelling occurs in an effort to extend the articular surface and this leads to the formation of osteophytes or enthesiophytes. He investigated the importance of the caudo-lateral curvilinear osteophyte (CCO) on the femoral neck in dogs for the first time. He also reported that CCO develops secondary to increase in stress on the joint capsule insertion in dogs with excessive hip joint laxity. He also found that the Morgan Line (ML) is a bone formation in the caudo-lateral region of the femoral neck indicative of joint instability considered as the first radiographic sign of Degenerative joint disease and there was association between ML and other signs of bone remodelling.

Mayhew *et al*. (2002) conducted a study on 25,968 dogs including 3,729 German Shepherd Dogs; 4,545 Golden Retrievers; 6,277 Labrador Retrievers and 1,191 Rottweilers and determined the prevalence of radiographic caudo-lateral curvilinear osteophyte (CCO) on the femoral neck in various breeds and age groups of dogs and evaluated its contemporaneous relationship with degenerative joint disease (DJD) and distraction index (DI). They concluded that contemporaneous association between the CCO and DJD and passive hip laxity, measured by use of the DI, is associated with both the CCO and DJD.

Powers *et al*. (2004) conducted a study on 48 Labrador Retrievers from 7 litters to determine the relationship between the caudo-lateral curvilinear osteophyte (CCO) and osteoarthritis associated with hip dysplasia in dogs and concluded CCO is an important early radiographic indication of osteoarthritis associated with canine hip dysplasia.

Ventrodorsal hip distraction view

Distraction index

Smith *et al*. (1990) reported that the distraction index (DI) and dorsolateral subluxation score (DLS) are negatively correlated. They also observed dogs with tight hips (DI $\leq$0.3 or DLS score $\geq$55%) are significantly less likely to develop canine hip dysplasia than dogs with loose hips (DI >0.7 or DLS score <45%). He also reported that the PennHIP method is used in dogs older than 4 months of age and calculated the distraction index (DI). The DI measured the relative displacement of the geometric centre of the femoral head from the center of the acetabulum when lateral stress is applied to the proximal femur by a PennHIP distractor. He also reported that DI is calculated from the distance between the geometric centres of the acetabulum and the femoral head is divided by the radius of the femoral head. The DI ranged from 0 to >1, with 0 representing full congruency of the hip joint and 1 representing complete luxation.

Lust *et al*. (1993) observed that 87% of Labrador Retrievers with a distraction index less than 0.4 at the age of 4 months developed normal hips whereas 57% of dogs with a distraction index 0.4 or greater became dysplastic.

Popovitch *et al*. (1995) compared susceptibility of degenerative joint disease on the basis of distraction index in different dogs. They reported that German Shepherd Dogs were 6.3 times more prone for degenerative joint disease than Rottweiler breeds having the similar distraction index.

Farese *et al*. (1998) developed a radiographic procedure to measure dorsolateral subluxation (DLS) of the femoral head in a weight-bearing position. They examined total of 24 dogs of varying ages which included Labrador retriever, Greyhound, and Labrador-greyhound crossbreeds. For each hip, the DLS score was determined by measuring the percentage of the femoral head medial to the lateral most point of the cranial acetabular rim on the dorso-ventral radiographic projection and the lateral most point of the central, dorsal acetabular rim on the CT image. Higher DLS scores indicated better coverage of the femoral head by the acetabulum. DLS scores were compared with the distraction index (DI) by grouping joints according to their probability of developing osteoarthritis (OA) as predicted by the DI. There was a strong correlation ($r = 0.89$ for both hips) between the DLS score measured on the weight bearing radiograph and the CT image. They also observed a strong correlation between the DLS score and the DI ($r = 0.37$). The DLS scores for OA unsusceptible joints and joints with a high probability of developing OA were significantly different ($P < 0.05$).

Ohlerth *et al*. (2003) conducted a study on 12 pure bred Labrador retrievers in which the Comparison of three distraction methods (two radiographic distraction techniques and one ultrasonographic distraction method) and conventional radiography for early diagnosis of canine hip dysplasia was done and concluded that the ultrasonographic distraction method only appears to be reliable in predicting true negatives and cannot be recommended as a screening method for early diagnosis of CHD in the present dog colony.

Kapatkin *et al*. (2004) did a prospective study on 500 clinically normal dogs of 10 breed to evaluate hip joint laxity by two radiographic techniques and concluded that Distraction radiography detected the greatest range and magnitude of passive hip laxity in the 10 breeds of dogs. The difference in values between breeds known to have high prevalence of canine hip dysplasia and those in Borzois was greater for Distraction Index than for Hip Extended Index.

Different methods of Radiographical hip scoring of hip dysplasia

British Veterinary Association (BVA)/Kennel Club (KC)

Wood and Lakhani (2003) documented a scheme that animals more than one-year-old should submit radiographs of hip joint to ensure adequate skeletal maturity. Under this scheme, nine radiographic features of each coxofemoral joint were considered collectively to define the condition of the hip joint, examined by a panel of veterinarians and awarded a numerical score.

Lewis *et al.* (2009) reported that according to BVA/KC method the total hip joint score ranged from 0 to 106. For the detection of pathological signs of osteoarthritis six of the nine features were scored exclusively such as (femoral head and neck exostoses, femoral head recontouring, dorsal acetabular edge, cranial effective acetabular rim, acetabular fossa and caudal acetabular edge,). For the detection of two of the features were scored exclusively on morphology (Norberg angle, subluxation) and one feature was scored on a mixture of both (cranial acetabular edge).

Dennis (2012) studied that in BVA/KC method of hip scoring, each coxofemoral joint (right and left) was observed for nine different anatomical features and a certain numerical score was given, with points being ascribed to abnormal features also and the total score for each hip joint was then calculated. Each hip joint score ranges between the range 0 to 53 and the total hip score ranges between the range 0 to 106. The nine anatomical features assessed in the BVA method were enlisted as Norberg angle, subluxation, dorsal acetabular edge, cranial acetabular edge, caudal acetabular edge, acetabular fossa, cranial effective acetabular rim, femoral head and neck exostoses and femoral head recontouring. The provided scores and the classification of the hip joint were as follows; 0 to 4 total score: Near to perfect or perfect hips, 5 to 10 total score: Borderline changes that were unlikely to deteriorate with age, 11 to 20 total score: Mild changes that deteriorated with age, sometimes developing into osteoarthritis, 21 to 50 total score: Moderate to obvious hip dysplasia in which osteoarthritis was already a noticeable feature, or severe hip dysplasia before arthritic variation, Above 50: Severe to very severe osteoarthritic changes secondary to hip dysplasia.

Penn hip Method

Smith *et al*. (2001) noticed that Labrador Retrievers with a distraction index (DI) < 0.3 to 0.4 at 8 months of age were classified as unaffected by hip dysplasia (HD) with a > 80 per cent probability of not advancing to secondary osteoarthritis in hip joints. Labrador Retrievers with a distraction index DI > 0.7 were classified as affected with Hip Dysplasia and thought to have a high probability of developing osteoarthritis in hip joints.

Choi *et al*. (2008) described the grading of scores in the Penn hip method according to distraction index as follows: Grade 1, if the distraction index is below 0.3. Grade 2: if the distraction index is 0.3. Grade 3, if the distraction index is 0.7 and Grade 4, if the distraction index is greater than or above 0.7. In a dog, hip dysplasia could be confirmed if the distraction index (DI) have a grade 4 or any grade with a degenerative joint disease lesion.

Ginja *et al*. (2010) portrayed the procedure for Penn hip method as follows. Three radiographic views of the hip joint were obtained with the animal kept in the supine position or ventrodorsal recumbency with both the femurs extended. Another two views were compression and distraction view. The multiple radiographic views and manual restraint requisite were main disadvantages of Penn hip method of radiography. The distraction view was taken using the Penn HIP distracter with hips in a neutral position and maximally displaced laterally. To calculate the DI, the distance between the geometric centres of the femoral head and acetabulum were divided by the radius of the femoral head. The distraction index ranged from 0 to > 1, 0 describing full congruency of the hip joint and 1 describing complete luxation.

Federation Cynologique Internationale (FCI)

Fluckiger (2007) reported that according to FCI method of hip scoring, grades have been assigned on the basis of size of Norberg angle, shape and depth of the acetabulum, degree of subluxation and signs of secondary joint disease. The Grade E for severe hip dysplasia, Grade D for moderate hip dysplasia, Grade C for mild hip dysplasia, Grade B for near normal hip joints and Grade A for no signs of hip dysplasia.

Orthopedic Foundation for Animals (OFA)

Fluckiger (2007) reported that OFA was one of the hip scoring methods used mainly in dogs above two-year age. In this system seven-point scoring is used, normal hip joint conformation was divided into excellent, good and fair score. In dysplastic hip conformation mild, moderate or severe hip dysplasia was scored. Re-examination after six months was suggested if the hip joints could not be allocated to any of these groups and were termed undetermined or borderline type.

Smith *et al.* (1995) reported that dogs suffering from passive hip laxity best estimated on a hip radiograph or by palpation under anaesthesia or heavy sedation. In 1961, American veterinary medical association made first effort to establish a uniform radiographic diagnostic criterion for diagnosis of CHD. Later on, this effort become the conventional radiographic technique and is used worldwide (Kapatkin *et al.,* 2002). In 1961, University of Pennsylvania (OFA) introduced 7-point subjective scoring system for diagnosis of Canine hip dysplasia at 2 years of age or older (Gibbs, 1997). In Great Britain, 106v point subjective scoring system with 53 points for each hip is used to score the hips on hip extended radiograph at 1 year of age. In 1985, Morgan and Stephens reported that norberg angle can be determined on Ventro-dorsal view by measuring the angle relationship between femoral head centre and dorsal cranial acetabular rim.

Ultrasonographic examination

O'Brien *et al.* (1997) correlated the ultrasonography distraction distant with Penn hip distraction index. The ultrasonography distraction distance was measured on the modified dorsal long axis view. He found that at the same age in young dogs it was correlated to the Penn hip DI. At one year of age, the correlation of radiographic evidence of canine hip dysplasia to ultrasonography distraction index was mild.

Adams *et al.* (2000) reported that Ultrasonographic measurement for Labrador retriever and Golden retriever was not a reliable predictor of canine hip dysplasia with or without degenerative joint disease.

Ohlerth *et al.* (2003) for early detection of canine hip dysplasia, he correlated conventional radiograph and three distraction methods. He reported that ultrasonography distraction method was moderately reliable in the detection of canine hip dysplasia.

Rocha and Torres (2007) reported that ultrasonography examination was t proved to be reliable method for earlier detection of passive hip laxity of coxofemoral joint in dogs aging 14 to 15 days.

Synovial fluid:

Lipowitz and Newton (1985) opined that the normal volume of synovial fluid in joint varied from joint to joint, ranging from 0.01 ml to 1.0 ml. In the dogs, the average volume of synovial fluid was found to be 0.24 ml. The author reported that in degenerative joint disease the volume of the synovial fluid might not be appreciably increased but with the increased inflammatory signs, the volume of synovial fluid in joint was increased.

Houlton (1994) stated that there was significant decrease in synovial fluid transparency of affected joint in dogs. He reported that the turbid nature of the synovial fluid was due to the inflammatory joint disease and the presence of cellular debris or fibrin in the synovial fluid.

Parry (1999) reported that physical examination of synovial fluid could be easily assessed at the time of collection. Colour, turbidity, and viscosity of synovial fluid could easily be examined immediately after collection. The volume obtained could vary from a few drops to more than 1 ml.

Herrero-Beaumont *et al.* (2001) and Gossec and Dougados (2004) reported that synovial fluid had a low viscosity in osteoarthritic condition, because of decreased concentration of hyaluronan (HA), chain length and consequently its molecular weight.

Kumar and Agarwal (2001) stated that the synovial fluid analysis could be used in differentiating inflammatory from non-inflammatory diseases. In addition, they reported that with few drops of synovial fluid it might rule out specific diagnosis for septic, crystalline and immune mediated arthritis. Nucleated cell count, differential cell count and a gross evaluation might provide useful information for prognosis and treatment of arthritic joint diseases.

Jacques *et al*. (2002) found that about 0.1 to 0.25 ml volume of synovial fluid routinely was obtained during aseptic arthrocentesis in small animal. During arthritic joint disease, synovial fluid volume was usually increased due to any cause of non-inflammatory or inflammatory joint diseases. Normal appearance of synovial fluid when examined was transparent and highly viscous. Particulate matter in the fluid, such as erythrocytes, leukocytes, organisms, and fibrin caused Cloudiness in the synovial fluid. Viscosity might be mildly decreased with osteoarthritic degenerative joint disease. Usually it decreased severely due to inflammatory joint diseases. In dogs, 3000 nucleated cells/μL was considered normal, although most healthy dogs had counts lower than this count.

Gerwin *et al.* (2006) stated that composition of synovial fluid was almost identical to plasma, a clear, viscous liquids in diarthrodial joints, with the exception of the large polymers like fibrinogen or larger globulins. Due to the sieving action of the synovial capillary walls these large polymers and globulins were reduced or not found at all in synovial fluid.

Xu *et al.* (2009) reported that there was an increased number of mononuclear cells (mainly macrophages and T lymphocytes) in synovial fluid of most osteoarthritic patients. He reported that there was increased levels of immunoglobulins, complement and inflammatory cytokines.

Weeren and DeGrauw (2010) opined that synovial fluid acted both as a lubricant for frictionless joint motion and the medium for transport of nutrients and waste products from the avascular articular cartilage. Synovial fluid was a key component in joint homeostasis.

Vishal (2011) reported that the total nucleated cell count was significantly higher in joint affected dogs as compared to control dogs. The cause for increased nucleated cell count may include non -inflammatory arthropathies (mildly increased nucleated cell count) and inflammatory arthropathies which have a normal.

TREATMENT

Conservative medication

The aim of using conservative management in dogs is to reduce or eliminate pain by a combination of exercise restriction, weight control, analgesics (NSAIDs) and physical therapies (Anderson, 2011).Nutraceuticals as a dietary supplement that bears or contains one or more of the following dietary ingredients such as a mineral, a vitamin, an amino acid, a medical herb or other botanical or a concentrate, metabolite, constituent, extract, or combinations of these ingredients (Nasri *et al.,* 2014a) Nutraceuticals shows promising results in various pathological complications such as diabetes (Baradaran *et al.,* 2013; Nasri, 2013) arthrosclerosis, (Madihi *et al.,* 2013; Setorki *et al.,* 2013) cardiovascular diseases (CVDs) (Khosravi *et al.,* 2012 & 2013), cancer (Shirzad *et al.,* 2011) and neurological (Akhlaghi *et al.,* 2011; Roohafza *et al.,* 2013) disorders.

Baradaran *et al.* (2014) and Nasri *et al.* (2014) have opinion that nutraceuticals have antioxidant activity with the ability to counteract these situation (Parsaei *et al.,* 2013; Kafash-Farkhad *et al.,* 2013). They are considered as healthy sources of health promotion, especially for prevention of life threatening diseases such as diabetes, (Mirhoseini *et al.,* 2013; Akbari *et al.,* 2013) infection, (Bahmani *et al.,* 2013; Karimi *et al.,* 2013) renal, (Rafieian *et al.,* 2013) and gastrointestinal disorders (Hosseini *et al.,* 2002; Kiani *et al.,* 2013).

McNamara *et al.* (1996) observed no clinical changes in hematological and hemostatic variables in young, clinically normal dogs treated with chondroprotective agents for 30 days and hence opined that it could be used safely for the treatment of dogs with osteoarthritis.

Hulse (1998) studied that dogs with osteoarthritis when administered with the combination of chondroitin sulphate and glucosamine subjectively, more normal locomotion and joint movement was observed.

Canapp *et al.* (1999) found that prophylactic dose of chondroitin sulphate in combination with glucosamine, decreases inflammatory condition in dogs with induced arthritis.

Dassler (2003) opined that the dogs those were expected to develop hip dysplasia due to genetic condition, when confined to a small cage of $1m^3$ dimensions where dogs spent most of the time sitting on their haunches was reported to have lesser chance for the development of hip dysplasia.

Szabo *et al.* (2007) conducted a study on 48 Labrador retriever dogs fed with controlled diet (25% less) and the dogs fed with ad libitum diet. He studied the formation of circumferential femoral head osteophytes in 48 Labrador retriever. He observed that the median age for the formation of circumferential femoral head osteophytes was 9 years in dogs fed with controlled diet as compared to dogs which were fed with ad libitum diet. It was evident that in 3 years dogs with circumferential femoral head osteophytes developed osteoarthritis at mean age of 11 years in dogs fed controlled diet compared to 6.5 years in dogs fed ad libitum.

Christensen *et al.* (2006) opined that when glucosamine sulphate 800 mg in addition with 1800 mg omega-3 fatty acid was fed to dogs with experimentally induced osteoarthritis, the proteoglycan content in the femoral condoyle was found to be significantly higher as compared to the control group.

Doig *et al.* (2000) discovered that once osteoarthritis became clinically evident there was no curative treatment for it. The main aim of medical therapy in the treatment of osteoarthritis was to renovate and conserve normal joint function by eliminating joint pain and subsidiary inflammation as well as cartilage protection from any further damage. He reported that it could be achieved with a combination of anti- inflammatory medications in combination with chondroprotective agents, moderation of activity and weight control.

Aragon *et al.* (2007) discovered a high level of comfort by administering meloxicam and moderate level of comfort with caprofen, etodolac, green-lipped mussels, pentosan polysulphate, polysulfated glycosaminoglycan and a combination of glucosamine hydrochloride, chondroitin sulfate, and manganese ascorbate for management of osteoarthritis in dogs. He also observed extremely low level of comfort existed with hyaluronan.

Corr (2007) reported that canine hip dysplasia could be conservatively managed with nonsurgical means by weight reduction, pain control, nutraceuticals, exercise management,

physiotherapy, and chondroprotective drugs which delays the development of osteoarthritis in canine.

Gladstein (2010) suggested the management of canine hip dysplasia by using prolotherapy and stem cell injections (ACell‟s MatriStem®). Prolotherapy solution composed of equal amount of Heels‟ traumeel, Vitamin B_{12}, Dextrose 50% and 2 per cent lignocaine. It was injected into the lateral and dorsal aspect of hip joint and after three treatments at an interval of two weeks and one stem cell therapy, the animals showed improved condition near to normal.

Kirkby and Lewis (2012) reported that hip dysplasia could be prevented by managing body weight. He also reported that the use of polysulfated glycosaminoglycan, mesenchymal stem cells or possibly extracorporeal shockwave therapy might also be beneficial in managing osteoarthritis as well as modulation of joint disease.

Surgical management

Conventional Surgical techniques

Olmstead (1998) and Vasseur (2008) advised total hip replacement and excision arthroplasty as salvage procedures for irreducible coxofemoral luxation, degenerative joint disease, Perthe`s disease, septic arthritis, osteoarthritis, femoral capital physeal separation and femoral neck fracture in canine.

(Slocum and Slocum, 1998) indicated the corrective procedures recommended for hip dysplasia were triple pelvic osteotomy, inter trochanteric osteotomy, femoral neck lengthening, shelf arthroplasty and pectineal myectomy.

Iamaguti *et al.* (2009) performed deepening of acetabular cavity and re-establishment of the femoral head ligament and the joint capsule for treatment of severe canine hip dysplasia surgically.

Anderson (2011) studied that juvenile pubic symphysiodesis, triple pelvic osteotomy, double pelvic osteotomy, femoral neck lengthening, denervation of the hip joint capsule, shelf arthroplasty, total hip replacement, intertrochanteric femoral osteotomy, pectineus myotomy or

myectomy, dorsal rim acetabuloplasty and femoral head and neck excision were the available surgical options for the management of hip dysplasia and osteoarthritis in coxofemoral joint.

Raghuvir *et al.* (2013) noted that femoral neck lengthening, pectineal myectomy, and corrective osteotomies might be adopted as surgical procedures in young animals for management of hip dysplasia. He also performed biocompatible osteoconductive polymer/shelf arthroplasty, total hip arthroplasty and femoral head and neck excision in adult animals with hip dysplasia and osteoarthrosis.

Denervation of Hip Joint

Braun *et al.* (2003) mentioned that overall 96.15 (%) per cent hip dysplastic animals showed clinical improvement after dorsal acetabular denervation surgically. Among these dogs 69.23 (%) per cent had no lameness or pain and 26.92 (%) per cent dogs had slight lameness or pain. They also mentioned that 83.6 per cent of the dogs showed significant increase in the musculature of the hind limbs. It was reported that marked improvement in muscle atrophy with an average of 88 days due to decrease in pain and rapid increased motor activity was observed. This improvement in musculature was accelerated by the administration of anabolic drugs such as nandrolone @ 1.5 mg/kg body weight. Finally, they concluded that prognosis for success of operation depended on the age of animal and degree of muscle atrophy, increased age and advanced muscular atrophy were considered to be unfavorable for outcome of surgery.

Whiteside *et al.* (2006) reported denervation of the coxofemoral joint as a novel surgical procedure for the treatment of degenerative osteoarthritis in a Bengal Tiger (*Panthera tigris tigris*). He observed that the tiger could walk more comfortably after one week of surgery with greater extension of the hind limbs along with increased activity and considerably less limping. Two weeks after the surgery, the tiger came back to its normal activity and remained to do well at 17 months post-surgery.

Ferrigno *et al.* (2007) reviewed 97 cases of canine hip dysplasia which were surgically treated by removal of the periosteum of both the cranial and dorsal region of the acetabulum (acetabular denervation). He reported that denervation resulted in remission of clinical signs with reduced lameness and pain after two days post-surgery. There was reduced muscle atrophy after

60 days post-surgery and overall improvement in quality of life was 95 % success rate from the perspective of veterinarians and owners up to 360 days postoperatively. They observed 72.16 per cent improvement in pain after two days of surgery, 78.35 per cent after 14 days, 100 per cent after 30 days, 97.46 per cent after 60 days, 96.29 per cent after 180 days and 95.23 per cent after 360 days of surgery. They also discovered that there was a significant decrease in lameness of about 63.91 per cent after seven days, 86.07 per cent after 60 days, 96.29 per cent after 180 days and 95.23 per cent after 360 days post-surgery.

Collard *et al.* (2008) opined laxity as the major cause of pain in hip dysplasia, which was responsible for the stretching of the joint capsule. The capsule of the hip joint was described to be innervated by many sensory nerve branches which ran on the periosteum around the joint. He noted that removing periosteum around the craniodorsal aspect of the joint relieved the pain. Based on 16 hip denervation surgeries, he discovered that mean surgical duration was 28.5 minutes and an overall improvement in clinical signs was noticed in 87 (%) per cent.

Silva *et al.* (2012) performed percutaneous and open approach for capsular denervation methods to treat hip dysplasia. Both approaches gave good results with significant decrease in lameness, pain, increased thigh girth and range of motion. They also noted increased degree of extension and flexion of hip joint. An overall 90 (%) per cent clinical improvement was observed. They suggested that thigh muscle mass could be measured immediately distal to the inguinal fold by using inelastic measuring tape.

POSTOPERATIVE CARE AND COMPLICATIONS ASSOCIATED WITH VARIOUS SURGICAL TECHNIQUES

Penwick (1992) reported that femoral head and neck ostectomy might take six to eight months in adult patients for full recovery and in younger dogs it recover faster.

Schulz and Dejardin (2003) suggested that for cemented total hip replacement or femoral head and neck ostectomy a patient must be free from persistent urinary tract infections and persistent pyoderma as it might lead to the risk of infection. They also observed functional abnormalities like decreased range of motion, particularly in abduction and extension, caudal or

dorsal displacement of femur, decreased angulations of the stifle and hip, muscle atrophy and limb shortening after femoral head and neck osteotomy.

Andrews *et al.* (2008) reported that the patients after undergoing total hip replacement might show common complications associated with included hip luxation, femur fracture, aseptic loosening of the acetabulum and or femoral stem, patellar luxation, infection, pulmonary embolism, incision granuloma, medullary infarction and sciatic neuropraxia. As there was no major complication in denervation process whereas, minor complication like seroma might be reported Collard *et al.* (2008).

Hummel *et al.* (2010) classified the complications of Zurich cementless total hip replacement as intraoperative, short-term and long-term. The intraoperative complications were fracture of femoral diaphysis, fracture of greater trochanter, lost screw in soft tissues, excessive hemorrhage and immediate revision of acetabular cup placement. The overall intraoperative complication rate was 11 (%) percent. The Short term complications include coxofemoral luxation, transient neuropraxia, fracture of femoral diaphysis and fracture of acetabulum and the overall short term complication rate was 6.75 % per cent. The long term complications include septic loosening, coxofemoral luxation, implant failure and fracture of femoral diaphysis and overall long term complication rate was 10.4 (%) per cent.

Silva *et al.* (2012) performed denervation of hip joint in 25 dogs and he reported that no major complications were observed but minor complications like suture dehiscence were observed in three dogs out of 25 dogs.

Rocha *et al.* (2013) performed surgical denervation of hip joint in dogs, in which hip dislocation was noticed one animal. They mentioned that because of surgery there was relief in pain in the joint capsule which helps the animal to original physical activities, including running, which, along with pre-existing muscular atrophy, contributed to the dislocation. So, they advised to provide kennel rest for seven days and gradual return to normal activities.

Stem Cell Therapy

Stem cell biology is gaining popularity in treatment of many incurable diseases. Very broadly they can be classified as embryonic/foetal stem cells and adult stem cells. The best

example of a stem cell is bone marrow derived stem cell. Though undifferentiated it can specialize into or can be induced into functional blood/bone cells under different environmental cues (Jaiswal *et al*., 1997).

In young animals' periosteum and bone marrow are the richest sources of MSCs and as age advances their numbers are seen to decrease (Kraus and Kirker-Head, 2006). Nauta *et al.,* 2013 have opinion that genetic manipulation of MSCs to overcome the hostile wound environment is emerging as a novel technique to enhance cell survival and proliferation, ultimately accelerating wound healing in animal models, this is particularly important in the treating diabetic and vascular wound, in which local cytokine levels are inadequate to achieve normal healing.

MSCs are capable of forming osteoblasts, chondrocytes and adipocytes both *in vitro* (Muraglia *et al.,* 2000) and *in vivo* (Aslan *et al.,* 2006). Genetic stability is one of the reasons why MSCs are preferred for regenerative use. MSCs maintain their diploid karyotype without aneuploidy, polyploidy or chromosomal structural abnormalities (Zhang *et al*., 2007), another reason for their use in cell transplantation is their low immunogenicity due to the absence of immunologically relevant cell surface markers. Bone marrow derived mesenchymal stem cells are also known to inhibit the proliferation of T lymphocytes, B lymphocytes, dendritic cells and natural killer cells (Kim *et al*., 2008). In 1998, James Thomson and his co-workers, at the University of Wisconsin–Madison, obtained the first human embryonic stem cell line (Thomson *et al*., 1998).

Centeno *et al.* (2008) reported first successful study of cartilage regeneration in the human knee, suffering from degenerative joint disease, using per-cutaneously implanted autologous adult mesenchymal stem cells.

Kim *et al.* (2009) reported that under chemically controlled conditions, human embryonic stem cells have been cultivated for the first time without the use of any animal substance, which is essential for future clinical uses and also they have sought out a way to manipulate skin cells to generate patient specific "induced pluripotent stem cells" (iPS), claiming it would be the "ultimate stem cell solution."

Freed *et al.* (2001) reported functional improvement in patients with severe Parkinson's disease through foetal cell transplants resulting in clinical benefit in younger but not in older patients.

Vidal *et al.* (2006) reported that in equine regenerative medicine, bone marrow collection from the sternum is likely preferred for cell-based therapies due to the reliable isolation, easy preparation procedure and separation of MSC in horses.

Kisiday *et al.* (2008) reported a superior chondrogenesis of Bone Marrow-MSCs relative to Adipose Derived Progenitor cells in response to TGF-β1. Wagner *et al.,* 2005; Colleoni *et al.,* 2009; Vidal *et al*. 2011) reported that adipose-derived MSC appears to have greater potential for proliferation and lower senescence relative to other sources of MSC. Berg *et al*. (2009) reported that MSCs obtained from umbilical cord blood have superior chondrogenic differentiation as compared to bone marrow MSCs.

Guest *et al.* (2008) reported that when an equine model was injected with both autologous and allogeneic marked MSC into artificially created superficial digital flexor tendon lesions then no significant variations were noted either in the number, distribution or leukocyte density of autologous and allogeneic cells at the injection point. No signs of external and histological inflammation were detected at the injection site.

Carrade *et al.* (2011) reported intraarticular injection of placental derived allogeneic MSC in comparison with autologous MSC injection both caused inflammation within the synovial fluid. However, no significant differences between the degree and type of inflammation were detected.

Crovace *et al.* (2007& 2010) and Lacitignola *et al.* (2008) reported that similar improvement was noticed when compared the healing effects of autologous expanded bone marrow derived MSC and bone marrow-derived mononuclear cells in tendon disorders characterized by significant improvement in ultrasonography, histology scores, higher COMP expressions and lowered type III collagen contents in relative to control group.

Zscharnack *et al.* (2010) reported that superior results were seen, in chondrogenesis, in an osteochondral defect of the femoral condyle in sheep, when pre-differentiated MSC were implanted into a collagen-I hydrogel.

Wilke *et al.* (2007) reported that after 30 days and 8 months, there was improvement in tissue regeneration, due to fibrous tissue formation, when undifferentiated MSC embedded in a fibrin gel, were implanted into the articular defects in equine model. No characteristic difference was detected with respect to collagen II and proteoglycan content of the tissue.

Murphy *et al.* (2003) showed decrease in the progression of Osteoarthritis by direct intra-articular stem cell injection into artificially induced knee joint disorder in caprine model there was marked regeneration of the medial meniscus and implanted fluorescent-labelled MSCs were detected in the newly formed tissue. Black *et al.* (2007 & 2008) reported improvement in clinical parameters of Dogs suffering from elbow and hip joint OA when injected with adipose derived mesenchymal stem. Yamada *et al.* (2004) reported that superior results were seen in regeneration of bone when stem cells are used in combination with platelet-rich plasma.

Marx *et al.* (2014) observed the effect of autologous stromal vascular fraction (SVF) or allogeneic cultured adipose-derived stem cells (ASCs) in dogs suffering from hip dysplasia and showing weak response to medicinal therapy. In both group patients treated with autologous SVF and dogs treated with allogeneic ASCs, clinical assessment showed marked improvement after the first week of therapy. On days 15 and 30, all dogs showed significant progress in range of motion, lameness at trot, and pain on palpation of the joints. Positive results were more evident in the SVF-treated group.

Kretlow *et al.* (2010) reported significant results when uncultured bone marrow mononuclear cells (bmMNCs) embedded within fibrin glue hydrogels are seeded into porous scaffolds to regenerate bone in rat model. Culture and expansion of MSCs consumes more time, cost and probable increase in the risk associated with treatment.

Samdani *et al.* (2009) found that bone marrow mononuclear cells (bmMNCs) treated group offered a greater protective benefit and decreased scar formation when compared to culture-expanded MSCs treated group in a model of spinal cord injury.

Platelets Rich Plasma

Platelet-rich plasma (PRP) is an autologous product that concentrates a large number of platelets in a small volume of plasma. PRP accelerates endothelial, epithelial, and epidermal regeneration, stimulates angiogenesis, enhances collagen synthesis promotes soft tissue healing, decreases dermal scarring, enhances the haemostatic response to injury, and reverses the inhibition of wound healing caused by glucocorticoids. The high leukocyte concentration of PRP has an added antimicrobial effect.

Platelet-rich plasma (PRP) is increasingly used for its ability to affect tissue regulation from the growth factors present in elevated platelet levels, and thereby reduce pain associated with OA (Halpern *et al.,* 2013). It is widely used in tissue repair and promotes a strong cicatrization stimulus (Barbosa *et al.,* 2008; Frykberg *et al.,* 2010 and Mehta *et al.,* 2010). Till date, seven different growth factors (GF) present in the PRP were identified which significantly contribute to the cicatrization process. Besides, the PRP is a low cost and easy access product, excluding the need for special equipments (Barbosa *et al.,* 2008 and Saad-Setta *et al.,* 2011).

Batista *et al.* (2011) reported that after four weeks of treatment, radiographic assessment, tomographic assessment, histomorphometry indicated a higher bone density in the platelet-rich plasma group compared to the centrifuged bone marrow group.

Cheng *et al.* (2008) observed that bone marrow derived stromal cells (BMNCs) delivered from PRP gel can repair bony defect in immunocompetent animals, the tissue engineered bone in BMNCs/PRP group is comparable to autogenous particulate cancellous bone group for the repair of critical sized bone defect in rabbit cranium model.

Haematobiochemical changes

Nganvongpanit *et al.* (2008) reported that dogs suffering with osteoarthritis showed elevated levels of Chondroitin sulfate (CS) and hyaluronan (HA), the most important cartilage biomolecules, in blood serum. The ELISA technique can be used to detect the serum CS and HA levels by using monoclonal antibodies against CS epitope 3B3 and WF6.

Lipowitz and Newton (1985) reported that no significant changes were observed in hematological parameters of dogs suffering with degenerative joint disease. They observed that in a few cases there was a non -significant increase in total leukocyte count which might indicate a low grade inflammatory process.

Mala (2006) reported that hematologically there were no significant changes between disease conditions and pre-and post-arthroscopic examination was revealed in the values of haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, neutrophils and lymphocytes. He reported that significant decrease in erythrocyte sedimentation rate in osteochondritis dissecans was seen in both pre and post- arthroscopic period and in degenerative joint disease, ligament injury and synovitis during post arthroscopic period alone.

Whiteside *et al.* (2006) reported that all hematological parameters like complete blood count and serum biochemical profile were within the reference ranges before and after surgical denervation of coxofemoral joint for the treatment of degenerative osteoarthritis in a Bengal Tiger (*Panthera tigris tigris).*

Serum Biochemistry

Ranganath and Subin (2006) observed that dogs suffering with hip dysplasia revealed no significant difference in the serum calcium and phosphorous levels as compared to healthy dogs. They reported that the serum alkaline phosphatase levels were found to be significantly higher in dogs with hip dysplasia and assumed that this might be due to increased osteoblastic activity associated with micro fractures healing in the subchondral bones of acetabular rim and femoral head.

3 MATERIALS AND METHODS

The study was conducted on clinical cases of dogs with the history of hind limb(s) lameness brought to the Department of Surgery and Radiology, Bihar Veterinary College, Patna for treatment. Eighteen dogs of either sex screened through radiographs for hip dysplasia were utilized for this study. A written consent was taken from the owners to include their animals under this study.

Anamnesis

History of each cases were recorded regarding the breed, age and sex, body weight, type of breeds viz. small, medium and large, usefulness of animal i.e. whether working or non-working, duration of lameness, difficulty in climbing staircase, vaccination, deworming schedule and food habits.

Physiological Parameters

Rectal temperature (ºF), heart rate (beats/min.), respiratory rate (breaths/min.) and colour of mucous membrane viz. pink, pale or congested were recorded.

Clinical signs

Clinical signs like degree of lameness i.e. weight bearing or non-weight bearing lameness, swaying of back, bunny hopping gait, sitting down after walking few distance, crossing of hind limbs while lying down and straightening of stifle and hock joint, if any were recorded.

Bone marrow collection and separation of nucleated cells

The dogs were kept off-feed for 12 hours before the procedure. The dogs were pre-anaesthetized by intramuscular administration of glycopyrrolate @ 0.01 mg/kg body weight, xylazine @ 1mg/kg body weight and general anaesthesia induced with propofol @ 4 -6 mg/kg body weight. The animals were placed in lateral recumbency. The skin over the dorsal surface of iliac crest (Hip bone) and around the hip joint were clipped and aseptically prepared by using

alcohol. For bone marrow aspiration from iliac crest, the greater prominence of the wing of ilium bone were palpated. A small tap incision was made with the help of surgical blade (no.15) over intended site to make easy penetration of 13 gauze Jamshidi biopsy needle in the ilium bone. The needle was introduced through the skin incision into the iliac crest. Once the needle touched with the wing of ilium bone, the needle was advanced further into the marrow cavity by continuously rotating it slowly. On reaching marrow cavity, stylet was removed and a 10 ml disposable syringe containing 0.5 ml heparin (5000 IU/ml) was placed onto the end of Jamshidi needle. Five milliliter bone marrow was aspirated from the iliac crest in a 10 ml disposable syringe under negative pressure (Fig. 1a). Bone marrow aspirate was transferred to pre-sterilized 15ml centrifuge tube and diluted with 10ml of RPMI-1640 (Roswell park memorial institute) medium. The aspirate was mixed well with the medium using a pipette. The diluted aspirate was distributed equally and gently layered over histopaque (1.077g/ml) in two centrifuge tube, each containing 3ml histopaque at the bottom. The tube was centrifuged at 2000 rpm for 30 minutes. Translucent ring containing nucleated cells was harvested with the help of pipette and washed twice in RPMI-1640 medium at 1000 rpm for 10 minutes and cell pellet was resuspended in 0.5 ml RPMI-1640 and kept at room temperature till implantation (Fig. 1c & d) (Granthos, and Zannettino, 2008; Harris *et al.,* 2004).

Bone marrow nucleated cell count and test of cell viability

The cell number were assessed by counting the cells using a hemocytometer under a light microscope and viability of BMNCs was tested using Trypan blue dye exclusion (Fig. 1e & f).

Evaluation of viability by Trypan blue exclusion test

The viability of mesenchymal stem cells was assessed by 'trypan blue exclusion test' using trypan blue dye (Strober, 2001). Trypan blue solution (Himedia TCL046) was filtered and 10µl of the single cell suspension was taken in eppendorf tube and mixed with 10µl of 0.4% trypan blue dye solution (1:1 ratio, dilution factor 2). After keeping for 5 min at room temperature, 10µl of the suspension was loaded into hemocytometer chamber. The cells focused at 40x under a microscope and cells in the four WBC squares were counted. The viable (unstained) and non-viable (blue stained) cells were counted separately. The number of the cells per ml and percentage of the viable cells were given by the formula (Gunetti *et al*., 2012)

Number of the cells per ml = (Total no. of cells/4) x dilution factor x 10^4

Viable cell percent = $\frac{No.\ of\ \ the\ viable\ cells\ per\ ml}{Total\ no.of\ cells\ per\ ml}$ x 100

Preparation of Activated Platelets

A 5 ml of whole blood were collected from saphenous vein in tubes that contain sodium citrate anticoagulant. A double centrifugation process of PRP separation was used. The first spin was performed at 1500 rpm for 5 minutes to separate RBCs from the remaining whole blood volume for the production of pure PRP (P-PRP), supernatant plasma excluding buffy coat was aspirated and then transferred to an empty sterile tube containing 5ml RPMI medium. The second spin was performed at 1000 rpm for 10 minutes, the supernatant plasma was discarded and final volume of 0.25ml of RPMI containing concentrate plasma was activated by adding 0.2ml of 10% calcium gluconate with slight modification (Mazzoca *et al.,* 2012). The platelets were counted manually using the Neubauer's chamber (Fig. 1g & h).

The isolated BMNCs suspended in 1.0 ml RPMI were used for treatment under group B whereas 0.5 ml activated platelets mixed with 0.5 ml RPMI and BMNCs were used for treatment in group C. The ultrasound guided injection of RPMI, BMNCs or BMNCs plus activated platelets were given in the hip joint to get accuracy of hip joint injection (Fig. 2a, b & c).

Experimental design

Eighteen dogs of either sex diagnosed for hip dysplasia by radiology were randomly distributed into three groups A (Control) fed with nutraceuticals (Canitone Joint Support Virbac Animal Health India Pvt. Ltd.) for three months and intra-articular RPMI once, group B treated with BMNCs (Mean number of 4.35 (± 0.07) x 10^6) suspended in 0.5 ml RPMI once and group C with 0.5 ml activated platelets (Mean number of 2.54 (±0.12) x 10^8) suspended in 0.5ml RPMI plus BMNCs (Mean number of 4.35 (± 0.07) x 10^6) suspended in 0.5 ml RPMI that were mixed together before implantation once as per designed below.

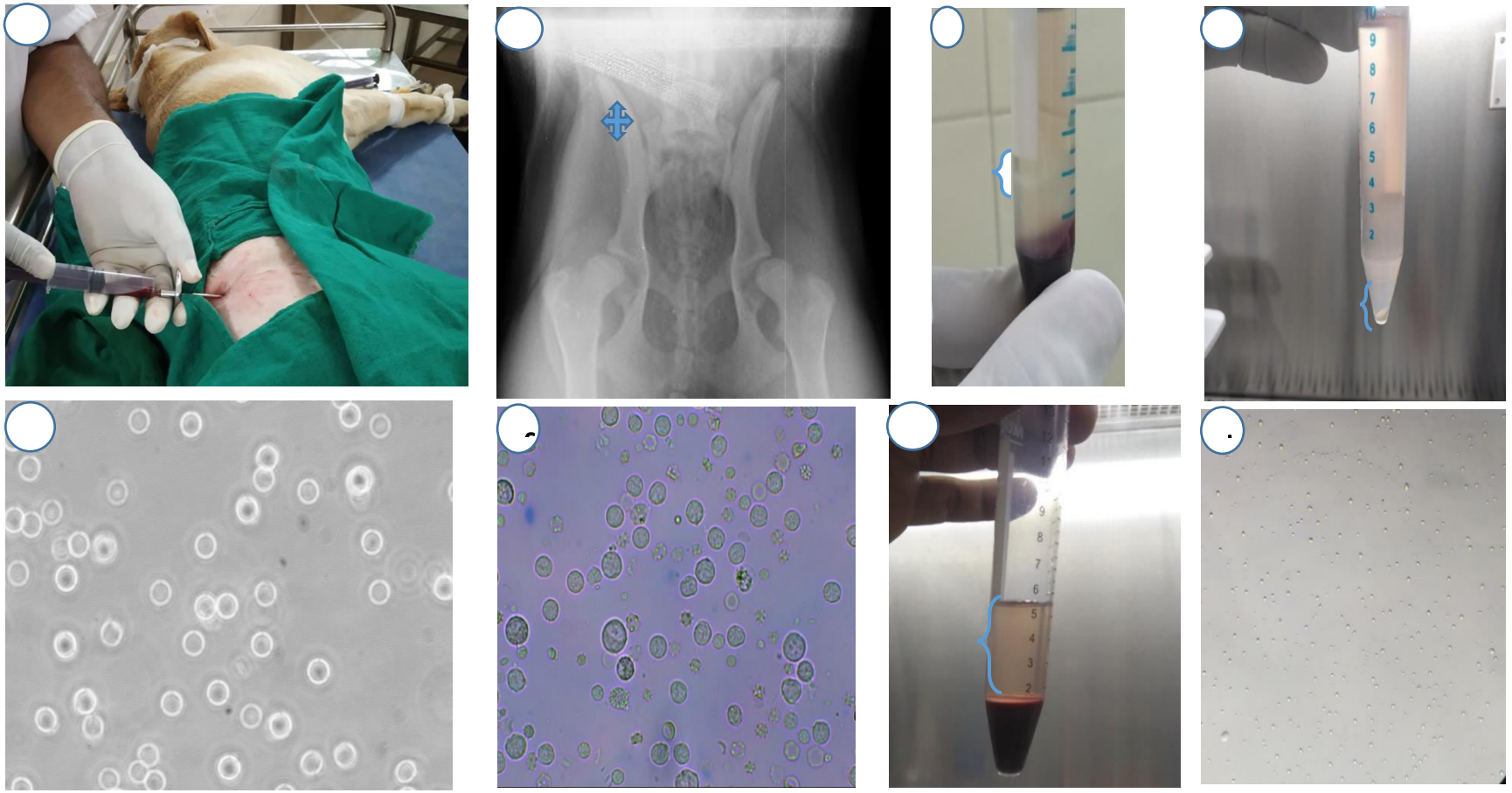

Fig. 1:Bone marrow mono-nuclear cells (BMNCs) and Platelets isolation (a) Bone marrow collection from iliac crest of a dog with Jamshidi biopsy needle, (b) Radiographic image of ilium the site for bone marrow collection ( ), (c) Translucent ring showing BMNCs after centrifugation, (d) BMNCs pellets ready for implantation, (e) Isolated uncultured BMNCs under microscope 40x, (f) Cell viability test (Trypan blue dye exclusion) dead cells having blue stain, (g) Plasma containing platelets. (h) Platelets under microscope 10x.

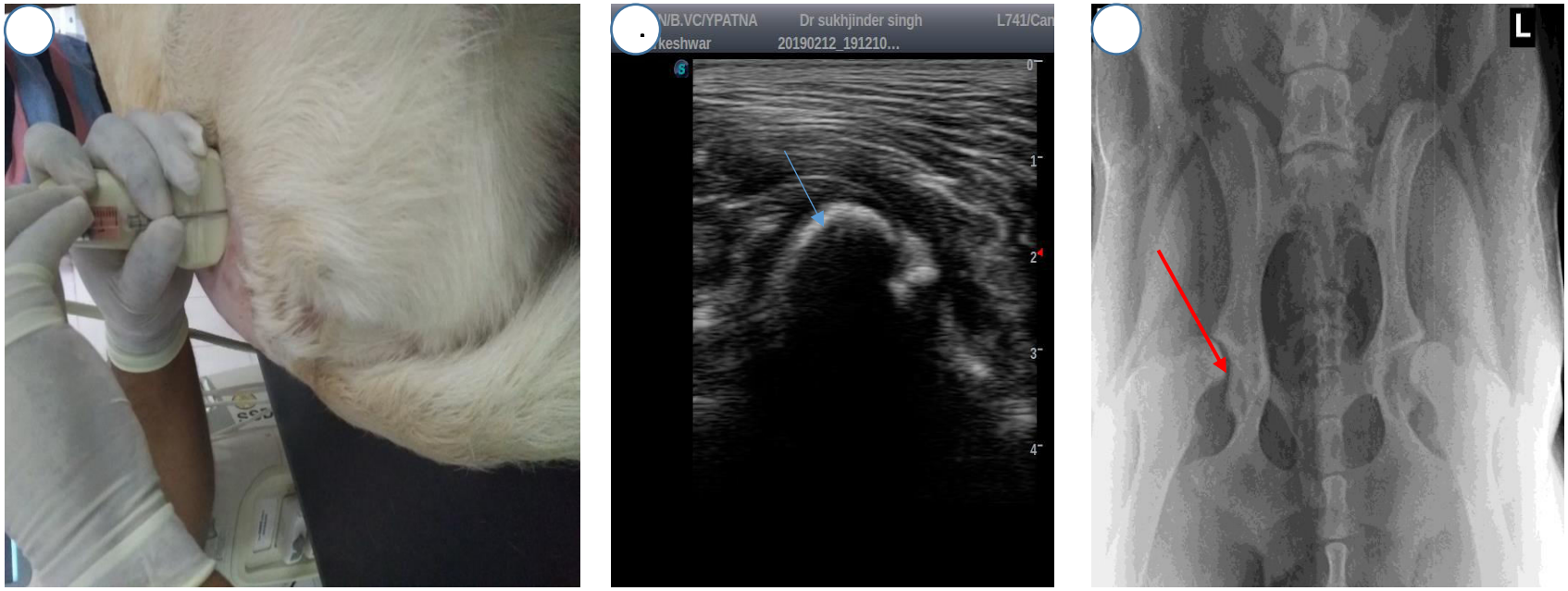

Fig. 2: (a) Ultrasound guided intra articular implantation of BMNCs, (b) Ultrasonography showing joint space and hyperechoic femoral head ( ), (c) Radiographic image showing joint space for intra articular BMNCs implantation ( ).

Groups	Treatment	Route of administration	No. of animals under treatment
A	Nutraceuticals+ RPMI (1.0 ml)	Orally (3M)+ Intra-articular (once)	6
B	Autologous Uncultured BMNCs suspended in 0.5ml RPMI	Intra-articular (once)	6
C	Autologous Uncultured BMNCs + Activated Platelets 0.5 ml each	Intra-articular (once)	6

3M: Three Months

The viability of cells were 95.89 percent (%) at the time of implantation. All animal under treatment groups were given Gabapentin @ 10 mg/kg BW for 10 days and meloxicam @ 0.2mg/kg body weight on days first.

Observations

Pre and Post treatment animals were evaluated at 4 weeks interval for minimally twelve weeks. Grading for pain, lameness, ability to jump, ability to climb stairs, Ortolani test, radiographically for norberg angle, Percent femoral head coverage, and distraction index as well as for C- reactive protein and oxidative stress parameters were recorded as per standard protocols.

Clinical Scoring System for assessing dogs

The severity of clinical signs was measured by using an ordinal scoring system which included pain on palpation, the ability to jump and climb stairs, lameness, and stiffness of movements (Nganvongpanit *et al.,* 2013)

Table no. 1: Clinical score for pain assessment.

Criterion	Grade	Clinical evaluation
Pain on palpation	0	No signs of pain on palpation of affected joint
	1	Slight signs of pain on palpation of the affected joint, the dog turns its head in recognition
	2	Moderate signs of pain on palpation of the affected joint, the dog pulls the limb as a defense reaction
	3	Severe signs pain on palpation, the dog vocalizes or becomes aggressive
	4	The dog does not allow palpation

Table no. 2: Clinical score for lameness assessment.

Criterion	Grade	Clinical evaluation
lameness	0	Normal, no lameness
	1	Mild lameness, not very difficult to move
	2	Clear lameness, not moving freely
	3	Obvious lameness when walking
	4	Severe lameness preventing the dog from supporting weight on the affected limb

Table no. 3: Clinical score for assessment of ability to jump.

Criterion	Grade	Clinical evaluation
Ability to jump	0	Jumps normally
	1	Jumps with care
	2	Jumps with some difficulty
	3	Jumps or rises with great difficulty
	4	Does not try because of difficulty / pain

Table no. 4: Clinical score for assessment of ability to climb stairs.

Criterion	Grade	Clinical evaluation
Ability to climb stairs	0	Goes up and down the stairs normally
	1	Slightly careful, uses both paws successively
	2	Sometimes uses both feet at the same time, evidently does not move freely
	3	Goes up the stairs like a rabbit at all times, goes up the stairs with great difficulty
	4	Does not try to climb because of the difficulty/pain

Ortolani test

The Ortolani test were performed by placing the dogs in lateral recumbency with the affected limb kept on upper most position by standing behind the dog facing towards the pelvis.

In lateral recumbency the femur was positioned at 90º to the long axis of pelvis and stifles were grasped with the palm over the patella. Simultaneously pressure was applied gently, but firmly, to the stifle to push femur towards hip joint to elicit subluxation (Fig. 3a & b). The stifles were then slowly abducted to bring the luxated femur head in acetabulum of hip joint. Dogs were consider to have a positive Ortolani sign if an audible "click" was observed during hip joint abduction (Fig. 3c & d) (Ortolani, 1976).

Radiography

The ventro-dorsal leg extended radiographic view (OFA view) were taken in awake animals. In non-cooperative animals, sedation were given using Glycopyrrolate (@ 0.01 mg/kg body weight IM) after five minutes xylazine (@ 1 mg/kg body weight IM) combination. The dogs were placed into supine position and the hind limbs were pulled straight back until the stifle and hocks get fully extended. The limbs then were adducted until the femurs became parallel to each other. Both the femur bone was rotated medially (inward) until the patella appeared cantered dorsally (Fig. 4a & b) (Farese *et al.,* 1998). Another radiograph was also taken with distracting object placed between the femur bones to record distraction view of hip joint (Fig. 4e).

Norberg angle (Morgan and Stephens, 1985) and Percentage femoral head coverage was recorded from normal radiograph whereas distraction index was recorded from radiographs taken on distraction view.

Norberg angle

The Norberg angle measurements were performed by using inbuilt digimizer software in the iCRo 3600 digital X-ray machine. The Norberg angle was measured in degrees between a line drawn to connect the centres of both femoral heads and a line connecting the center of the femoral head and the craniolateral aspect of the ipsilateral acetabular rim (Fig. 4c) (Henrigson *et al.,* 1966; Comhaire and Schoonjans, 2011). A Norberg angle of greater than 105° was considered to be normal. Norberg angles less than 105° were consistent with hip laxity as scored (Willis, 1997).

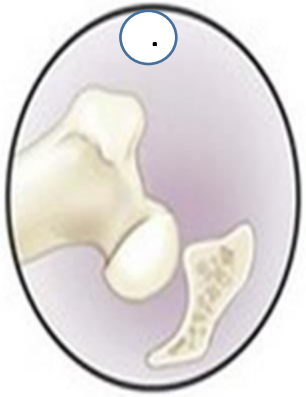

Fig. 3: Ortolani test (a) pressure applied along the axis of femur (arrow), (b) subluxation of femoral head from acetabulum, (c) Abduction of the femur by maintaining pressure, (d) reposition of femoral head back into acetabulum with palpable click.

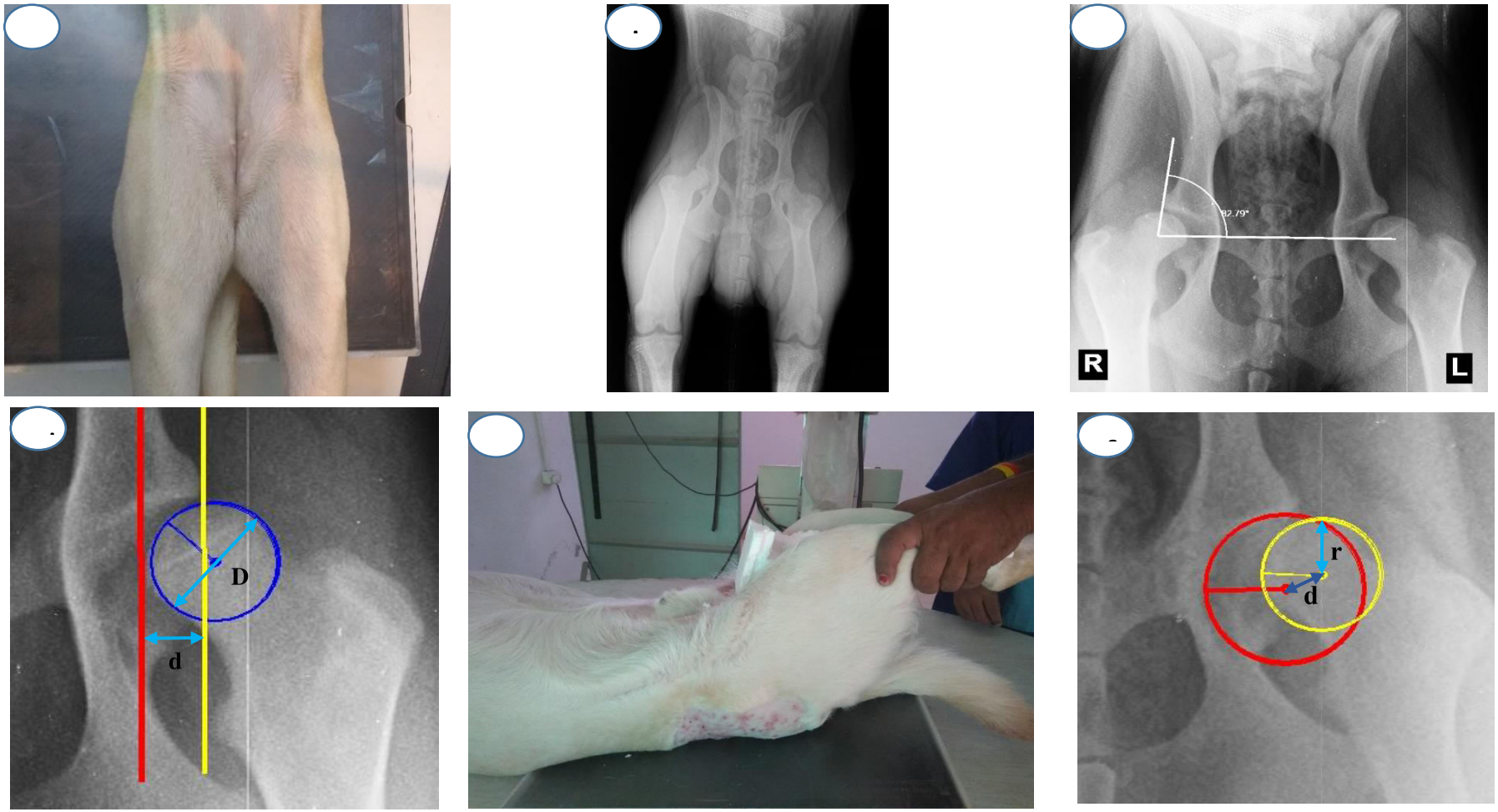

Fig. 4: Showing (a) Positioning of animal in ventro-dorsal view, (b) Ventro-dorsal view radiograph of pelvis, (c) Norberg angle, (d) Percent femoral head coverage (d), (e) Positioning of animal for radiography in distraction view, (f) Distraction index measurement on radiograph.

Table No. 5: Radiographic score for assessment of Norberg angle. (Fluckiger, 2007)

GRADE	CONDITION	SCORE	EVALUATION
A	No signs of Hip Dysplasia	**0**	▪ Femoral head and the acetabulum will be congruent. ▪ The craniolateral acetabular rim will appears sharp and slightly rounded. ▪ The joint space will be narrow and even. ▪ The Norberg angle will be about 105°. ▪ In excellent hip joints the craniolateral rim will encircle the femoral head somewhat more in caudolateral direction.
B	Near normal hip joints	**1**	▪ The femoral head and the acetabulum may be slightly incongruent and the Norberg angle will be about 105° or ▪ The femoral head and the acetabulum may be congruent and the Norberg angle will be less than 105°.
C	Mild hip dysplasia	**2**	▪ The femoral head and the acetabulum will be incongruent, ▪ the Norberg angle will be about 100° ▪ There will be slight flattening of the craniolateral acetabular rim. ▪ Not more than slight signs of osteoarthrosis on the cranial, caudal, or dorsal acetabular edge or on the femoral head and neck will be present.
D	Moderate hip dysplasia	**3**	▪ There will be obvious incongruity between the femoral head and the acetabulum with subluxation. ▪ The Norberg angle will be more than 90°

			▪ Flattening of the craniolateral rim and/or osteoarthrotic signs will be present.
E	Severe Hip Dysplasia	**4**	▪ Marked dysplastic changes of the hip joints, such as luxation or distinct subluxation will be present. ▪ The Norberg angle will be less than 90°. ▪ Flattening of the cranial acetabular edge, deformation of the femoral head (mushroom shaped, flattening) or other signs of osteoarthrosis will be noted.

Percent femoral head coverage (PFHC)

For femoral head coverage calculation, the digimizer software (version 5.3.5) was used to draw straight line from cranial acetabular rim towards caudal acetabular rim then a parallel line was drawn on the inner aspect of femoral head, then the distance between line drawn on inner femoral head and the line drawn from cranial acetabular rim was calculated (d). The distance obtain was divided by the diameter of femoral head (D) and then multiply by 100. This gave the value of percent femoral head coverage (Fig. 4d) (Tomlinson and Johnson, 2000). Normal joint overlap was considered to be 50% with values less than this being consistent with joint incongruity (Smith *et al.,* 2012).

Formula used to calculate percent femoral head coverage:

$$\% \text{ FHC} = d/D*100$$

Distraction index

The relative degree of femoral head displacement from the acetabulum were quantified by calculating the distraction index (DI) from radiographic view. The stress radiographic method of measuring passive hip laxity was done under sedation. Dogs were pre-medicated with

glycopyrrolate @ 0.01mg/kg body weight I/M, 10 mins later by xylazine @ 1mg/kg body weight I/M.

The distraction view of hip was made by placing sedated dogs in supine position, with the hips in neutral orientation (femur approximately perpendicular to the radiographic table), prior to applying stress to the hip. The foam was used as distraction device placed between the legs and firmly pressing it down to pelvis. While grasping hocks, the knees were pushed together using device as fulcrum to improve a lateral distractive force on the hip joints (Fig. 4e). Distraction was maintained for short period of time sufficient to permit exposure of radiographic film.

The digimizer software (version 5.3.5) was used to draw the circles on femoral head and one on acetabulum of respected side, then a line was drawn from femoral head center to the acetabular center. The distance between these two centres were measured as distraction length. The distraction index was calculated by dividing the distraction length (d) by the radius of circle (r) used for femoral head (Fig. 4f) (Ginja *et al.,* 2007).

Formula used to calculate distraction index.

$$\mathbf{DI = d/r}$$

Haemato-Biochemical tests

After clinical examination, a total of 5 ml blood (4 ml in plain vial and 1 ml in EDTA) was collected from the peripheral vein for the estimation of different Haemato-biochemical parameters. Blood collected in anticoagulant was used for complete blood count (CBC) estimation and serum were separated for C-reactive protein estimation and oxidative stress parameters.

Complete Blood Count (CBC)

The heparinized blood was utilized for the estimation of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count, total leukocyte count (TLC) and differential leukocyte count (DLC) using standard procedures.

Haemoglobin (Hb)

Haemoglobin was estimated by cyanomethemoglobin method using spectrophotometer as per standard protocol.

Total Leukocyte Count (TLC)

The number of leukocytes are calculated per µl of blood using hemocytometer as per Becton-Dickinson (1998).

Total Erythrocyte Count (TEC)

Estimated by hemocytometer as described by Math *et al.* 2016.

Packed Cell Volume (PCV)

PCV was estimated by micro hematocrit technique as described by (Murray *et al.,* 1983) and the volume was expressed in volume percent (%).

Different Leukocyte Count (DLC)

Blood smears was prepared from fresh blood and stain with Wright's stain (Lucas and Jamroz, 1974) percent of different leucocyte were determined by examining the smear under the oil immersion of light microscope.

C-reactive protein

Quantitative determination of C- reactive protein from serum was done by Turbidimetric immunoassay kit (TURBILYTE –CRP, Tulip Diagnostics Pvt. Ltd.).

Oxidative Stress parameters

Estimation of Lipid peroxidation (LPO)

Membrane per oxidative damage of RBCs in serum due to free radicals were determined in terms of malondialdehyde (MDA) production by standard protocol Stock and Dormandy, 1971

Estimation of Superoxide dismutase (SOD)

Superoxide dismutase activities in serum were estimated as per the method described by Madesh and Balasubramanian (1998). It involves generation of superoxide by Pyrogallol autoxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye MTT 3-(4-5 dimethyl thiazol 2-xl) 2, 5 diphenyl tetrazolium bromide] to its formazan, measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to

solubilize the formazan formed. The colour evolved is stable for many hours and is expressed as SOD Units (one unit of SOD is the amount (µg) of serum required to inhibit the MTT reduction by 50 %). The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helped to solubilize the formazan formed and colour evolved was stable for many hours.

Estimation of Catalase (CAT)

Activities of catalase enzymes were estimated by spectrophotometric method as described by Bergmeyer (1983) and were expressed as mM H_2O_2 utilized /min /mg serum.

Glutathione peroxidase (GPx)

Quantitative determination of C- reactive protein from serum was done using commercial kit (GRSA, Sigma Aldrich)

Data Analysis:

The means of parameteric observations was compared by analysis of variance (ANOVA) as described by Snedecor and Cochran (1989), while non-parametric observations was compared using a kruskal-wallis one-way ANOVA (Petric and Watson, 2006). For each treatment comparison, differences between groups was considered significant at $P<0.05$.

4 RESULTS

A total of 217 cases of canine of different breeds, age and sex having problem for hind quarter affections had been recorded during the period from February 2017 to August 2019 and radiographed for pelvic bone in ventro-dorsal view at the Department of Surgery and Radiology, Bihar Veterinary College, Patna. The ventro-dorsal views of the recorded pelvic radiographs were screened for hip dysplasia based on the visible radiographic changes present in the hip joints, out of 217 recorded cases during this period 109 cases (51 %) were diagnosed for hip dysplasia and remaining 108 cases (49%) were of different affection related to pelvic bone and neurological disorders. Among the cases diagnosed for canine hip dysplasia males were forty-three percent (47/109; 43%) whereas, rest fifty-seven percent were of female (62/109; 57%) (Fig. 5 & 6).

Animals

For this study a total 18 dogs diagnosed for hip dysplasia based on the visible radiographic changes in the hip joint at the time of admission were included. The descriptions of these animals are shown in Table no. 6.

Breeds

Out of 18 dogs selected for treatment under this study for hip dysplasia Labrador retriever were (11/18; 61 %) followed by Rottweiler (3/ 18; 17%), German shepherd (2/18; 11%) and Golden retriever (2/18; 11%) respectively (Fig. 7).

Age

The age of the animals suffering from hip dysplasia varied from 4 to 24 months (Median age 10 months) with the maximum numbers of animals recorded between 6 to 12 months (12/18; 67 %) of age groups followed by 0-6 months (2/18; 11%), 12-18 months (2/18; 11%) and 18-24 months (2/18; 11%) respectively (Fig. 8).

Table no. 6: Detail history of all eighteen dogs suffering from hip dysplasia

Case no.	Breed	Age	Sex	Body weight in Kg	Type of breed	Purpose of dog	Duration of lameness	Difficulty in climbing stairs
1.	German shepherd	15M	Female	45	Medium	Non-working	4 months	Yes
2.	German shepherd	15M	Female	45	Medium	Non-working	4 months	Yes
3.	Labrador retriever	7M	Male	14	Medium	Non-working	10 days	No
4.	Rottweiler	5M	Female	22	Medium	Non-working	3 days	Yes
5.	Rottweiler	8M	Female	27.5	Medium	Non-working	4 days	No
6.	Rottweiler	8M	Female	27.5	Medium	Non-working	4 days	No
7.	Golden retriever	9M	Female	23.9	Medium	Non-working	1 month	Yes
8.	Labrador retriever	2Y	Male	35	Medium	Non-working	12 days	Yes
9.	Labrador retriever	2Y	Male	35	Medium	Non-working	12 days	Yes
10.	Labrador retriever	1Y	Female	26	Medium	Working	5 days	Yes
11.	Labrador retriever	8M	Male	29	Medium	Non-working	15 days	No
12.	Labrador retriever	8M	Male	29	Medium	Non-working	15 days	No
13.	Labrador retriever	4M	Female	12	Medium	Non-working	20 days	Yes
14.	Golden retriever	1Y	Female	27	Medium	Non-working	1 month	Yes
15.	Labrador retriever	11M	Male	24	Medium	Non-working	7 days	No
16.	Labrador retriever	11M	Male	24	Medium	Non-working	7 days	No
17.	Labrador retriever	7M	Male	26	Medium	Non-working	3 days	Yes
18.	Labrador retriever	7M	Male	26	Medium	Non-working	3 days	Yes

M= month, Y= year

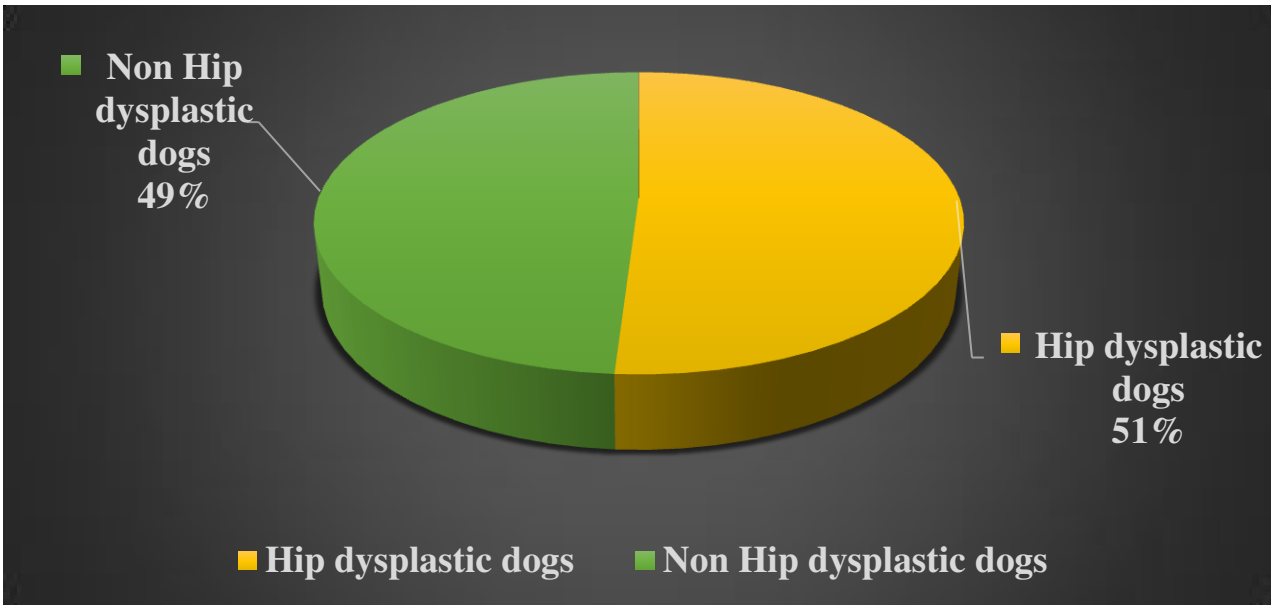

Fig. 5: Percent distribution of Canine hip dysplasia out of 217 cases of dogs suffering from hindlimb(s) affections.

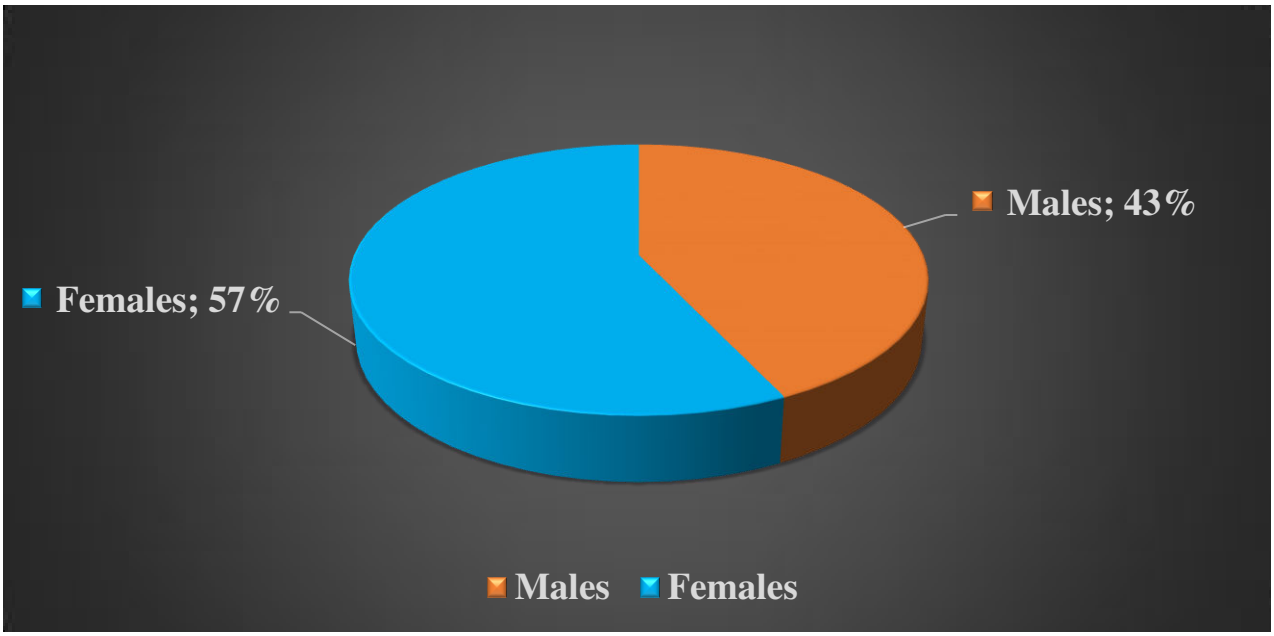

Fig. 6: Sex wise distribution of hip dysplastic dogs out of 109 cases.

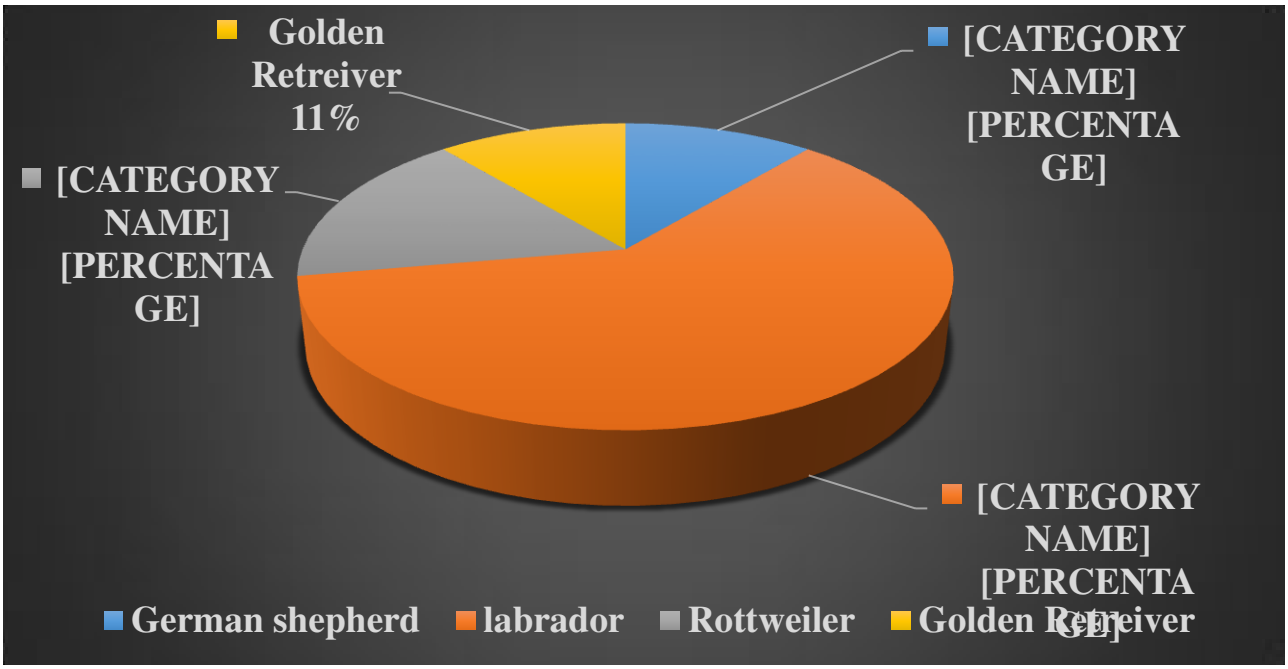

Fig. 7: Breed wise distribution of dogs suffering from hip dysplasia.

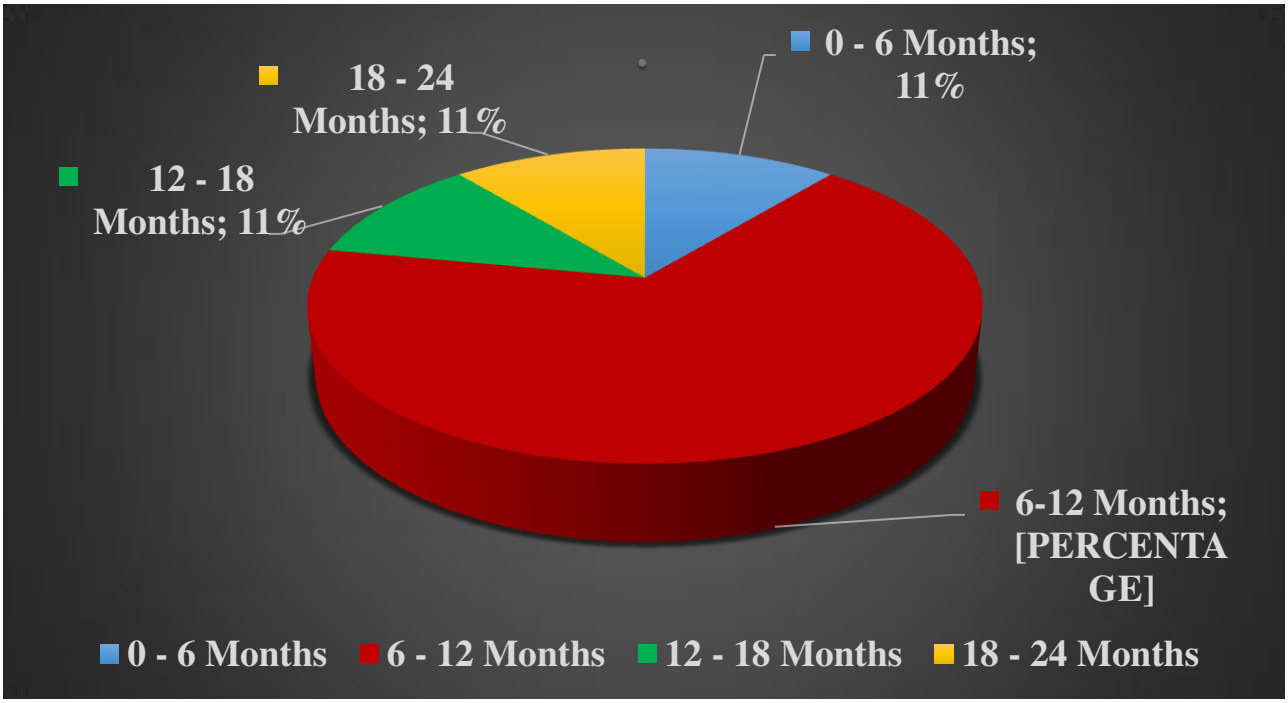

Fig. 8: Age wise distribution of dogs suffering from hip dysplasia.

Sex

Accidentally there were equal number of males nine (9/18; 50%) and nine females (9/18; 50%) (Fig. 9).

Body weight

The body weight of the animals varied from 12 to 45 kg (median wt. 26 kg) with maximum numbers of animals were belongs to 20-30 Kg (12/18; 67%), followed by 40-50 Kg (2/18; 11%), 30-40 kg (2/18;11%), and less than 20 Kg (2/18;11%) respectively (Fig. 10).

Type of breeds

The dogs were categorized on the basis of their body size. All the animals were medium sized breeds (18/18; 100%).

Duration of lameness

The animals were brought with the complaint for pain while sitting and getting up, showed lameness while walking, swaying hind quarter and reluctant to climb staircase. The duration for these clinical signs in animals suffering as reported by the owners were varied from 3 days to 4 months. The maximum number of cases brought to treatment for these clinical signs were from 0-10 days (9/18; 50%) followed by 10-20 days (5/18; 28%), 20-30 days (2/18; 11%) and 120 days (2/18; 11%) respectively (Fig. 11).

Purpose of dog

Seventeen dogs were belonging to non-working (17/18; 99%) whereas, rest one was as working (1/18; 1%) in police force.

Difficulty in climbing stairs

The difficulty in climbing the stairs were recorded in the history as climbing puts pressure on the hip joints and animals reluctant to climb due to pain. Out of eighteen dogs, eleven animals (11/18; 61.11) showed reluctance to climb the staircase whereas, rest 7 dogs (7/18; 39%) were normally climbed the stairs.

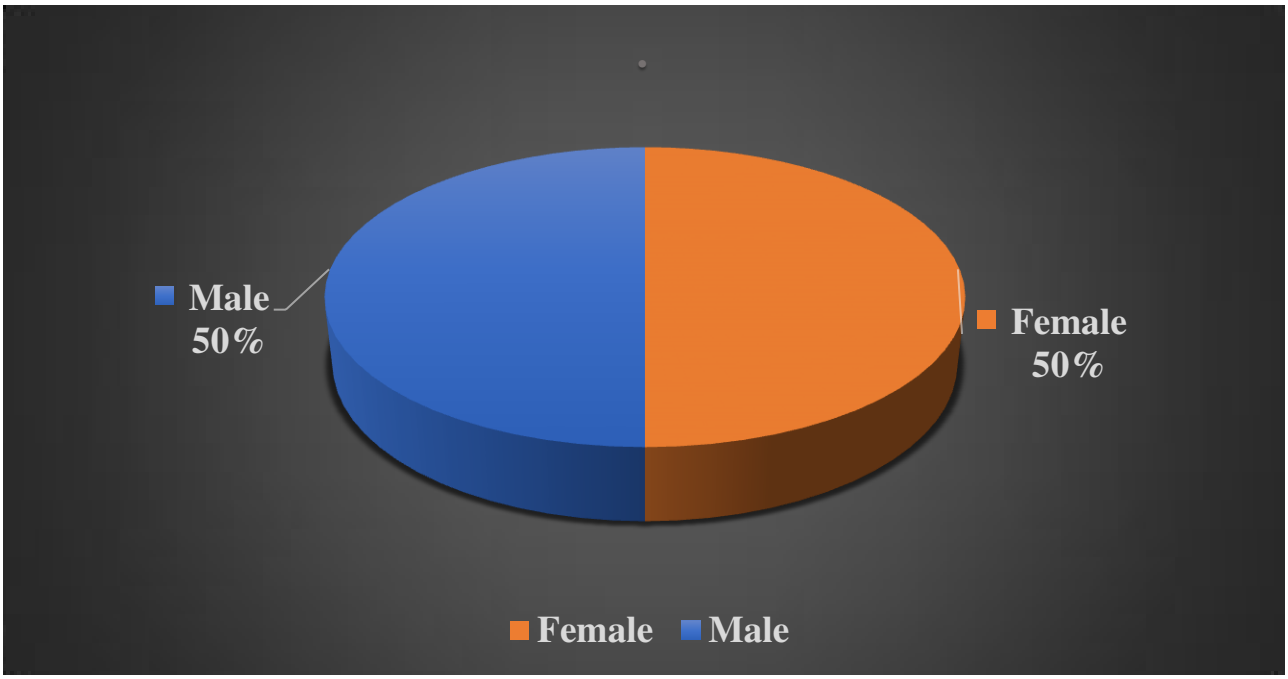

Fig. 9: Sex wise distribution of dogs suffering from hip dysplasia.

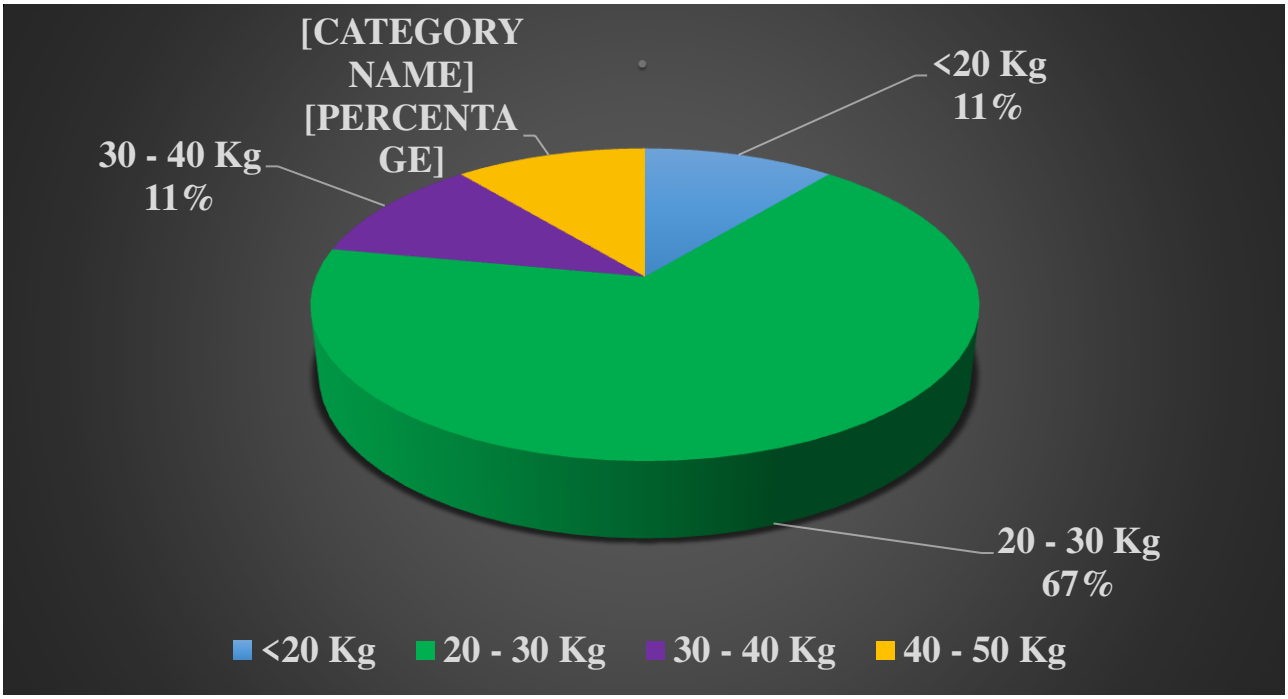

Fig. 10: Body weight wise distribution of dogs suffering from hip dysplasia.

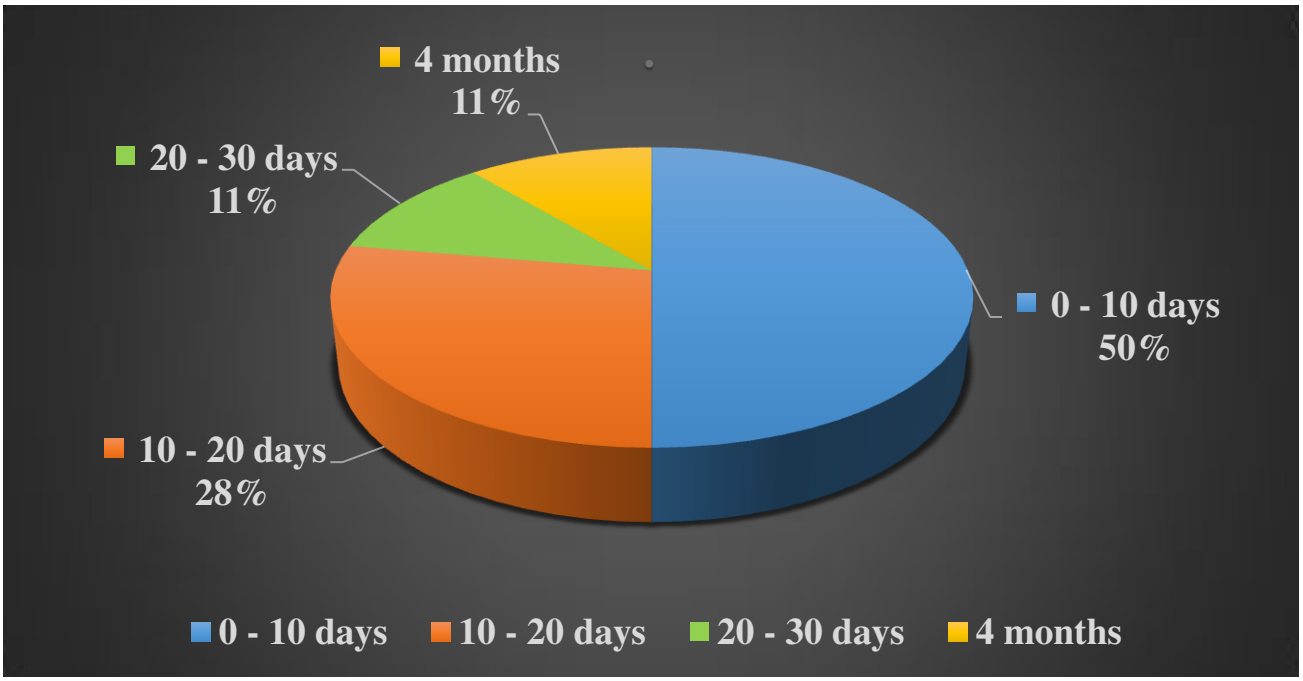

Fig. 11: Percent distribution of duration of lameness in dogs suffering from hip dysplasia.

Type of floor

The types of floor where animals used to keep were also recorded as slippery to rough to predict the chances of slipped injury that may predisposed to injury in hip joint. As per reported by the owners, thirteen animals (13/18: 72%) were kept on floor with tiles and remaining five (5/18; 28%) had plaster floor.

Vaccination and Deworming

All animals were vaccinated as per the scheduled for specific vaccine and dewormed with suitable anthelmintic agents.

Feeding habits

The feeds offered by the owners to their pets were also recorded and it had been observed that most of the animals (15/18; 83%) were fed upon a combination of two or more feeds that includes rice, milk, boiled eggs, curd and commercial food and rest (3/18; 17%) were strictly kept on vegetarian diet.

Physiological Parameters

The animals were examined for clinical parameters like rectal temperature, heart rates and respiratory rates and results of these parameters among the groups at different time intervals were as follows

Rectal temperature (°F)

The Mean ±SE values of rectal temperature of animals in different groups at various time intervals are shown in Table no. 7, Fig. 12). The normal value of rectal temperature in small to large breeds of dogs varied from 100.2 °F to 102.5 °F. The rectal temperature values of animals among the groups A, B and C at different time intervals were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Table no. 7: Mean ± S.E. values of rectal temperature (°F) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	102.06 ± 0.13	102.01 ± 0.14	101.95 ± 0.12	101.75 ± 0.17
Group B	101.91 ± 0.17	101.93 ± 0.25	101.86 ± 0.25	101.21 ± 0.27
Group C	101.61 ± 0.39	101.76 ± 0.26	101.56 ± 0.26	101.78 ± 0.13

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

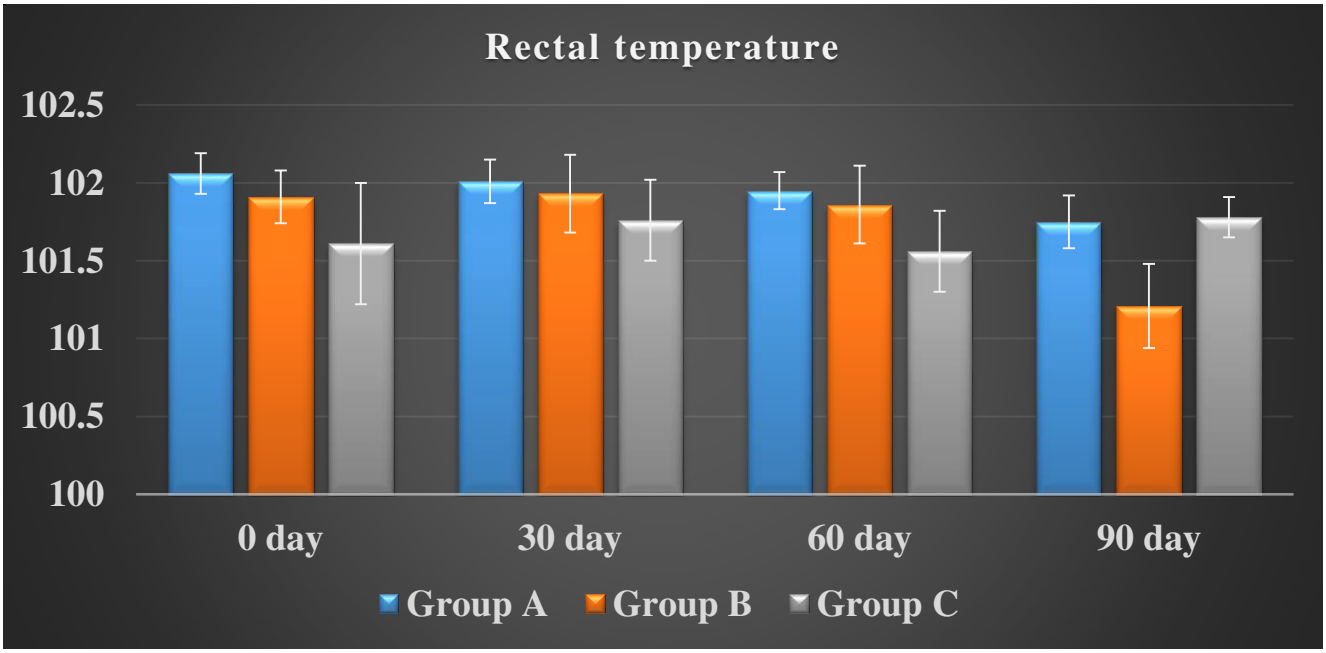

Fig. 12: Histogram showing Mean ± S.E. of rectal temperature (°F) of animals not significant in different groups at various time intervals

Heart rate (beats/min)

The Mean ±SE values of heart rates of animals in different groups at various time intervals are shown in (Table no. 8, Fig. 13). The normal value of heart rates in small to large breeds of dogs varied from 70 to 120 beats/min. The values of heart rates of animals among the groups A, B and C at different intervals of time were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Respiration rate (breaths/min)

The Mean ±SE values of respiration rates of animals in different groups at various time intervals are shown in (Table no, 9, Fig. 14). The normal value of respiration rates in small to large breeds of dogs ranges from 18 to 34 breaths/min. The respiration rate values of animals among the groups A, B and C at different intervals of time were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Colour of mucous membrane

Colour of mucous membrane of dog varies from pale (abnormal) to pinkish (normal) and congested (abnormal). The colour of the mucous membrane observed different animals in groups A, B and C at various time intervals were pinkish i.e. normal.

Clinical signs

In groups A, B and C, all the dogs exhibited signs of hind limb lameness, pain in hip joint, abnormal gait and difficulty in rising. Bunny hopping gait was noticed in one dog in group A. Swaying of back were noticed in three animals in group A, two in group B and one animal in group C. Partial weight bearing lameness were exhibited in two animals in group A and one each in groups B and C. Atrophy of thigh musculature was noticed in three animals in group A, two in group C and one in group B (Fig. 15a, b & c). Other clinical signs such as crossing of hind limbs while lying down and straightening of stifle and hock joint were not noticed.

Table no. 8: Mean ± S.E. values of heart rate (beats/min) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	98.50 ± 0.89	98.50 ± 0.42	98.66 ± 0.61	97.83 ± 0.30
Group B	98.66 ± 0.33	99.16 ± 0.30	99.33 ± 0.61	98.33 ± 0.55
Group C	99.50 ± 0.22	98.33 ± 0.48	99.50 ± 0.50	98.33 ± 0.33

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

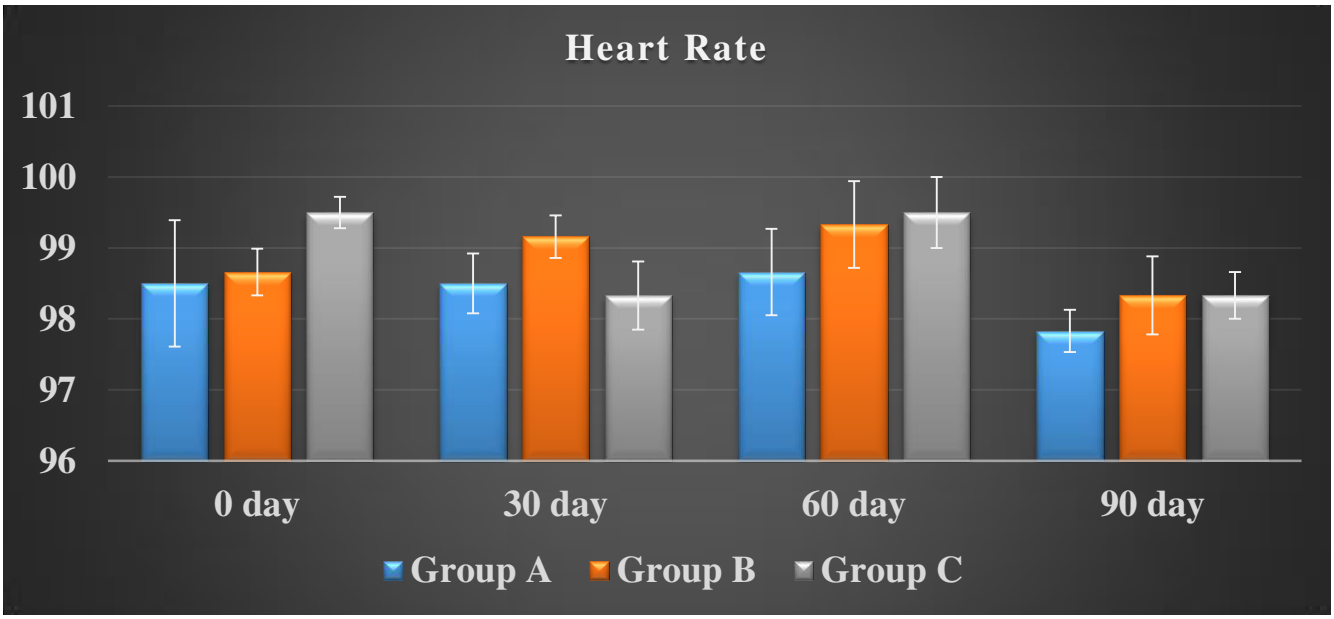

Fig. 13: Histogram showing Mean ± S.E. of heart rate (beats/min) of animals not significant in different groups at various time intervals

Table no. 9: Mean ± S.E. values of respiration rate (breaths/min) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	21.50 ± 0.56	21.00 ± 0.45	21.66 ± 0.50	21.50 ± 0.34
Group B	22.16 ± 0.30	21.33 ± 0.55	21.16 ± .40	21.66 ± 0.42
Group C	21.83 ± 0.48	21.33 ± 0.42	20.83 ± 0.30	20.83 ± 0.40

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

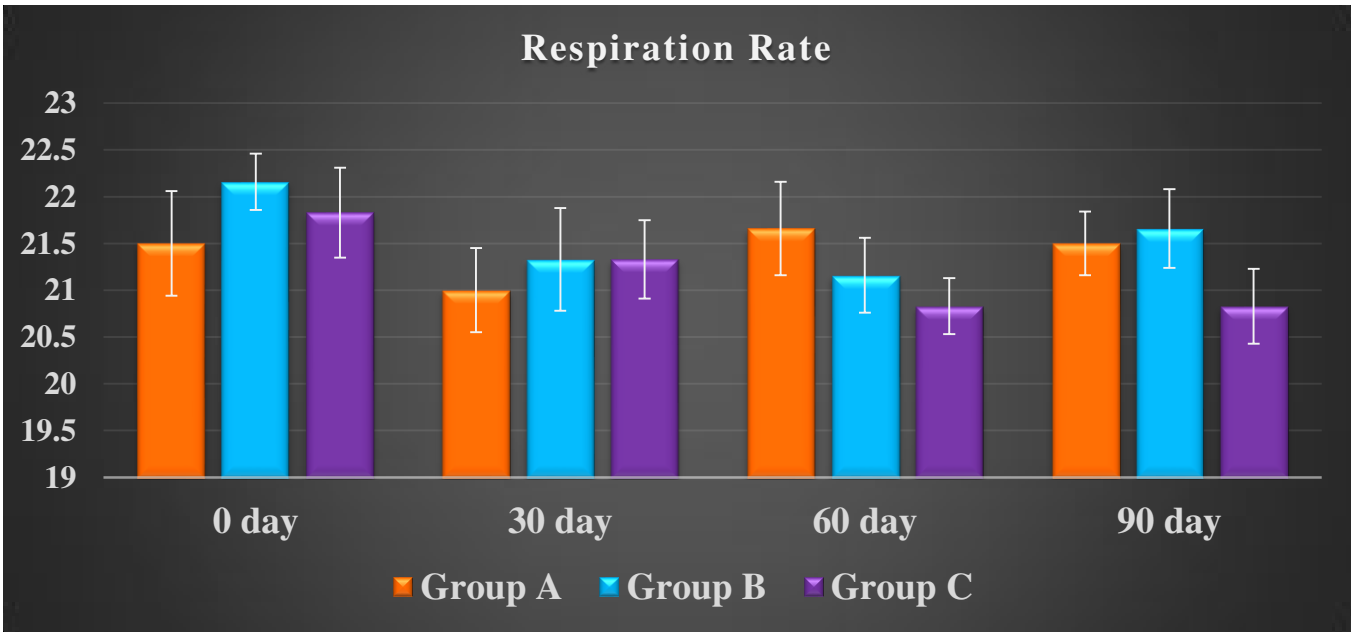

Fig. 14: Histogram showing Mean ± S.E. of respiration rate (breaths/min) of animals not significant in different groups at various time intervals.

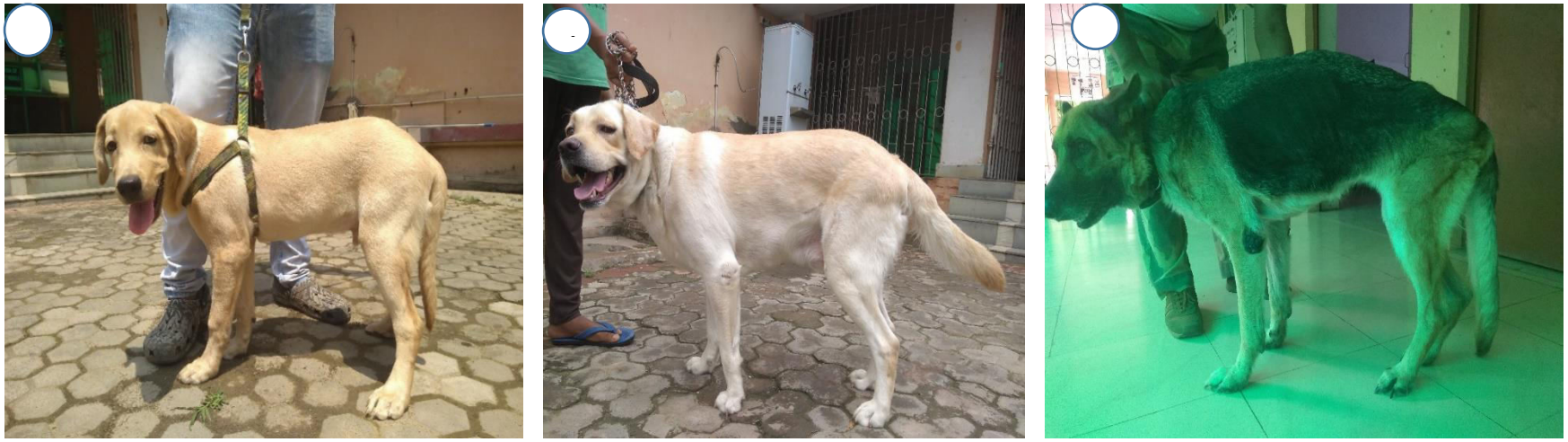

Fig. 15: Showing (a) mild (b) moderate and (c) severe thigh muscle atrophy in dogs suffering from canine hip dysplasia.

Score for assessing the clinical sign in dogs

Pain Score

The Mean ± SE values of scores for pain related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 10, Fig. 16). There were no significant differences in score value for pain observed among the groups A, B and C on days 0. On days 30^{th} and 60^{th} the score for pain in group C was significantly different from groups B and A. Though there were no significant differences observed between the groups B and A albeit lower value of score observed in group B as compared to A. On days 90^{th} the score value for pain were significantly differed amongst the groups A, B and C with slight pain in the animals of group B to complete abolished in pain in the animals of group C. The mean score for pain showed significantly different at respective intervals of time within the groups B and C whereas, in group A, the score for pain on days 30^{th}, 60^{th} and 90^{th} showed only significantly different from days 0. The improvement in the scores for pain in joint in respect to different intervals of time were best in the animals of group C followed by groups B and A.

Lameness score

The Mean ± SE values of scores for lameness related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 11, fig. 17). There were no significant differences in score value for lameness observed among the groups A, B and C on days 0. On days 30^{th}, the score for lameness in groups C and B were significantly different from group A. Though there were no significant difference observed between the groups B and C albeit lower value of score with improvement in the sign for lameness observed in the animals of group C (1.50 ± 0.22) as compared to B (1.83 ± 0.17). On days 60^{th} and 90^{th} the score for lameness in group C were significantly different from groups B which was also differ significantly from group A. In group A, the score value on days 90^{th} was significantly different from the respective intervals of time. In group B the lameness score on days 90^{th} was significantly different from rest of the days intervals. The score on days 30^{th} and 60^{th} were also differed significantly from days 0. Though there were no significant difference in score observed for lameness between days 30^{th} and 60^{th} in group B albeit lower score for lameness on days 60^{th}

(1.67 ± 0.21) noticed as compared to days 30th (1.83 ± 0.17). In group C the score value on days 60th and 90th differed significantly from rest

Table no. 10: Mean ± S.E. values of scores of animals for pain in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	2.50 ± 0.22^{a}	2.34 ± 0.21^{bx}	2.00 ± 0.00^{abx}	1.50 ± 0.22^{bx}
Group B	2.50 ± 0.22^{a}	2.00 ± 0.00^{bx}	1.50 ± 0.22^{cx}	1.00 ± 0.00^{dy}
Group C	2.17 ± 0.16^{a}	1.00 ± 0.00^{by}	0.50 ± 0.22^{cy}	0.00 ± 0.00^{dz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

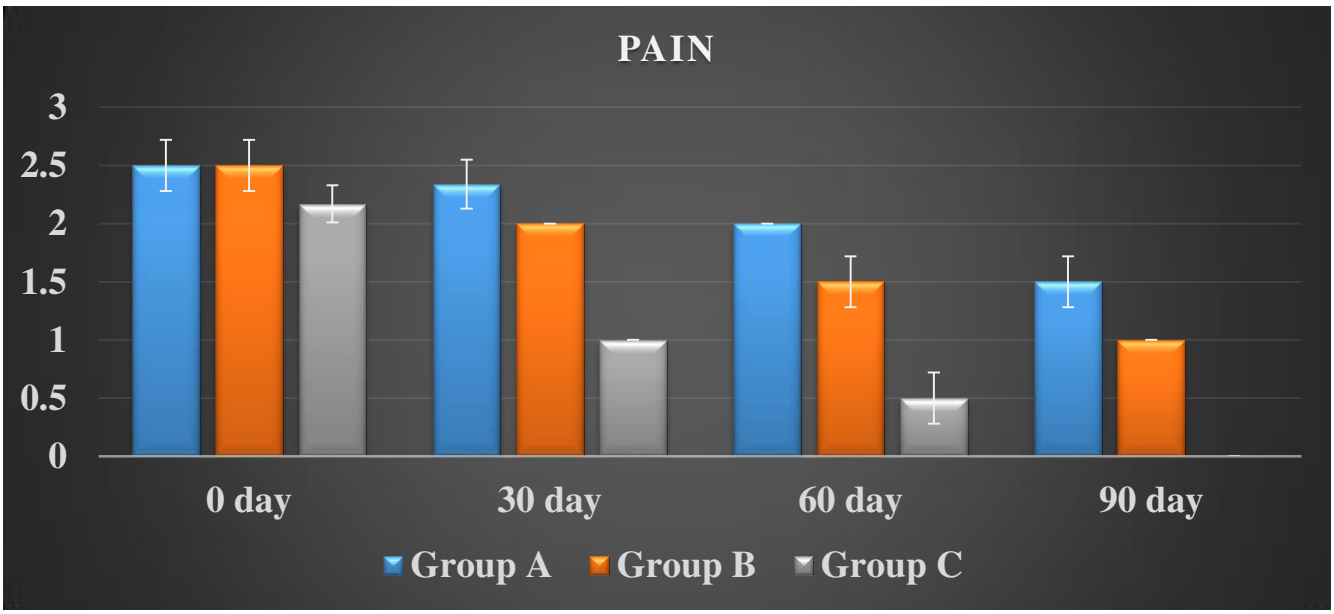

Fig. 16: Histogram showing Mean ± S.E. of scores of animals for pain in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).

Table no. 11: Mean ± S.E. values of scores of animals for Lameness in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	2.67 ± 0.21^{a}	2.50 ± 0.22^{abx}	2.33 ± 0.2^{abx}	2.00 ± 0.00^{cx}
Group B	2.83 ± 0.17^{a}	1.83 ± 0.17^{by}	1.67 ± 0.21^{by}	0.83 ± 0.17^{cy}
Group C	2.83 ± 0.30^{a}	1.50 ± 0.22^{by}	0.50 ± 0.22^{cz}	0.00 ± 0.00^{cz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

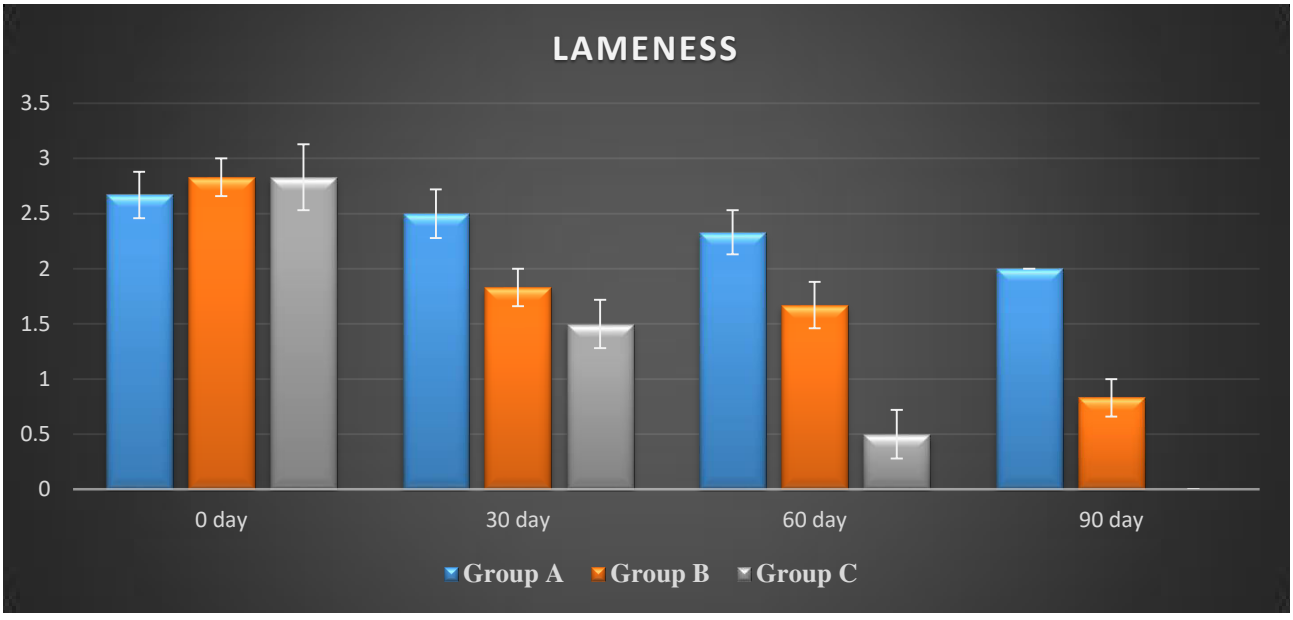

Fig. 17: Histogram showing Mean ± S.E. of scores of animals for Lameness in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).

of the time intervals and lameness score value on days 30^{th} (1.50 ± 0.22) also differed significantly from the mean score observed on 0^{th} day (2.83 ± 0.30). The score value amongst the groups at different intervals of time showed better improvement in clinical sign of lameness in group C followed by groups B and A.

Score for ability to jump

The Mean ±SE values of scores for ability to jump related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 12, Fig. 18). There were no significant differences in score value for ability to jump observed among the groups A, B and C on days 0. On 30^{th} day the score for ability to jump in group C was significantly different from groups B which was also differ significantly from group A. On days 60^{th} and 90^{th} the score for pain in group C were significantly different from groups B and A. Though there were no significant differences observed between the groups B and A albeit lower value of score observed in group B as compared to A in these particular intervals of time. In group A, there were no significant differences in score value for ability to jump observed at respective intervals of time. In group B, the score on days 30^{th}, 60^{th} and 90^{th} were also differed significantly from days 0. Though there were no significant difference in score observed for ability to jump between days 30^{th}, 60^{th} and 90^{th} albeit lower score for ability to jump on days 90^{th} (1.33 ± 0.21) noticed as compared to days 60^{th} (1.67 ± 0.21) followed by days 30^{th} (1.83 ± 0.17). In group C, significant difference in score values were observed for ability to jump on days 30^{th} and 90^{th} as compared to 0^{th} day. Though there were no significant difference observed on days 60^{th} albeit lower score for ability to jump on days 60^{th} (0.50 ± 0.22) noticed as compared to days 30^{th} (1.00 ± 0.00). The scores value amongst the groups at different intervals of time showed better improvement for ability to jump in group C followed by groups B and A.

Score for ability to climb stairs

The Mean ± SE values of scores for ability to climb stairs by the animals suffering from hip dysplasia in different groups at various intervals of time are shown in (Table no. 13, Fig. 19). There were no significant differences in score value for ability to climb stairs observed among the groups A, B and C on days 0, 30^{th} and 60^{th}. On days 90^{th} score values in groups C showed significantly different from group A. Though there were no significant difference in scores value

observed between the groups A and B similarly in groups C and B albeit lower value of score observed in group B (0.66 ± 0.21) as compared to A1.00 ± 0.44 and also the animals in group C did not find any reluctancy to climb stair in group C (0.00 ± 0.00) as compared to group B (0.66 ± 0.21). The score values to climb stairs within the group A and B did not show any significant difference various interval of time respectively. Though there were no significant difference observed in the score value within groups A and B albeit lower score for ability to climb stairs were observed on days 90th as compared to days 60th, 30th and 0 respectively. In group C, no significant difference in score observed for ability to climb stairs between days 30, 60 and 90 but on days 60 and 90 there were significant differences observed in the score value as compared to day 0. Though there were no significant difference in score between day 60 and 90 albeit there were slight difficulties in climbing stairs at days 60th to normal in climbing stairs observed on days 90th. Though there were no significant difference in score observed for ability to climb stairs between days 0 and 30 albeit lower score for ability to climb stairs on days 30th (0.83 ± 0.30) noticed as compared to days 0 (1.67 ± 0.61). The improvement in the scores for ability to climb stairs in respect to different intervals of time were best in the animals of group C followed by groups B and A.

Ortolani test

Ortolani test was performed in awake condition in 15 dogs, whereas in 3 dogs, Ortolani test as performed under Xylazine sedation @1 mg/ Kg body weight at 0th day, 30th day, 60th day and 90th day time interval. In Group A, days 0 all six dogs had showed positive sign for Ortolani test and further no improvement observed on subsequent intervals on days 30th, 60th and 90th. In Group B, on days 0 all six animals were positive for Ortolani sign and no improvement noticed on days 30th. On days 60th, out of six dogs, two were showed improvement and found negative for Ortolani's sign and remaining four animals did not show improvement i.e. showing positive sign for this test. On days 90th, improvement was noticed in all animals and found negative for Ortolani's sign. In Group C, on days 0 all six dogs exhibited positive Ortolani sign. On days 30th two animals found negative whereas rest four were positive for Ortolani's sign. On days 60th and 90th all animals were found negative and did not showed Ortolani's sign. The overall improvement in Ortolani's sign were better in group C followed by groups B and A.

Table no. 12: Mean ± S.E. values of scores of animals for ability to jump in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	2.50 ± 0.22	2.33 ± 0.21^{x}	2.17 ± 0.30^{x}	1.83 ± 0.40^{x}
Group B	2.83 ± 0.17^{a}	1.83 ± 0.17^{by}	1.67 ± 0.21^{bx}	1.33 ± 0.21^{bx}
Group C	2.33 ± 0.33^{a}	1.00 ± 0.00^{bz}	0.50 ± 0.22^{bcy}	0.00 ± 0.00^{cy}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

Fig. 18: Histogram showing Mean ± S.E. of scores of animals for ability to jump in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).

Table no. 13: Mean ± S.E. values of scores of animals for Ability to climb stairs in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	1.50 ± 0.67	1.33 ± 0.61	1.33 ± 0.61	1.00 ± 0.44^{x}
Group B	1.83 ± 0.60	1.33 ± 0.42	1.17 ± 0.40	0.66 ± 0.21^{xy}
Group C	1.67 ± 0.61^{a}	0.83 ± 0.30^{ab}	0.50 ± 0.22^{b}	0.00 ± 0.00^{by}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

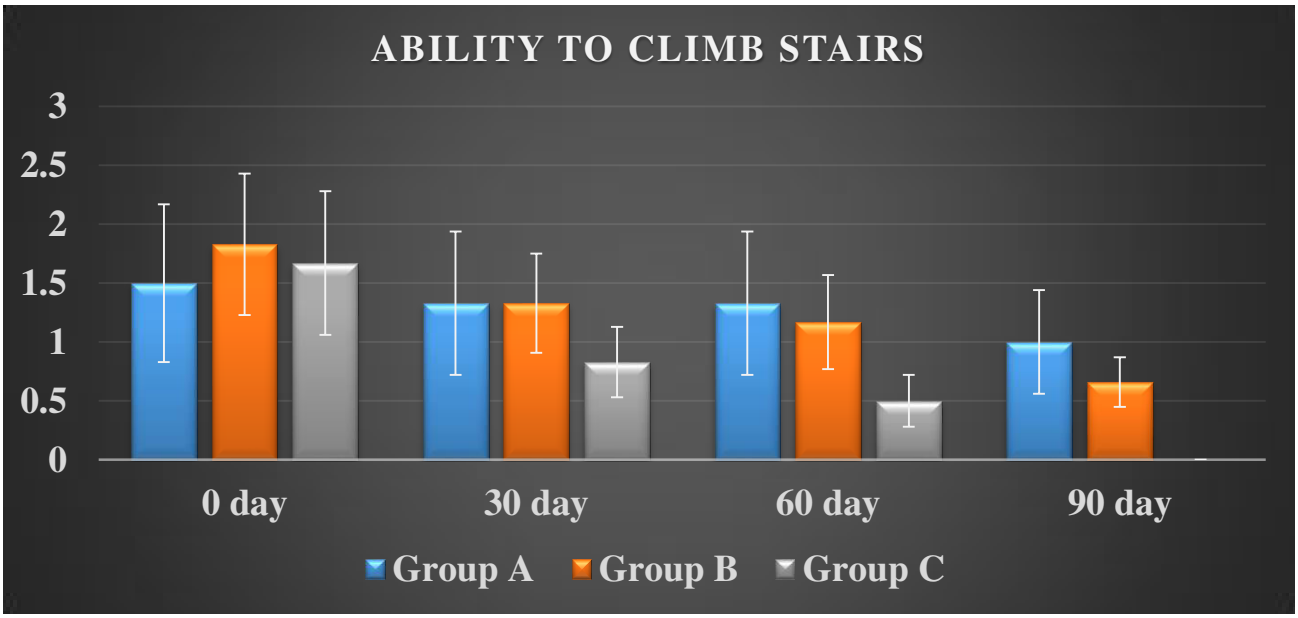

Fig. 19: Histogram showing Mean ± S.E. of scores of animals for ability to climb stairs in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).

Score for assessing the radiographic sign in dogs

Norberg angle (NA)

The Mean ± SE values of scores for norberg angle related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 14, Fig. 20). There were no significant differences in score value for norberg angle observed among the groups A, B and C on days 0. On days 30th there were no significant differences in score value for norberg angle between groups B and C but it was significantly differing from group A. Though there were no significant differences observed between groups B and C albeit lower value of score observed in group C (2.33 ± 0.33) as compared to B (3.50 ± 0.50). On 60th and 90th day, the score value for norberg angle were significantly differed amongst the groups A, B and C. The score value for norberg angle did not show significant difference between the groups A and B at various time intervals. In group C, no significant difference in score value for norberg angle were observed between on days 0 and 30th but it showed significantly differed on days 90th as compared to days 60th. Though there were no significant difference observed between day 0 and 30 albeit lower value of score observed on day 30 (2.33 ± 0.33) as compared to day 0 (3.00 ± 0.25). The score value amongst the groups at different intervals of time showed better improvement in group C followed by groups B and A.

Percent femoral head coverage

The Mean ± SE values of percent femoral head coverage (PFHC) related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 15, Fig. 21). There were no significant differences in mean value of percent femoral head coverage observed among the groups A, B and C on days 0. On 30th day, mean values of percent femoral head coverage in group C were significantly different from group B and A. Though there were no significant difference observed between the groups B and A albeit higher mean value of percent femoral head coverage observed in group B (33.51 ± 1.66) as compared to A (30.28 ± 1.33). On 60th and 90th day, the mean values of percent femoral head coverage were significantly differed amongst the groups A, B and C. In group A, there were no significant differences in mean value of percent femoral head coverage observed on days 0, 30th, 60th and 90th. In group B, there were no significant differences in mean value of percent femoral head coverage observed

between days 0 and 30th but it was significantly differed from day 60 and 90. Though there were no significant differences in mean value of percent femoral head coverage observed between days 0 and 30th albeit higher mean value of percent femoral head coverage observed on day 30th (33.51 ± 1.66) as compared to day 0 (29.46 ± 1.63). In group C, the mean value of percent femoral head coverage showed significantly different at respective intervals of time. The improvement in the mean value of percent femoral head coverage in respect to different intervals of time were best in the animals of group C followed by groups B and A.

Distraction index

The Mean ± SE values of distraction index (DI) related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 16, Fig. 22). There were no significant differences in mean value of distraction index observed among the groups A, B and C on days 0. On day 30, 60 and 90, there were significant difference in the mean values of distraction index amongst the groups A, B and C. In group B and C, significant differences were observed in the mean values of distraction index on days 0, 30th, 60th and 90th within the group but no significant differences were observed in the mean values of distraction index in group A at various interval of time. The mean value amongst the groups at different intervals of time showed better improvement in distraction index in group C followed by groups B and A.

Haemato-biochemical examinations

Haemoglobin (Hb) (g/dL)

The Mean ±SE values of haemoglobin (g/dL) of animals in different groups at various time interval is shown in (Table no. 17, Fig. 23). The values of haemoglobin in different groups A, B and C at various intervals of times were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Packed cell volume (PCV) (%)

The Mean ±SE values of PCV (%) of animals in different groups at various time intervals are shown in (Table no. 18, Fig. 24). The PCV values of different groups A, B and C at various

intervals of times were within the normal reference range and did not show any statistically significant difference (P≤0.05).

Table no. 14: Mean ± S.E. values of norberg angle scores of animals in different groups at various time intervals.

Norberg angle	0 day	30 day	60 day	90 day
Group A	3.83 ± 0.16	3.83 ± 0.16^{x}	3.83 ± 0.16^{x}	3.83 ± 0.16^{x}
Group B	3.50 ± 0.50	3.50 ± 0.50^{y}	2.67 ± 0.33^{y}	2.67 ± 0.33^{y}
Group C	3.00 ± 0.25^{a}	2.33 ± 0.33^{ay}	1.33 ± 0.21^{bz}	0.33 ± 0.21^{cz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at P < 0.05)

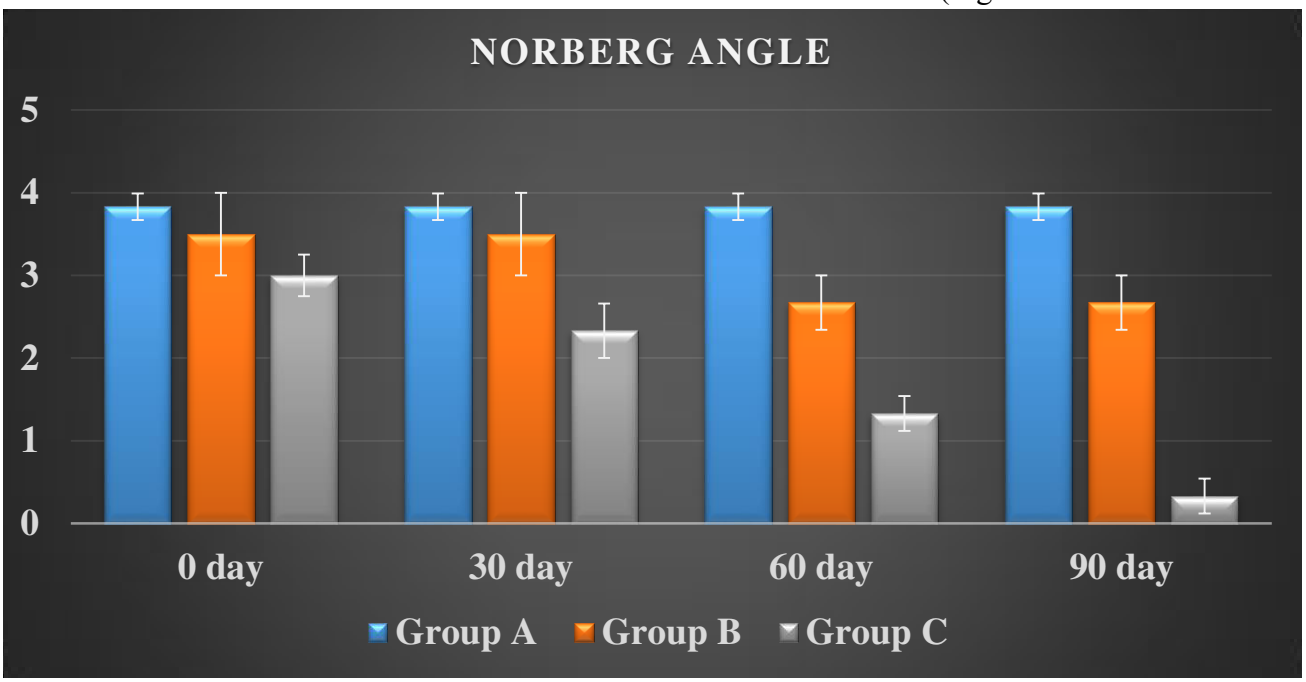

Fig. 20: Histogram showing Mean ± S.E. of Norberg angle (NA) of animals in different groups at various time intervals. The score value was lowest in group C followed by groups B and A (score 0-best, 4-worst).

Table no. 15: Mean ± S.E. values of percent femoral head coverage of animals in different groups at various time intervals

	0^{th} day	30^{th} day	60^{th} day	90^{th} day
Group A	30.00 ± 1.36	30.28 ± 1.33^{ax}	30.53 ± 1.31^{x}	32.30 ± 2.01^{x}
Group B	29.46 ± 1.63^{a}	33.51 ± 1.66^{ax}	38.75 ± 1.23^{by}	43.48 ± 1.46^{cy}
Group C	29.95 ± 1.18^{a}	40.96 ± 1.38^{by}	52.21 ± 1.20^{cz}	61.93 ± 1.45^{dz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

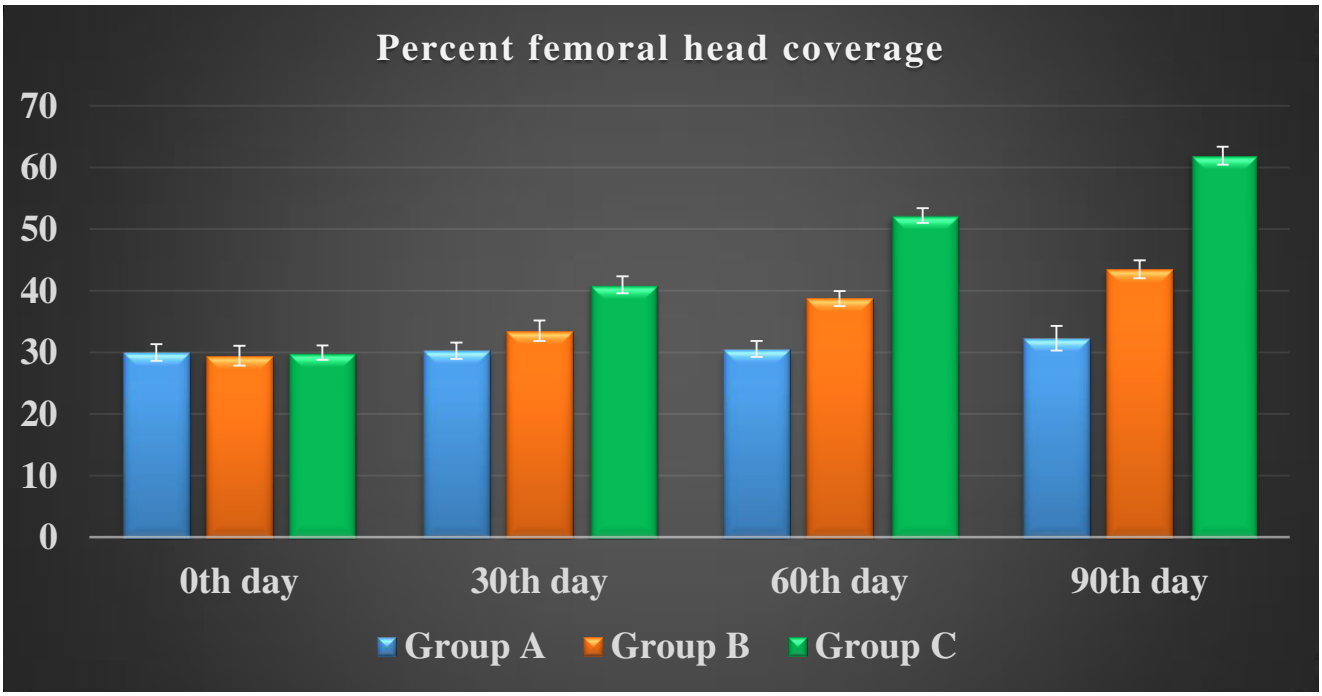

Fig. 21: Histogram showing Mean ± S.E. of Percent femoral head coverage (PFHC) of animals in different groups at various time intervals. The highest value for PFHC showed least luxation of hip joint in group C followed by groups B and A.

Table no. 16: Mean ± S.E. values of distraction index of animals in different groups at various time intervals

	0th day	30th day	60th day	90th day
Group A	0.45 ± 0.19	0.44 ± 0.02^{x}	0.44 ± 0.17^{x}	0.43 ± 0.17^{x}
Group B	0.44 ± 0.11^{a}	0.34 ± 0.01^{by}	0.26 ± 0.02^{cy}	0.18 ± 0.03^{dy}
Group C	0.38 ± 0.32^{a}	0.22 ± 0.03^{bz}	0.12 ± 0.02^{cz}	0.04 ± 0.01^{dz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

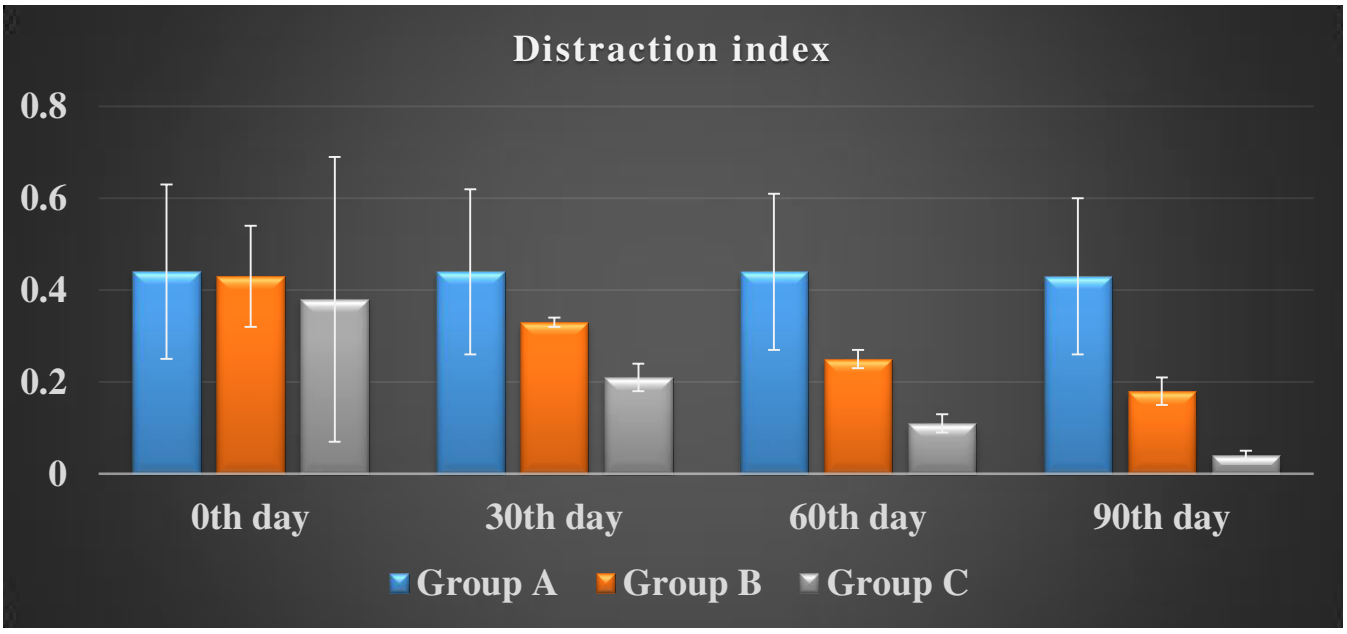

Fig. 22: Histogram showing Mean ± S.E. of Distraction Index (DI) of animals in different groups at various time interval. The lowest mean value for DI showed least luxation of hip joint in group C followed by groups B and A.

Table no. 17: Mean ± S.E. values of Haemoglobin (Hb) (g/dl) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	13.76 ± 0.28	14.30 ± 0.24	14.31 ± 0.14	14.21 ± 0.26
Group B	14.03 ± 0.17	14.21 ± 0.27	14.15 ± 0.43	14.36 ± 0.25
Group C	13.61 ± 0.33	14.00 ± 0.37	14.10 ± 0.39	14.48 ± 0.34

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

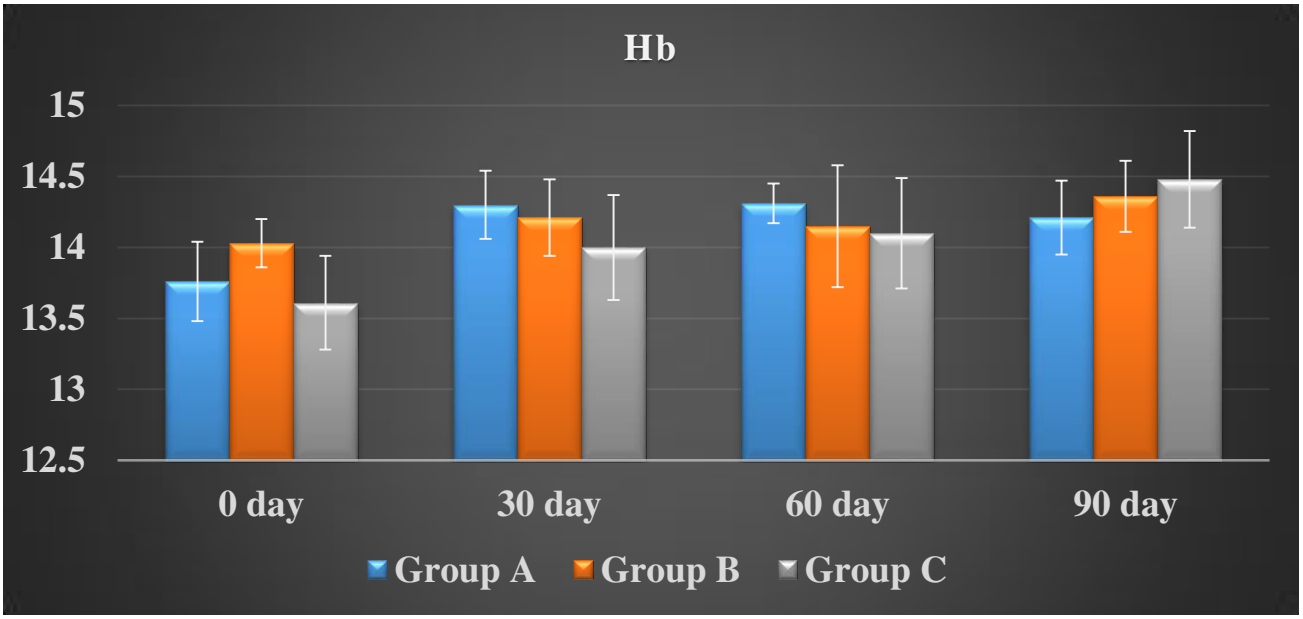

Fig. 23: Histogram showing Mean ± S.E. of Haemoglobin (Hb) (g/dl) of animals not significant in different groups at various time intervals

Table no. 18: Mean ± S.E. values of packed cell volume (PCV) (%) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	41.30 ± 0.84	42.90 ± 0.72	42.95 ± 0.42	42.65 ± 0.77
Group B	42.10 ± 0.50	42.65 ± 0.80	42.45 ± 1.29	43.10 ± 0.76
Group C	40.85 ± 0.99	42.00 ± 1.12	42.30 ± 1.17	43.23 ± 0.91

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

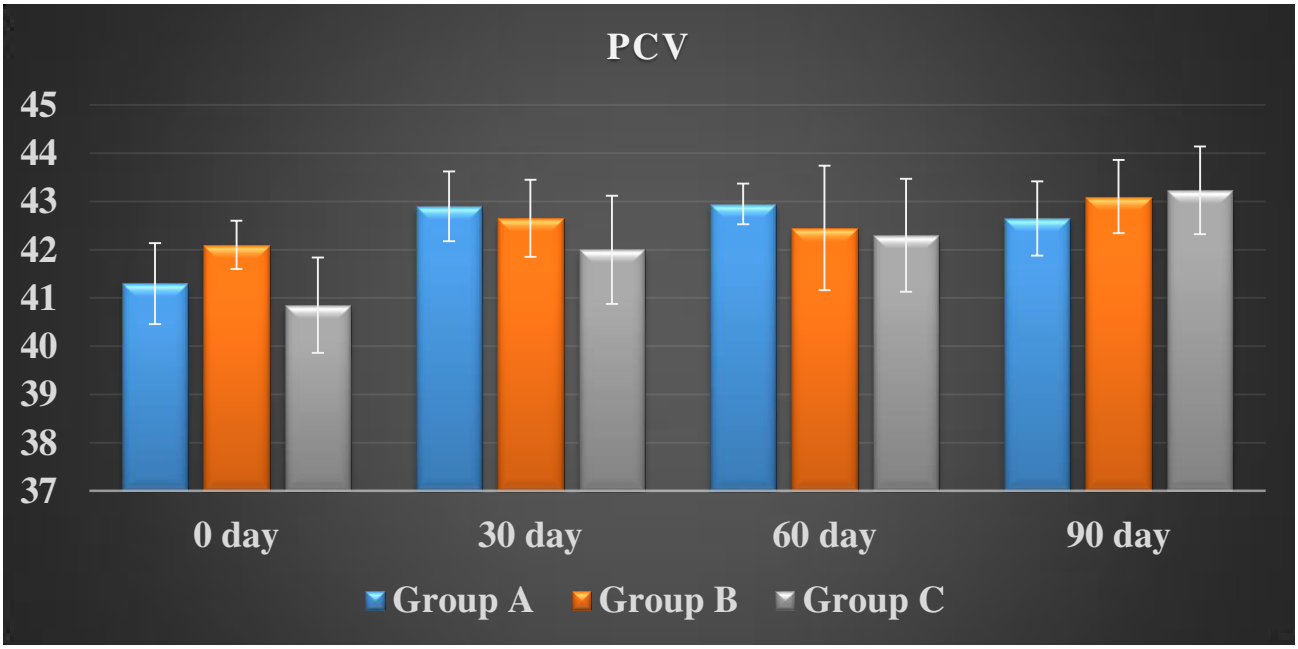

Fig. 24: Histogram showing Mean ± S.E. values of packed cell volume (PCV) (%) of animals not significant in different groups at various time intervals.

Total erythrocytes count (TEC) (10^6/µL)

The Mean ±SE values of total erythrocytes count of animals in different groups at various time intervals are shown in (Table no. 19, Fig. 25). The values of total erythrocyte count (TEC) in groups A, B and C at different interval of time were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Total leucocyte count (TLC) (10^6/µL)

The Mean ±SE values of total leucocytes count of animals in different groups at various time intervals are shown in (Table no. 20, Fig. 26). The values of total leucocytes count (TLC) in different groups A, B and C at various intervals of times were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Neutrophils (%)

The Mean ±SE values of neutrophils count (%) of animals in different groups at various time intervals are shown in (Table no. 21, Fig. 27). The values of neutrophils count in different groups of animals A, B and C at various interval of times were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Eosinophils (%)

The Mean ±SE values of eosinophils count (%) of animals in different groups at various time intervals are shown in (Table no. 22, Fig. 28). The values of eosinophil of groups A, B and C at different interval of time were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Basophils (%)

The Mean ±SE values of eosinophils count (%) of animals in different groups at various time intervals are shown in (Table no. 23). The values of basophils of groups A, B and C at different interval of time were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Table no. 19: Mean ± S.E. values of total erythrocyte count (TEC) (10^6/µL) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	6.88 ± 0.14	7.15 ± 0.12	7.15 ± 0.07	7.10 ± 0.13
Group B	7.01 ± 0.83	7.10 ± 0.13	7.07 ± 0.24	7.16 ± 0.13
Group C	6.80 ± 0.17	7.00 ± 0.19	7.05 ± 0.20	7.20 ± 0.15

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

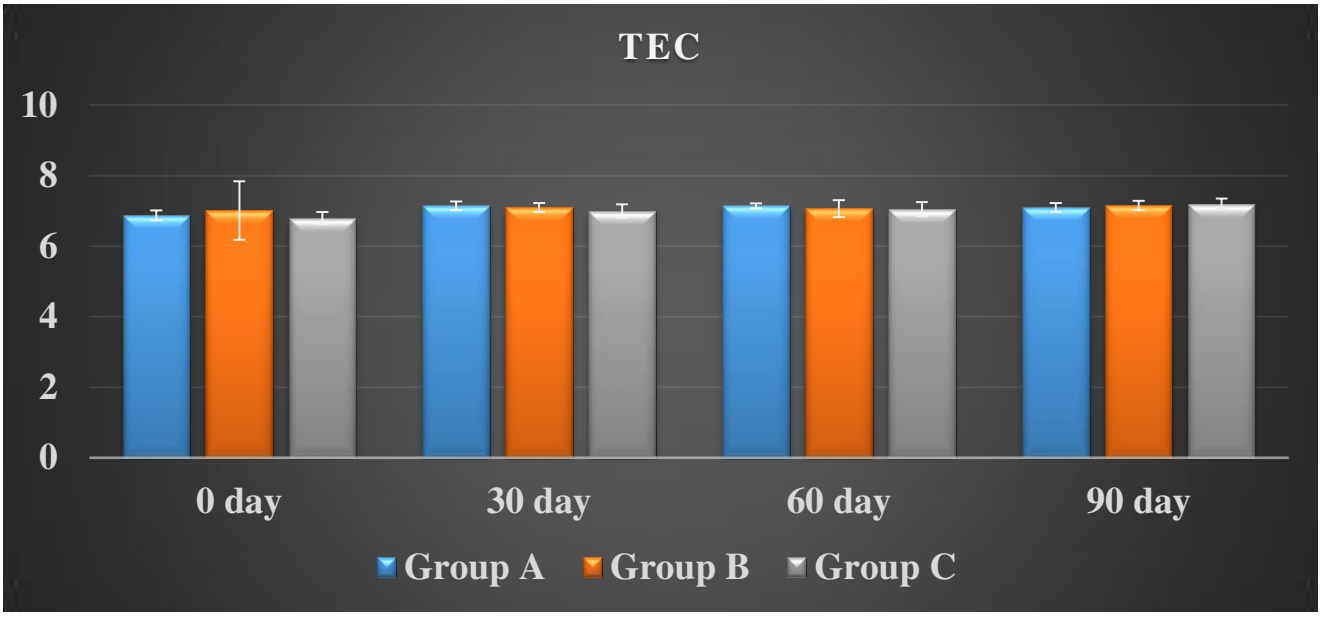

Fig. 25: Histogram showing Mean ± S.E. values of total erythrocyte count (TEC) (10^6/µL) of animals not significant in different groups at various time intervals

Table no. 20: Mean ± S.E. values of total leukocyte count (TLC) (10^3/µL) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	11085 ± 540.14	10417 ± 688.20	10295 ± 744.68	10039 ± 631.87
Group B	9433 ± 335.91	9999 ± 404.93	10546 ± 783.39	10855 ± 1033.63
Group C	11742 ± 927.57	11178 ± 842.31	11530 ± 912.75	10392 ± 479.58

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

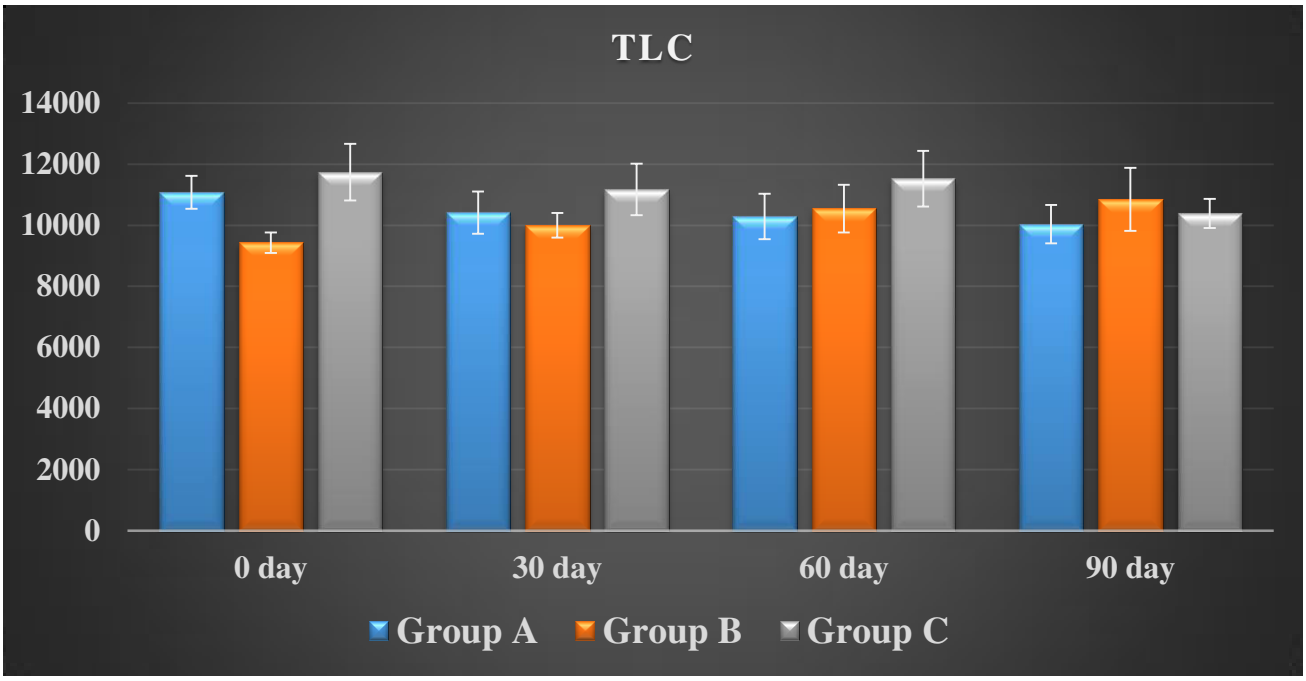

Fig. 26: Histogram showing Mean ± S.E. values of total leukocyte count (TLC) (10^3/µL) of animals not significant in different groups at various time intervals

Table no. 21: Mean ± S.E. values of Neutrophils (%) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	71.66 ± 0.62	71.83 ± 0.48	71.50 ± 0.22	70.83 ± 0.30
Group B	71.16 ± 1.01	72.16 ± 0.40	71.83 ± 0.94	71.83 ± 0.70
Group C	73.00 ± 0.36	73.16 ± 0.87	71.50 ± 0.56	71.83 ± 0.30

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

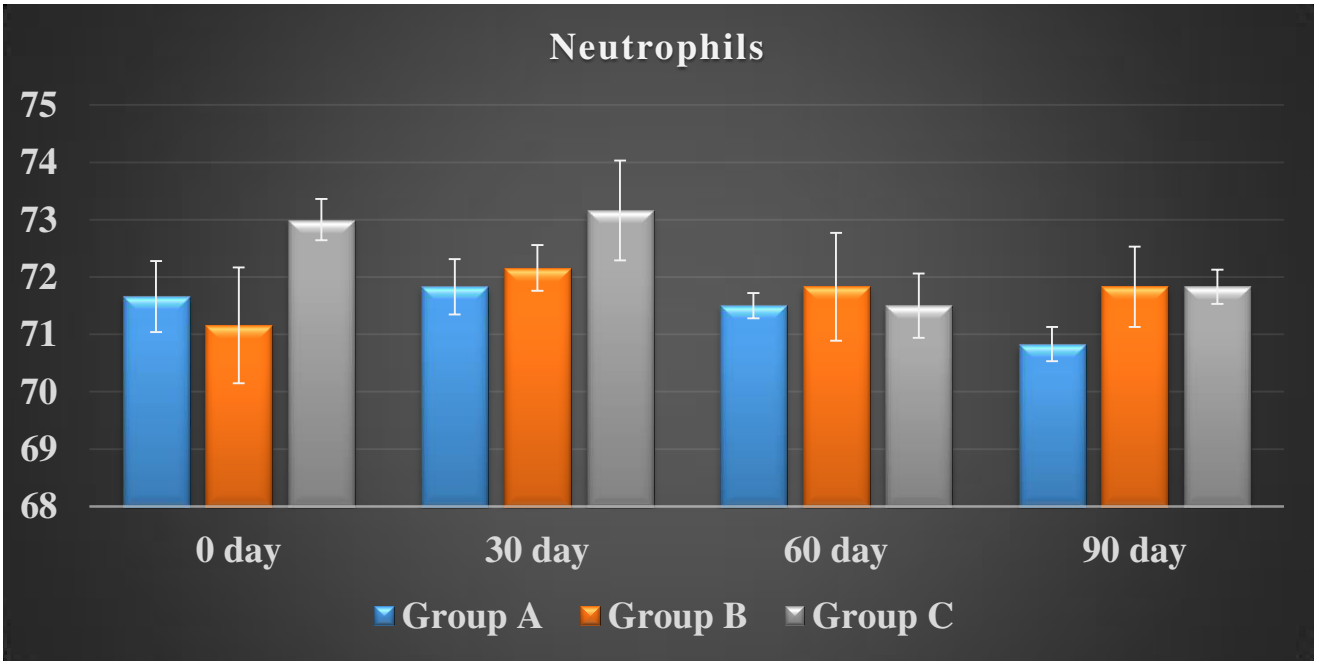

Fig. 27: Histogram showing Mean ± S.E. values of Neutrophils (%) of animals not significant in different groups at various time interval

Table no. 22: Mean ± S.E. values of Eosinophils (%) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	1.66 ± 0.33	2.00 ± 0.36	1.83 ± 0.40	2.00 ± 0.25
Group B	1.83 ± 0.30	1.66 ± 0.21	1.50 ± 0.22	1.83 ± 0.17
Group C	1.66 ± 0.42	1.33 ± 0.21	1.50 ± 0.43	1.33 ± 0.33

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

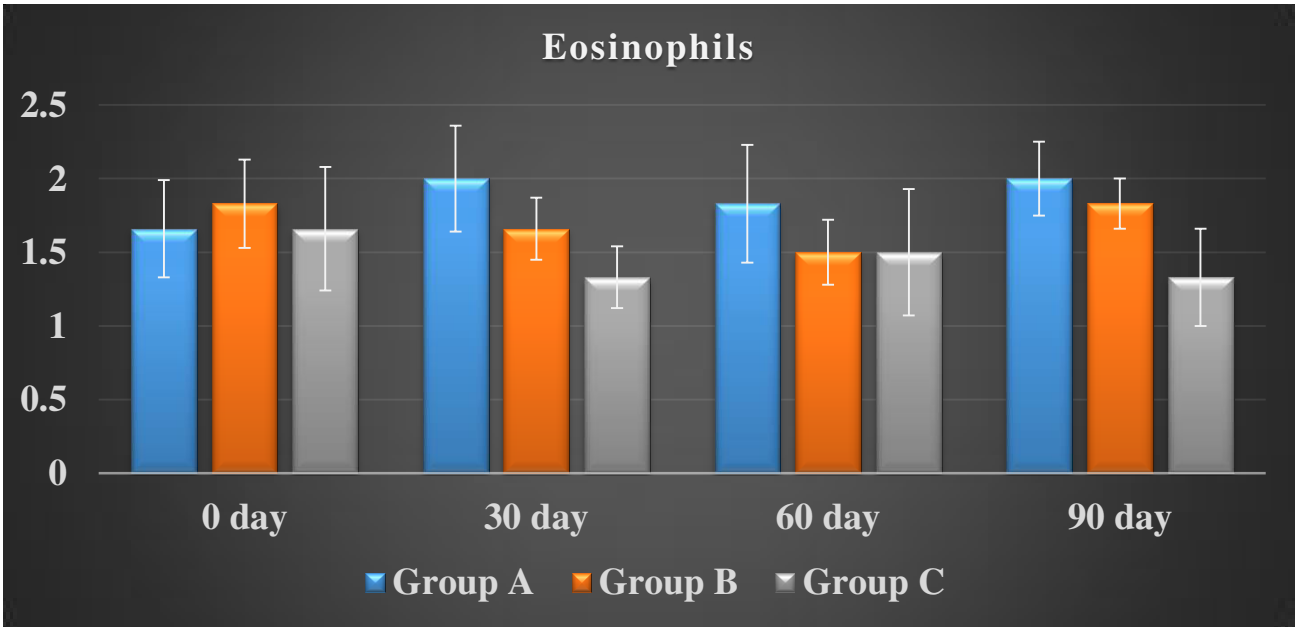

Fig. 28: Histogram showing Mean ± S.E. values of Eosinophils (%) of animals not significant in different groups at various time intervals

Table no. 23: Mean ± S.E. values of Basophils (%) of animals in different groups at various time intervals.

	0 day	30 day	60 day	90 day
Group A	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group B	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group C	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

Lymphocytes (%)

The Mean ±SE values of lymphocytes count (%) of animals in different groups at various time intervals are shown in (Table no. 24, Fig. 29). The values of lymphocytes of groups A, B and C at different interval of time were within the normal reference range and did not show any statistically significant difference ($P \leq 0.05$).

Monocytes (%)

The Mean ±SE values of monocytes count (%) of animals in different groups at various time intervals are shown in (Table no. 25, Fig. 30). The monocytes values of groups A, B and C at different interval of time were within the normal reference range and did not showed any statistically significant difference ($P \leq 0.05$).

C- reactive protein (CRP)

The Mean ± SE values of C - reactive protein (CRP) related with hip dysplasia in animals of different groups at various intervals of time are shown in (Table no. 26, Fig. 31). There were no significant differences in mean value of C - reactive protein observed among the groups A, B and C on days 0. On day 30th and 90th, the mean value of C - reactive protein were significantly differed amongst the groups A, B and C but on days 60th, the mean value of C - reactive protein in group C was significantly different from groups B and A. Though there were no significant difference observed between the groups B and A albeit lower mean value of C - reactive protein observed in group B (5.58 ± 0.23) as compared to A (8.61 ± 0.20). In group A, no significant differences were observed in the mean values of C - reactive protein at various interval of time. In group B and C, the mean values of C - reactive protein showed significantly different at respective intervals of time. The mean values of C-reactive protein showed minimal inflammatory reaction in hip joints of animals in groups C followed by groups B and A.

Table no. 24: Mean ± S.E. values of Lymphocytes (%) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	19.66 ± 0.76	19.83 ± 0.48	19.66 ± 0.34	19.83 ± 0.17
Group B	19.16 ± 0.60	19.16 ± 0.30	19.50 ± 0.67	20.33 ± 0.21
Group C	19.16 ± 0.17	19.33 ± 0.34	19.83 ± 0.40	20.00 ± 0.26

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

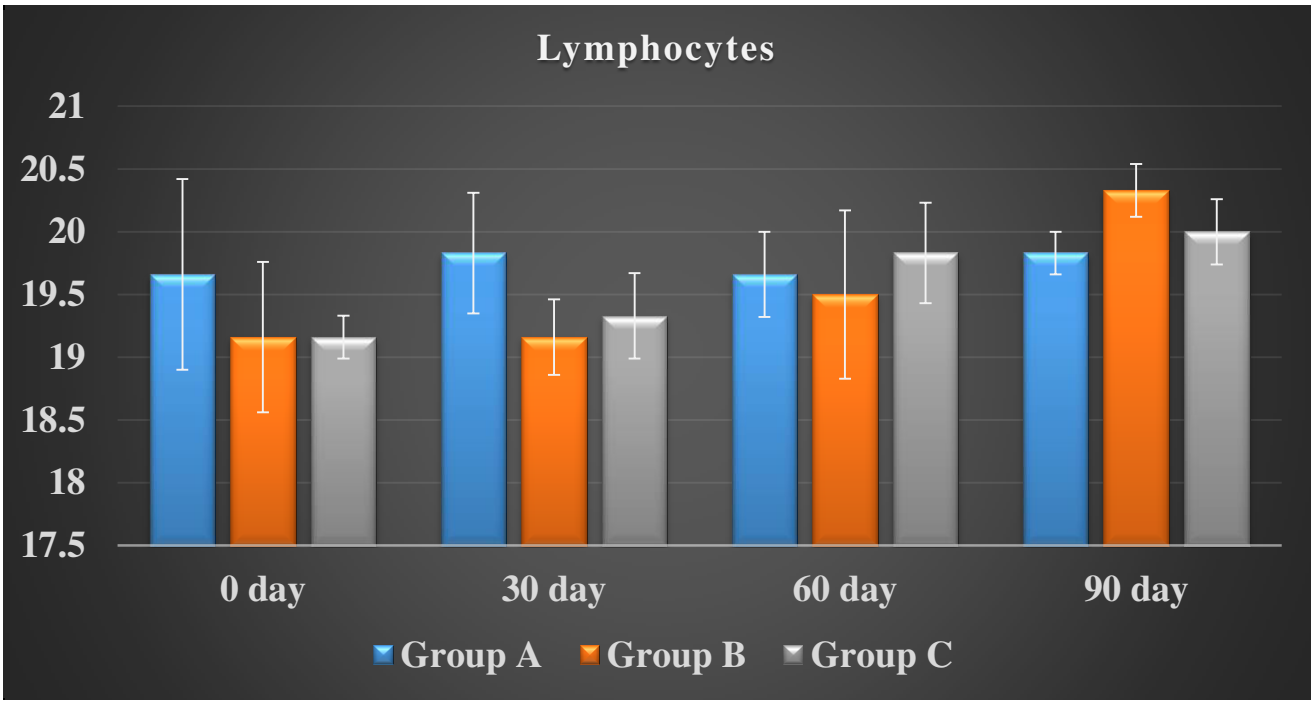

Fig. 29: Histogram showing Mean ± S.E. values of Lymphocytes (%) of animals not significant in different groups at various time intervals

Table no. 25: Mean ± S.E. values of Monocytes (%) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	3.50 ± 0.34	3.66 ± 0.21	4.00 ± 0.36	3.00 ± 0.45
Group B	3.50 ± 0.56	3.50 ± 0.43	3.16 ± 0.30	3.66 ± 0.56
Group C	3.33 ± 0.50	3.66 ± 0.42	3.33 ± 0.34	3.83 ± 0.30

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

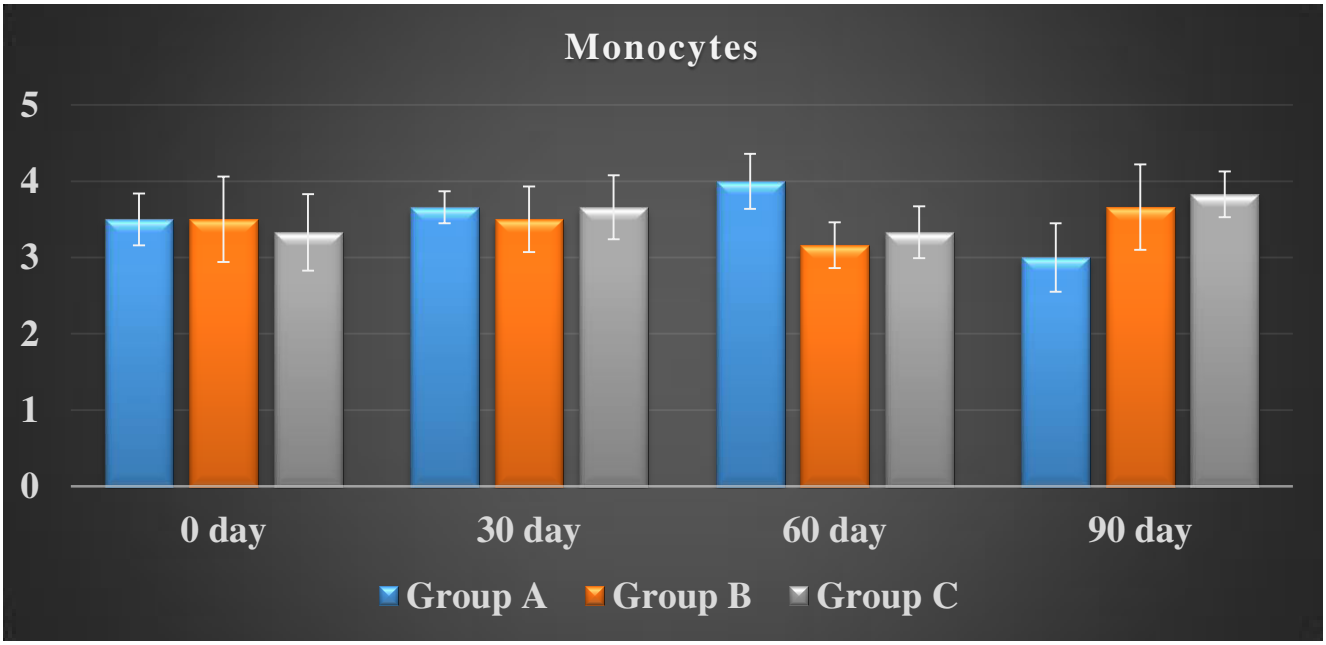

Fig. 30: Histogram showing Mean ± S.E. values of Monocytes (%) of animals not significant in different groups at various time intervals.

Table no. 26: Mean ± S.E. values of C - reactive protein (CRP) (mg/dl) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	9.25 ± 0.36	8.97 ± 0.21^{x}	8.61 ± 0.20^{x}	8.50 ± 0.20^{x}
Group B	9.07 ± 0.17^{a}	6.73 ± 0.20^{by}	5.58 ± 0.23^{cx}	4.60 ± 0.13^{dy}
Group C	9.73 ± 0.21^{a}	5.89 ± 0.25^{bz}	4.93 ± 0.27^{cy}	4.02 ± 0.14^{dz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

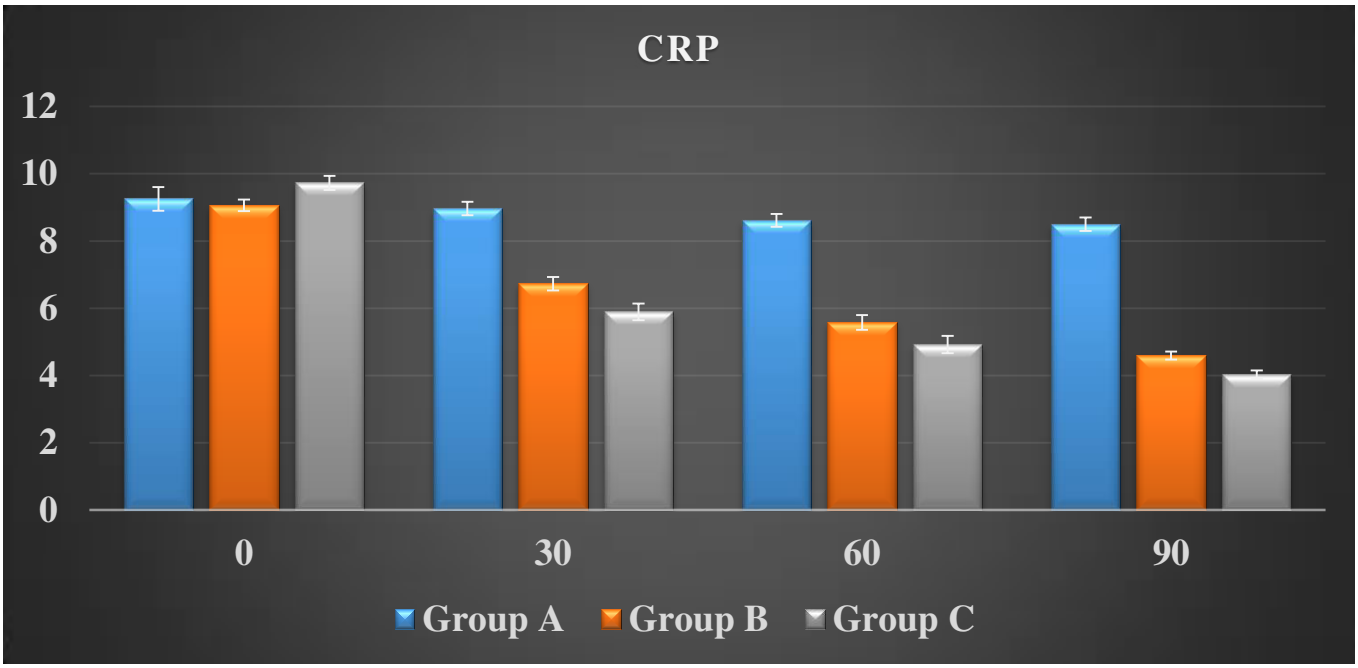

Fig. 31: Histogram showing Mean ± S.E. of C - reactive protein (CRP) (mg/dl) of animals in different groups at various time intervals. The lowest value of CRP suggested least inflammatory condition in group C followed by groups B and A.

Oxidative stress

Malondialdehyde (MDA) (n mol/ml of blood serum)

The Mean ± SE values of Malondialdehyde (MDA) related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 27, Fig. 32). There were no significant differences in mean value of Malondialdehyde observed among the groups A, B and C on days 0. On days 30^{th} and 60^{th}, the mean value of Malondialdehyde were significantly differed amongst the groups A, B and C. On days 90^{th}, no significant difference in the mean values of malondialdehyde were observed between groups B and C but it was significantly different from group A. Though there were no significant difference observed between the groups B and C albeit lower mean value of malondialdehyde observed in group C as compared to B. In group A, no significant differences were observed in the mean values of malondialdehyde at various interval of time. In group B and C, no significant difference was observed in the mean values of malondialdehyde between day 60^{th} and days 90^{th} but it was significantly differed from day 30^{th} and day 0. Though there were no significant difference observed between day 60^{th} and 90^{th} albeit lower mean value of malondialdehyde observed on days 90^{th} (11.07 ± 0.88) as compared to day 60^{th} (11.89 ± 0.59). Lower mean values of MDA with subsequent intervals of time showed less lipid peroxidation indicating least stress in the animals of group C followed by groups B and A.

Superoxide dismutase (SOD) (K/gram protein)

The Mean ± SE values of Superoxide dismutase (SOD) related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 28, Fig. 33). There were no significant differences in mean value of superoxide dismutase observed among the groups A, B and C on days 0. On days 30^{th}, 60^{th} and 90^{th}, the mean value of Superoxide dismutase was significantly differed amongst the groups C, B and A. In groups B and C, significant differences were in the mean values of Superoxide dismutase observed on days 0, 30^{th}, 60^{th} and 90^{th} whereas, significant differences were not observed in the mean values of Superoxide dismutase in group A at various intervals of time. Higher values of SOD with advancement of time showed least stress in the animals of group C followed by groups B and A.

Table no. 27: Mean ± S.E. values of Malondialdehyde (MDA) (n mol/ml of blood serum) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	12.42 ± 0.66	12.20 ± 0.68^{x}	11.89 ± 0.59^{x}	11.07 ± 0.88^{x}
Group B	13.15 ± 0.31^{a}	8.75 ± 0.60^{by}	7.17 ± 0.24^{cy}	6.13 ± 0.12^{cy}
Group C	12.55 ± 0.49^{a}	6.67 ± 0.27^{bz}	5.61 ± 0.27^{cz}	5.34 ± 0.24^{cy}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

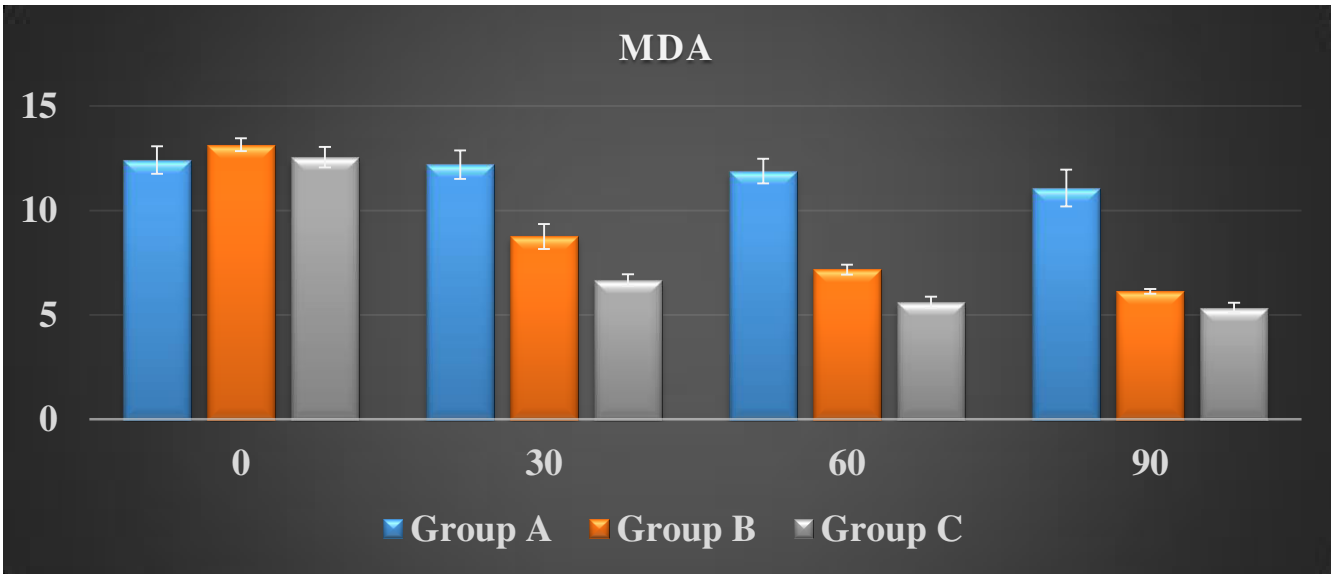

Fig. 32: Histogram showing Mean ± S.E. of Malondialdehyde (MDA) (n mol/ml of blood serum) of animals in different groups at various time intervals. The lowest value of MDA suggested least oxidative stress in group C followed by groups B and A.

Table no. 28: Mean ± S.E. values of Superoxide dismutase (k/gram of protein) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	184.83 ± 2.75	187.69 ± 2.61^{x}	189.47 ± 2.56^{x}	190.62 ± 2.53^{x}
Group B	184.21 ± 3.57^{a}	218.95 ± 3.07^{by}	230.60 ± 3.26^{cy}	244.63 ± 2.37^{dy}
Group C	180.40 ± 4.29^{a}	230.95 ± 3.64^{bz}	242.24 ± 2.10^{cz}	254.71 ± 1.65^{dz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

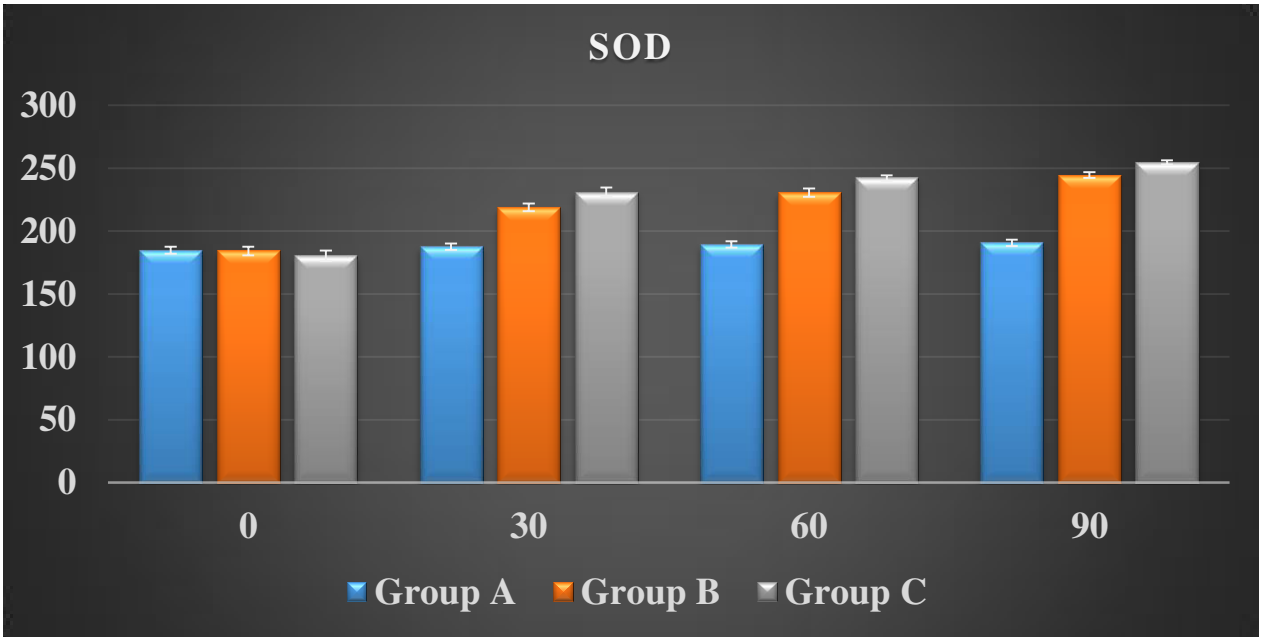

Fig. 33: Histogram showing mean ± S.E. of Superoxide dismutase (SOD) (K/gram protein) of animals in different groups at various time intervals. The highest value of SOD suggested least oxidative stress in group C followed by groups B and A.

Catalase (CAT) (k/gram of protein)

The Mean ± SE values of catalase (CAT) related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 29, Fig. 34). There were no significant differences in mean value of catalase observed among the groups A, B and C on days 0. On days 30^{th}, the mean value of catalase in group C was significantly different from groups B and A. Though there were no significant difference observed between the groups B and A albeit higher mean value of catalase observed in group B (3.39 ± 0.23) as compared to A (2.88 ± 0.20). On days 60^{th}, the mean values of catalase were significantly differed amongst the groups C, B and A. On 90^{th} day, the mean value of catalase in group B and C were significantly different from group A. Though there were no significant difference observed between the groups B and C albeit higher mean value of catalase observed in group C (5.02 ± 0.09) as compared to group B (4.91 ± 0.15). In group A, there were no significant differences in mean value of catalase observed at day different intervals of time. In group B, no significant differences were observed in the mean values of catalase between day 30^{th} and days 60^{th} but it significantly differed from days 0 and days 90^{th}. Though there were no significant difference observed between day 30^{th} and 60^{th} albeit higher mean value of catalase observed on day 60 (3.84 ± 0.23) as compared to days 30^{th} (3.39 ± 0.23). In group C, no significant differences were observed in the mean values of catalase between day 60^{th} and day 90^{th} but it significantly differed from days 30^{th} and days 0. Though there were no significant difference observed between day 60^{th} and 90^{th} albeit higher mean value of catalase observed on days 90^{th} (5.02 ± 0.09) as compared to day 60 (4.65 ± 0.15). Higher values of CAT with advancement of time showed least stress in the animals of group C followed by groups B and A.

Glutathione Peroxidase (GPx)

The Mean ± SE values of Glutathione peroxidase (GPx) related with hip dysplasia in animals of different groups at various time intervals are shown in (Table no. 30, Fig. 35). There were no significant differences in mean value of Glutathione peroxidase observed among the groups A, B and C on days 0. On days 30^{th}, 60^{th} and 90^{th}, there were significant differences in mean value of Glutathione peroxidase observed among the groups A, B and C. In group A, the mean values of Glutathione peroxidase on days 90^{th} were significantly different from the respective intervals of time. In group B and C, the mean value of Glutathione peroxidase showed

significantly different at respective intervals of time. The higher value of Glutathione peroxidase (GPx) with respect to time indicates least stress in the animals of group C followed by groups B and A.

Overall response to the treatment among different groups

Overall response to the treatment among different groups shown in (Table no. 31, Fig. 36). In group A, where all six dogs were provided Nutraceuticals orally for three months and 1.0 ml of RPMI intra-articularly once for the treatment only two out of six animals showed improvement in pain, lameness, ability to jump and climbing stairs. Although no appreciable improvement in the radiographs were noticed only two dogs recovered uneventfully. In group B, the animals were treated with autologous uncultured bone marrow mono-nucleated cells (BMNCs) suspended in 0.5 ml RPMI and treated once intra-articularly, all six animals showed appreciable improvement in gait, norberg angle, percent femoral head coverage and distraction index values without any signs of pain, lameness and recovered eventfully without any complications. In group C, the animals were treated with autologous uncultured bone marrow mono-nucleated cells (BMNCs) suspended in 0.5 ml RPMI mixed with activated platelets suspended in 0.5 ml of RPMI and treated once intra-articularly, all six animals under this group showed eventful improvement in gait, norberg angle, percent femoral head coverage, distraction index values without any complications.

Table no. 29: Mean ± S.E. values of Catalase (CAT) (k/gram of protein) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	2.74 ± 0.20	2.88 ± 0.20^{x}	3.07 ± 0.19^{x}	3.11 ± 0.23^{x}
Group B	2.79 ± 0.15^{a}	3.39 ± 0.23^{bx}	3.84 ± 0.23^{by}	4.91 ± 0.15^{cy}
Group C	2.79 ± 0.14^{a}	4.24 ± 0.14^{by}	4.65 ± 0.15^{cz}	5.02 ± 0.09^{cy}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

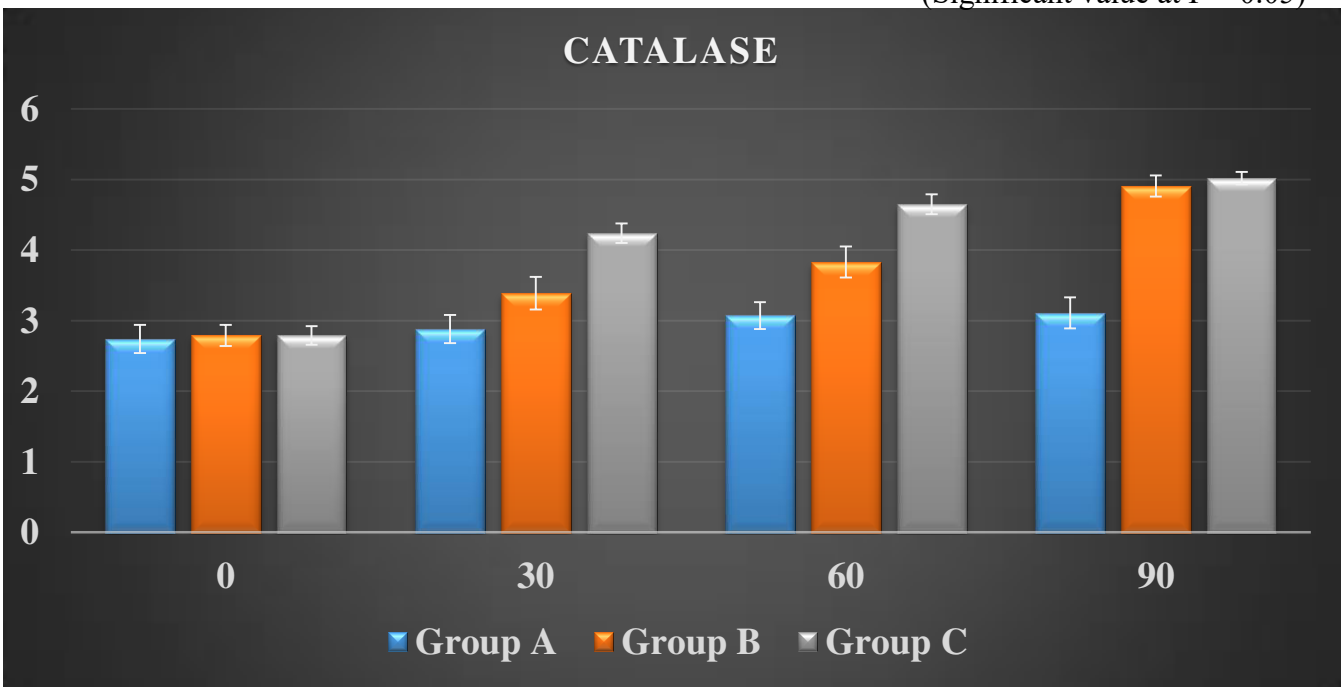

Fig. 34: Histogram showing Mean ± S.E. of Catalase (CAT) (k/gram of protein) of animals in different groups at various time intervals. The highest value of Catalase suggested least oxidative stress in group C followed by groups B and A.

Table no. 30: Mean ± S.E. values of Glutathione peroxidase (U/ml) of animals in different groups at various time intervals

	0 day	30 day	60 day	90 day
Group A	0.078 ± 0.002^{a}	0.0812 ± 0.001^{abx}	0.083 ± 0.001^{abx}	0.0850 ± 0.001^{cx}
Group B	0.081 ± 0.001^{a}	0.0905 ± 0.001^{by}	0.11 ± 0.000^{cy}	0.124 ± 0.000^{dy}
Group C	0.083 ± 0.001^{a}	0.100 ± 0.000^{bz}	0.122 ± 0.001^{cz}	0.148 ± 0.001^{dz}

Superscripts a, b, c and d in rows indicates significant difference within the group from pre treatment
Superscripts x, y, z in columns indicates significant difference between the groups within days.

(Significant value at $P < 0.05$)

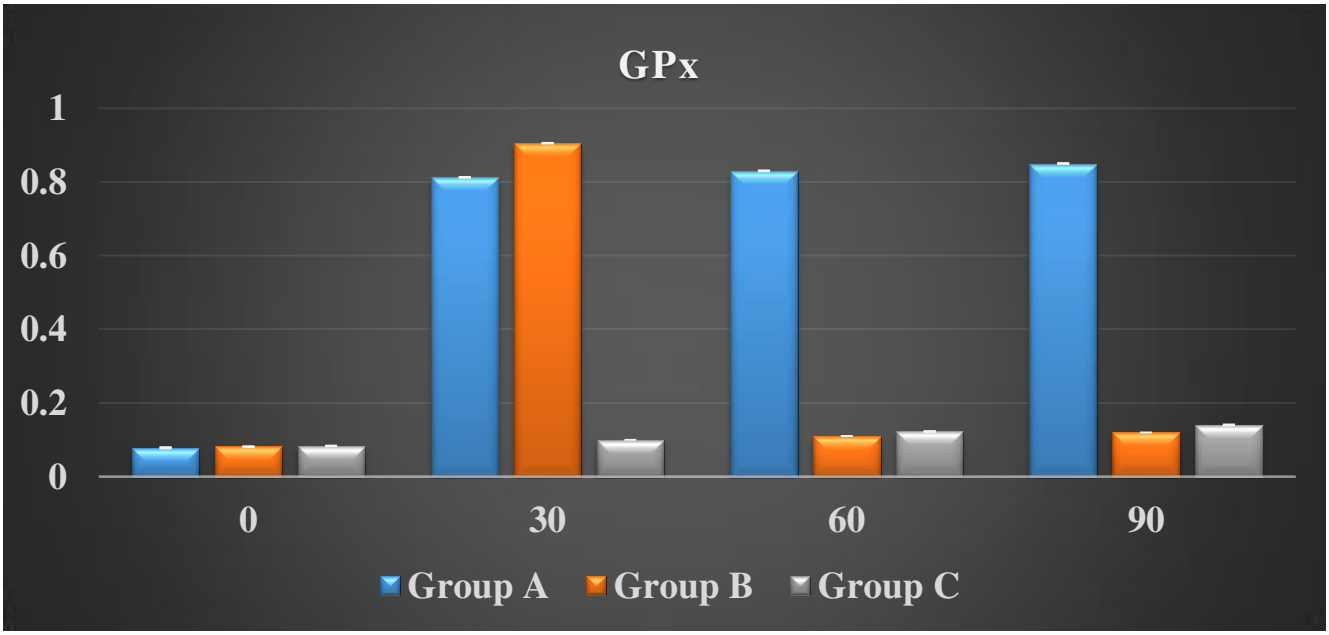

Fig. 35: Histogram showing mean ± S.E. of Glutathione peroxidase (U/ml) of animals in different groups at various time intervals. The highest value of GPx suggested least oxidative stress in group C followed by groups B and A.

Table no. 31: Overall response of treatment among different groups.

Groups	Treatment	Route	No. of animals	Responded to treatment
A	Nutraceuticals+ RPMI (1.0ml)	Orally (3M) + Intra-articular (Once)	6	33.34% (2/6)
B	Autologous Uncultured BMNCs suspended in 1.0ml RPMI	Intra-articular (Once)	6	100% (6/6)
C	Autologous Uncultured BMNCs+ Activated Platelets each suspended in 0.5ml RPMI	Intra-articular (Once)	6	100% (6/6)

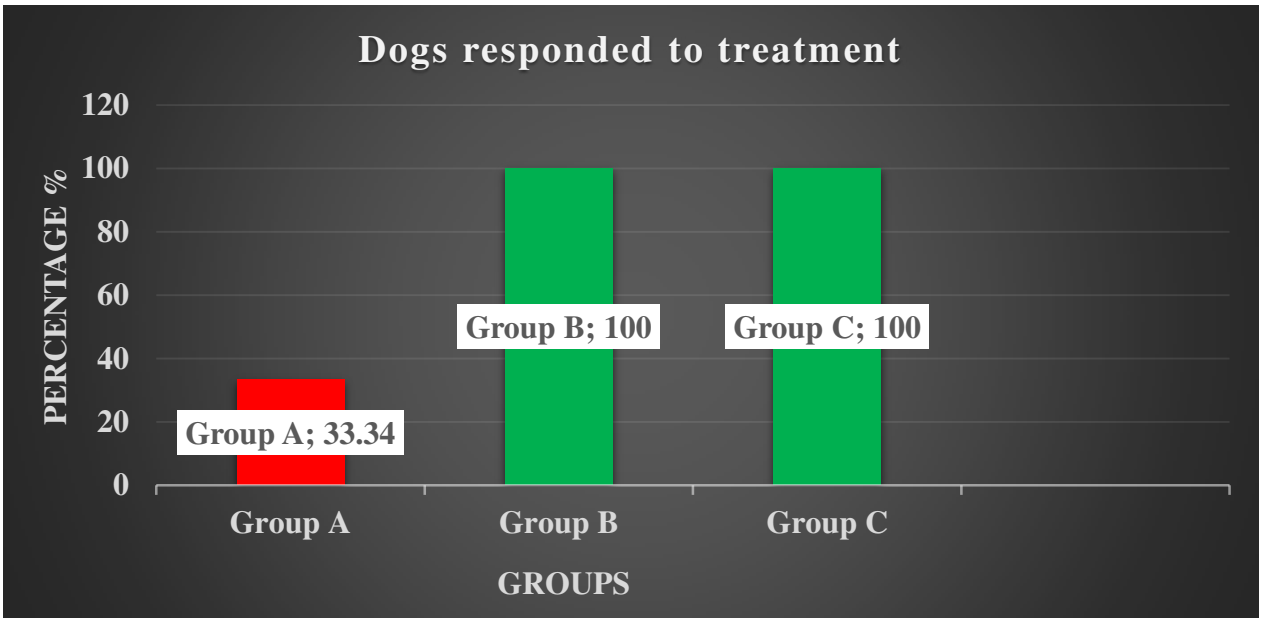

Fig. 36: Histogram showing the response of treatment in animals suffering from hip dysplasia on the basis of observation on days 90. Group A (Control), Group B (Bone marrow mono-nuclear cells-BMNCs), Group C (BMNCs + Activated platelets).

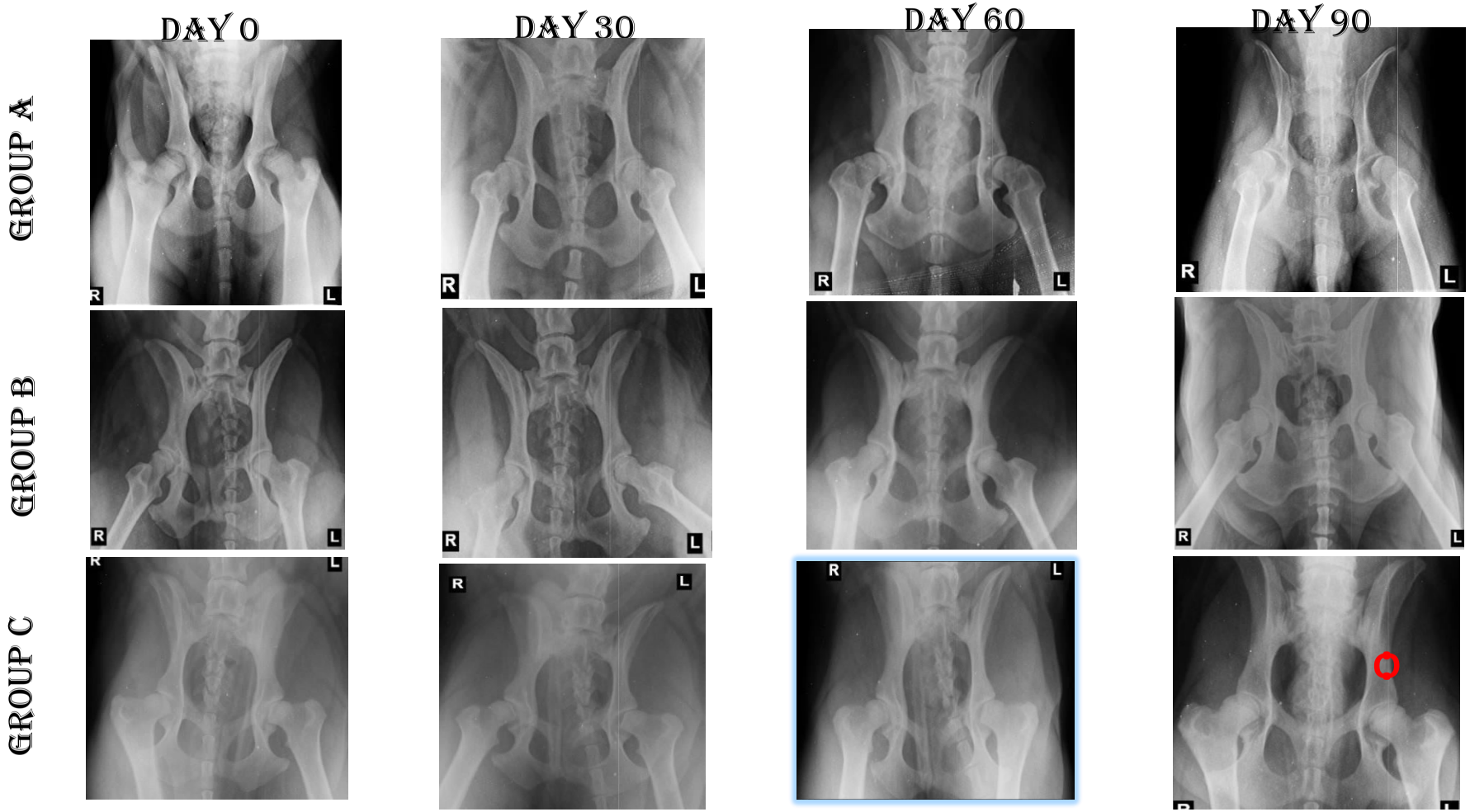

Fig. 37: Ventro-dorsal view radiographs of pelvic bone in dogs at different time intervals. The radiographs showed better response in group C (O) followed by groups B and A in terms of disappearance of osteophytes, clearly visibility of joint space, norberg angle, percent femora head coverage and distraction index. Group A (Control), Group B (Bone marrow mono-nuclear cells-BMNCs), Group C (BMNCs + Activated platelets).

Discussion

One of the most frequently discussed areas in clinical studies is related to the selection of the animals. The key issue in the selection of an appropriate animal is availability of cases in the clinics and literature consulted. Prior to deciding the problem of this study, the ventro-dorsal view radiographs of pelvic bone of dogs brought with problem in hind quarter during last one and half years were screened and observed good number of cases of hip dysplasia based on visible radiographic changes present in the hip joints. There were 217 pelvic radiographs of dogs recorded during February 2017 to August 2019 and out of that 109 cases (51 %) were diagnosed for hip dysplasia and remaining 108 cases (49%) were of different affections related to pelvic bone and neurological disorders. It has been observed that cases of hip dysplasia coming to our teaching veterinary hospital are enough to design this study. Hip dysplasia (HD) is an inherited, non-congenital orthopaedic disease with highest incidence and heritability of up to 95% in the canine species that particularly prevalent in large and giant breeds of dog (Mäki *et al.,* 2004; Janutta and Distl, 2006; Ginja *et al.,* 2008; Guo *et al.,* 2011; Sanchez-Molono *et al.,* 2015). It is characterized by degenerative joint disease that can progressively trigger the development of osteoarthritis (OA) of the affected joint (Smith *et al.,* 2001), characterized by articular cartilage lesions, bone remodeling, presence of osteophytes and inflammation in hip joint (Johnston *et al.,* 2008). Two approaches of canine HD management have been described, which include conservative management and surgery (Anderson, 2011). In dogs, one of the principal conservative therapeutic approaches involves prolong oral administration of nutraceuticals, whose formulation is primarily composed of glucosamine and chondroitin sulfate together with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) (McCarthy *et al.,* 2006; Sauvé *et al.,* 2003). However, prolonged use of NSAIDs can be associated with side effects, especially in the digestive system and kidneys (Luna *et al.,* 2007). Even prolong oral administration of medications are major challenge in canine due variation in temperament of animals and palatability of the medicaments. The objective of the study was to evaluate whether one-time implantation in hip joint with uncultured autologous BMNCs can stimulate cartilage repair and

whether combined use of BMNCs and activated autologous platelets derived growth factors has any advantage over BMNCs alone for *in-vivo* treatment in clinical cases of canine hip dysplasia.

The age of the animals observed under this study varied from 4 to 24 months (Median age 10 months) with the maximum numbers of animals recorded between 6 to 12 months (12/18; 67 %) of age groups followed by 0-6 months (2/18; 11%), 12-18 months (2/18; 11%) and 18-24 months (2/18; 11%) respectively. Hip dysplasia is a juvenile-onset condition with clinical signs often first evident at 4 to 12 months of age. The laxity joints are not present at birth but may be detectable as early as 7 weeks of age. (Riser *et al*., 1985; Wallace *et al*., 1995; Piermattei *et al.,* 2006).

In present study equal number of males (9/18; 50%) and females (9/18; 50%) were recorded. The findings of the present study differ from the findings of Shiju Simon *et al*. (2010) who reported male dogs are more vulnerable to hip dysplasia than female and bilateral hip dysplasia are more common as compare to unilateral. This could be due to less number of samples included in this study. However, multiple large prevalence studies have shown that predilection of hip dysplasia is not associated with sex (Rettenmaier *et al.,* 2002; Hou *et al.,* 2010 and Runge *et al.,* 2010; Krontveit *et al.,* 2012a and Hou *et al.,* 2013).

Most of the animals affected from hip dysplasia recorded from Labrador retriever breed (11/18; 61%) in present study followed by Rottweiler (3/ 18; 17%), German shepherd (2/18; 11%) and Golden retriever (2/18; 11%) respectively that belongs to medium-large category. Our findings are supported with the findings of Dyce *et al*. (2000) in which 285 dogs for breed incidence of coxofemoral joint for hip dysplasia recorded and found highest incidence in Labrador Retriever (21%), German Shepherd Dogs (20%), Golden retriever (15%), , Rottweiler (6%) and cross bred (16%). LaFond *et al.,* (2002) conducted epidemiological study to determine breeds at risk for canine hip dysplasia (CHD) and reported that Labrador retriever, German Shepherd, Mastiff, Rottweiler, Saint Bernard, Golden Retriever breeds are more prone to hip dysplasia.

The feeds offered by the owners to their pets in our study were a combination of two or more, that includes rice, milk, boiled eggs, curd and commercial foods and calcium supplements (15/18; 83%) and rest (3/18; 17%) were strictly kept on vegetarian diet. Kasstrom (1975) and

Kealy *et al*. (1992) studied the relation of feed offered to the animals that develop CHD and conclude that over fed dogs grow faster than dogs fed a restricted diet and hence are more prone to the development of CHD. Fries and Remedios (1995) opined that excess dietary calcium and vitamin D may contribute to the development of canine hip dysplasia in genetically predisposed animals and should be avoided in young, rapidly growing dogs. Large-breed dog puppies fed with food high in calcium or high in calcium and phosphorus during growth that leads to disturbed endochondral ossification and delayed skeletal maturation and growth of bone length (Nap and Hazewinkel, 1994; Richardson *et al.,* 2010).

The animals were examined for clinical parameters like rectal temperature, heart rates and respiratory rates and results of these parameters among the groups at different time intervals did not show any significant difference. These findings are in accordance with the findings of Ranganath and Subin (2006) and Vishal (2011) who reported that there were no significant differences observed in the rectal temperature, heart rate and respiratory rate in dogs with and without hip dysplasia. Similarly, the haematological parameters also did not show any significant changes among the groups at different intervals of time. Similar findings were observed by Lipowitz and Newton (1985), Mala (2006) and Whiteside *et al.* (2006) in dogs suffering from degenerative joint disease.

In groups A, B and C, all the animals exhibited signs of hind limb lameness, pain in hip joint, abnormal gait and difficulty in rising. Bunny hopping gait was noticed in one dog in group A. Swaying of back were noticed in three animals in group A, two in group B and one animal in group C. Partial weight bearing lameness were exhibited in two animals in group A and one each in groups B and C. Atrophy of thigh musculature was noticed in three animals in group A, two in group C and one in group B. Other clinical signs such as crossing of hind limbs while lying down and straightening of stifle and hock joint were not noticed. The most common symptoms of CHD are joint pain, gait abnormalities, such as stiffness, reduced height of step, shortened stride length, bunny hopping, difficulty in rising, climbing stairs or in jumping over obstacles (Fry and Clark., 1992; Ginja *et al.,* 2008).

On days 90th the scores value for pain, lameness, ability to jump, ability to climb stairs and Ortolani sign were significantly differed amongst the groups A, B and C with best improvement shown by the animals in group C followed by groups B and A. Animals suffering

from hip dysplasia have stiffening of limb, loss of muscle over the thigh, pain and creaking (crepitus) on movement and limping could be due to osteoarthritis in hip joint. Multipotent stem cells secret various types of growth factors and anti-inflammatory proteins against inflammatory molecules (e.g. IL-1, IL-2, IL-12, TNF and INF-g) (Aggrawal and Pittenger, 2005). Intra-articular stem cell injection into artificially induced injury in knee joint showed decrease in the progression of osteoarthritis in caprine model (Murphy *et al.,* 2003). The better scores value for the clinical sign shown by the animals in group B could be due to intra-articular injection of BMNCs. Campbell *et al.,* (2015) reported better improvement in pain score with intra-articular injection of activated platelets as therapy for cartilage degenerative condition as compared to hyaluronic acid (HA). Studies have also report that PRP contributes to an anti-inflammatory effect through HGF and TNF-α reduction of NF-κB trans activating activity and target gene expression in chondrocytes, while preventing monocyte chemotaxis by expression of TGF-β1 countering the effect on chemokine transactivation by TNF-α. HGF plays a predominant role in the anti-inflammatory effect exerted by PRP, by inhibiting NF-κB activity, which, upon the blockade of HGF by the competitive inhibitor NK4, the inhibition on NF-κB activity almost nullified Bendinelli *et al.* (2010). In group C the animals were treated with the combination of BMNCs and activated platelets intra-articularly therefore, best results in improvement in clinical sign observed in the animals under this group could be due to combined effects of the therapeutic regimens.

On days 90th the scores value for Norberg angle, mean value of percent femoral head coverage and distraction index were significantly differed amongst the groups A, B and C with best improvement shown by the animals in group C followed by groups B and A. Mesenchymal stem cells (MSCs), a component of mononuclear cells are highly chondrogenic cells capable of self-renewal and multilineage differentiation into bone and cartilage. (Yoo *et al.,* 1998; Pittenger *et al.,* 1999). In the present study cells were directly implanted into hip joint cavity, better score for radiographic parameters in group B in the present study could be direct contact of implanted cells with synovial fluid of implanted hip joints that contains numerous cytokines and growth factor that influence better proliferation and differentiation of cells in the joint cavity. Our hypothesis corroborated with the findings of Chen *et al.,* (2005) who reported that components of the joint cavity, like synovial fluid or synovial cells, induce chondrogenesis in BMNCs *in vitro*. Another possible reason for better radiographic scores in group B as compared to group A could

be the interaction of implanted BMNCs with normal as well as injured chondrocytes in dysplastic hip joint, as chondrocytes might have been liberated in synovial fluid in dysplastic hip joint. The injured chondrocytes may liberate different cytokines that could promote better proliferation as well as differentiation of BMNCs towards chondrogenic lineage. In an *in vitro* study by Lettry *et al.,* (2010) it was proved that MSCs differentiates rapidly into chondrocytes when grown in co-cultures containing chondrocytes as compared to when grown alone. It has been reported that most of the intra-articularly injected MSCs migrated into the chondral defect and regenerate neocartilage, while residual MSCs migrate to synovium in the joints and produce trophic factors that plays chondroprotective effects. The immuno-modulatory effects of MSCs in dog joints have also been reported in several studies (Park *et al.,* 2013; Yun *et al.,* 2016 and Zhang *et al.,* 2018).

Platelet rich plasma (PRP) can be easily obtained on the day of surgery from autologous whole blood having a platelet concentration above baseline (Marx, 2001). It is a rich source of autologous growth factors, such as PDGF, TGF- b, IGF, VEGF, epithelial cell growth factor (ECGF) as well as other factors (Gotterbarm *et al.,* 2006; Ham *et al.,* 2012). In present study it is used as an alternative option to recombinant growth factors in group C. PRP promoted adipose-derived stem cells (ADSC) proliferation and differentiation into chondrogenic cells that strongly expressed collagen II, Sox9 and aggrecan. PRP-pre-treated ADSCs improved healing of injured articular cartilage in murine models compared with that of untreated ADSCs (Van-Pham *et al.,* (2013). In group C BMNCs were pre-treated with activated platelets prior to implantation into hip joint therefore, best results in improvement in radiographic scores observed in the animals under this group could be due to combined effects of the therapeutic regimens. The combination of MSCs and platelet rich plasma when injected intra-articularly, better histological score for healing of cartilage defect has been reported as compared to MSCs alone (Yun *et al.,* 2016).

C-reactive protein (CRP) is an acute phase protein synthesized in the liver. Its rate of synthesis increases within hours of acute injury and onset of inflammation reach as high as 20 times the normal levels. A rapid fall of CRP is an indication for recovery. The degree of elevation of CRP level directly reflects the activity of inflamed tissue and its ability to fall to normal levels on resolution of the condition renders quantified CRP values to be a good indicator in several rheumatic diseases. In present study, no significant differences were observed in the mean values

of C- reactive protein at various interval of time in group A. In group B and C, the mean values of C - reactive protein showed significantly different at respective intervals of time. The mean values of C-reactive protein showed minimal inflammatory reaction in hip joints of animals in groups C followed by groups B and A. The non-significant difference in CRP value in group A could be consistent arthritic changes in the hip joints of animals under this group which was also radiographically changes in hip joint visible in the animals under this group whereas, significant difference could be the results of anti-inflammatory cytokines released by the BMNCs in group B and combination of BMNCs and activated platelets in group C. CRP is a sensitive and specific marker for the presence or absence of systemic inflammatory activity (Ceron *et al.,* 2005; Kjelgaard-Hansen and Jacobsen, 2011). Additionally, it has been suggested that canine CRP could serve as a quantitative marker of systemic inflammation, as the observed serum concentrations have been reported to reflect the severity of an inflammation (Caspi *et al.,* 1987). CRP changes in systemic inflammation are not significantly influenced by NSAIDs. This makes sense as CRP production is mainly regulated by pro-inflammatory cytokines and NSAID only modulates prostaglandin synthesis (Hulton *et al.,* 1985).

Oxidative stress in the body represents an imbalance between the production of reactive oxygen species (ROS) and the ability of the antioxidant defense mechanisms in the body to detoxify the reactive intermediates. The greater the oxidative stress, the more severe the resulting cellular damage during that may cause poor outcome in tissue injury (Sies, 1997), and therefore minimization of oxidative stress is therefore very important.

Lipids are one of the most susceptible substrates to free radical damage and biomarkers of lipid peroxidation are considered the best indicators of oxidative stress (Georgieva, 2005). Malondialdehyde (MDA) is one of the several low-molecular-weight end-products formed during the radical induced decomposition of polyunsaturated fatty acid (Janero, 1990). MDA readily reacts with thiobarbituric acid producing a red pigment that can be easily measured by spectrophotometry in the form of thiobarbituric acid reactive substances (TBARS) (Janero, 1990).

There were no significant differences in mean value of Malondialdehyde observed among the groups A, B and C on days 0. On days 30th and 60th, the mean value of Malondialdehyde were significantly differed amongst the groups A, B and C. On days 90th, no significant

differences in the mean values of malondialdehyde were observed between groups B and C but it was significantly different from group A. Though there were no significant differences observed between the groups B and C albeit lower mean value of malondialdehyde observed in group C (5.34 ± 0.24) as compared to B (6.13 ± 0.12). Increase level of serum MDA have been reported in human patient with developmental dysplasia of the hip joint (Altay *et al.,* 2017). Nutraceutical supplementation decrease IL-6 and increase IL-10 which plays important role in regulation of inflammatory process and minimize the lipid peroxidation (Aggarwal and Shaheen*,* 2007; Popa *et al.,* 2007). In group A less value of MDA as compared to days 0 could be due to oral supplementation of nutraceuticals in the animals of this group. Platelets rich plasma is a potential therapeutic target for the treatment of osteoarthritis with or without the addition of stem cells, as it significantly decreases MMP3, MMP13, and ADAMDTS-5, IL-6 and COX-2 while simultaneously increase TGF-β, aggregan, collagen, TIMPs and intracellular anti-inflammatory cytokines IL-4, IL-10, and IL-13 (Moussa *et al.,* 2017). The lowest value of MDA in group C could be due to incorporation of activated platelets having anti-inflammatory properties and less oxidative stress. In group B though activated platelets not used for treatment protocols but the uncultured BMNCs have and cytokines and other cells that could mimic the inflammatory process.

On days 90^{th}, the mean value of catalase in group B and C were significantly different from group A. Though there were no significant difference observed between the groups B and C albeit higher mean value of catalase observed in group C (5.02 ± 0.09) as compared to group B (4.91 ± 0.15). The mean value of superoxide dismutase (SOD) and catalase (CAT) were significantly different among the groups on days 90^{th}. The mean value of glutathione peroxidase (GPx) on days 90^{th} was also significant and highest in group C (0.148 ± 0.001) followed by groups B (0.124 ± 0.000) and A (0.0850 ± 0.001) that further support better improvement in clinical sign of animals suffering from hip dysplasia in groups C followed by groups B and A. In osteoarthritis (OA) glutathione provides resistance and resilience to damage of cartilage from oxidative stress (Zhu *et al.,* 2019).

5 SUMMARY AND CONCLUSION

The present study was conducted on clinical cases of dogs with the history of hind limb(s) lameness brought to the Department of Surgery and Radiology, Bihar Veterinary College, Patna for treatment. The ventro-dorsal views of the recorded pelvic radiographs were screened for hip dysplasia based on the visible radiographic changes present in the hip joints, out of 217 recorded cases 109 cases (51 %) were diagnosed for hip dysplasia and remaining 108 cases (49%) were of different affection related to pelvic bone and neurological disorders.

A written consent was taken from the owners to include their animals under this study. Eighteen dogs suffering from hip dysplasia of either sex were used in this study. The animals were randomly divided into three groups A, B and C consisting of six animals each to study the effect of biomaterials i.e. once in affected hip joint with uncultured autologous bone marrow derived mono-nuclear cells (BMNCs) (4.35 (±0.07) x 10^6) (group B), BMNCs (4.35 (±0.07) x 10^6) and activated platelets (2.54 (±0.12) x 10^8) (group C) and nutraceuticals orally for three months and 1ml RPMI intra-articularly once (group A-Control). The viability of mesenchymal stem cells was assessed by 'trypan blue exclusion test' using trypan blue dye and 95.89% of BMNCs were viable at the time of implantation.

Complete history regarding breed, age, sex, body weight, type of breeds viz. small, medium and large, usefulness of animal i.e. whether working or non-working, duration of lameness, difficulty in climbing staircase, vaccination, deworming schedule and food habits were noted.

Breed wise distribution of animals showed that maximum number of cases were reported in Labrador breed (61%) of dogs followed by Rottweiler (17%), German shepherd (11%) and Golden retriever (11%) respectively. Age wise distribution of animals showed that maximum number of animals were reported in young dogs in the age group of 6 to 12 months (67%) followed by dogs of by 0-6 months (11%), 12-18 months (11%) and 18-24 months (11%) respectively. The body weight of the animals varied from 12 to 45 kg with maximum numbers of animals were belongs to 20-30 Kg (67%). There were equal number of males nine (50%) and nine females (50%). All the animals were medium sized breeds.

Out of eighteen dogs, eleven animals (61.11%) showed reluctance to climb the staircase whereas, rest 7 dogs (39%) were normally climbed the stairs. Thirteen animals (72%) were kept on floor with tiles and remaining five (5/18; 28%) had plaster floor. All animals were vaccinated as per the scheduled for specific vaccine and dewormed with suitable anthelmintic agents. The most of the animals (83%) were fed upon a combination of two or more feeds that includes rice, milk, boiled eggs, curd and commercial food and rest (17%) were strictly kept on vegetarian diet.

Physiological parameters such as rectal temperature (ºF), heart rate (beats/min.), respiratory rate (breaths/min.) and colour of mucous membrane were recorded at four-week interval for twelve weeks. All these parameters at different time intervals were within the normal reference range and did not show any statistically significant difference.

The clinical signs showed by the animals included signs of hind limb lameness, pain in hip joint, abnormal gait, difficulty in rising, bunny hopping gait, swaying of back, partial weight bearing lameness and atrophy of thigh musculature.

The animals were scored for assessing clinical signs based on pain on palpation, lameness, ability to jump and ability to climb stairs at different time intervals. In groups A, B and C, all the animals exhibited signs of hind limb lameness, pain in hip joint, abnormal gait and difficulty in rising. On days 90^{th} the scores value for pain, lameness, ability to jump, ability to climb stairs and Ortolani sign were significantly differed amongst the groups A, B and C with best improvement shown by the animals in group C followed by groups B and A. The scores for Norberg angle, mean value of percent femoral head coverage and distraction index were significantly differed amongst the groups A, B and C on days 90th with best improvement shown by the animals in group C followed by groups B and A.

C-reactive protein (CRP) is an acute phase protein synthesized in the liver. Its rate of synthesis increases within hours of acute injury and onset of inflammation reach as high as 20 times the normal levels. A rapid fall of CRP is an indication for recovery. In present study, no significant differences were observed in the mean values of C- reactive protein at various interval of time in group A. In group B and C, the mean values of C - reactive protein showed significantly different at respective intervals of time. The mean values of C-reactive protein

showed minimal inflammatory reaction in hip joints of animals in groups C followed by groups B and A.

Oxidative stress in the body represents an imbalance between the production of reactive oxygen species (ROS) and the ability of the antioxidant defence mechanisms in the body to detoxify the reactive intermediates. The greater the oxidative stress, the more severe the resulting cellular damage during that may cause poor outcome in tissue injury, and therefore minimization of oxidative stress is therefore very important. Lipids are one of the most susceptible substrates to free radical damage and biomarkers of lipid peroxidation malondialdehyde (MDA) is considered the best indicators of oxidative stress formed during the radical induced decomposition of polyunsaturated fatty acid. On days 90^{th}, no significant differences in the mean values of malondialdehyde were observed between the groups B and C but it was significantly different from group A. Though there were no significant differences observed between the groups B and C albeit lower mean value of malondialdehyde observed in group C (5.34 ± 0.24) as compared to B (6.13 ± 0.12). The lowest value of MDA in group C could be due to incorporation of activated platelets having anti-inflammatory properties and less oxidative stress. In group B though activated platelets not used for treatment protocols but the uncultured BMNCs have and cytokines and other cells that could mimic the inflammatory process.

On 90^{th} day, the mean value of catalase in group B and C were significantly different from group A. Though there were no significant difference observed between the groups B and C albeit higher mean value of catalase observed in group C (5.02 ± 0.09) as compared to group B (4.91 ± 0.15). The mean value of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were significantly different among the groups on days 90^{th}. In osteoarthritis (OA) glutathione provides resistance and resilience to damage of cartilage from oxidative stress. On days 90^{th} the mean value of glutathione reductase was also significant and highest in group C (0.148 ± 0.001) followed by groups B (0.124 ± 0.000) and A (0.0850 ± 0.001) that further support better improvement in clinical sign of animals suffering from hip dysplasia in groups C followed by groups B and A. On the basis of the results of the study it was concluded that

1. The Nutraceuticals can ameliorate the clinical signs associated with canine hip dysplasia.
2. The implantation of uncultured autologous BMNCs in hip joint can improve the clinical signs and augment the healing of cartilage in canine hip dysplasia.

3. A combination of BMNCs and activated platelets further improve the clinical signs and healing of cartilage in canine hip dysplasia.

6 LITERATURE CITED

Adams, W. M., Dueland, R. T., Daniels, R., Fialkowski, J. P., & Nordheim, E. V. (2000). Comparison of two palpation, four radiographic and three ultrasound methods for early detection of mild to moderate canine hip dysplasia. *Veterinary Radiology & Ultrasound*, ***41*****(6),** 484-490.

Aebi, H. E., and Bergmeyer, H. O. (1983). Catalase, methods enzymology. *Academic, New York*, ***2***, 349-359.

Aggarwal, B. B. (2003). Signalling pathways of the TNF superfamily: a double-edged sword. *Nature reviews immunology*, ***3*****(9)**, 745.

Agarwal, S., & Shaheen, R. (2007). Stimulation of antioxidant system and lipid peroxidation by abiotic stresses in leaves of Momordica charantia. *Brazilian Journal of Plant Physiology*, ***19*****(2)**, 149-161.

Aggarwal, S., & Pittenger, M. F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*, ***105*****(4),** 1815-1822.

Akbari, F., Ansari Samani, R., Karimi, A., Mortazaei, S., Shahinfard, N., & Rafieian-Kopaei, M. (2013). Effect of turnip on glucose and lipid profiles of alloxan-induced diabetic rats. *Iranian Journal of Endocrinology and Metabolism*, ***14*****(5),** 1-7.

Akeda, K., An, H. S., Okuma, M., Attawia, M., Miyamoto, K., Thonar, E. M. & Masuda, K. (2006). Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. *Osteoarthritis and Cartilage*, ***14*****(12),** 1272-1280.

Akeda, K., An, H. S., Pichika, R., Attawia, M., Eugene, J. M. T., Lenz, M. E., Uchida A & Masuda, K. (2006). Platelet-rich plasma (PRP) stimulates the extracellular matrix metabolism of porcine nucleus pulposus and anulus fibrosus cells cultured in alginate beads. *Spine*, ***31***(9), 959-966.

Akhlaghi, M., Shabanian, G., Rafieian-Kopaei, M., Parvin, N., Saadat, M., & Akhlaghi, M. (2011). Citrus aurantium blossom and preoperative anxiety. *Revista Brasileira de Anestesiologia*, ***61*****(6),** 707-712.

Alexander, J. W. (1992). The pathogenesis of canine hip dysplasia. *Veterinary Clinics of North America: Small Animal Practice*, ***22*****(3)**, 503-511.

Altay, M. A., Ertürk, C., Levent, A., Çetin, B. V., & Aksoy, N. (2017). Serum prolidase activity and oxidative–antioxidative status in patients with developmental dysplasia of the hip and its relationship with radiographic severity. *Redox Report*, ***22*****(5)**, 227-234.

Anderson, A. (2011). Treatment of hip dysplasia. *Journal of Small Animal Practice*, ***52*****(4),** 182-189.

Andrews, C. M., Liska, W. D., & Roberts, D. J. (2008). Sciatic neurapraxia as a complication in 1000 consecutive canine total hip replacements. *Veterinary Surgery*, ***37*****(3)**, 254-262.

Aragon, C. L., Hofmeister, E. H., & Budsberg, S. C. (2007). Systematic review of clinical trials of treatments for osteoarthritis in dogs. *Journal of the American Veterinary Medical Association*, ***230*****(4)**, 514-521.

Aslan, H., Zilberman, Y., Kandel, L., Liebergall, M., Oskouian, R. J., Gazit, D., & Gazit, Z. (2006). Osteogenic differentiation of noncultured immunoisolated bone marrow-derived CD105+ cells. *Stem Cells*, ***24*****(7)**, 1728-1737.

Bahmani, M., Vakili Saatloo, N., Maghsoudi, R., Momtaz, H., Saki, K., Kazemi-Ghoshchi, B., & Emami, F. (2013). A comparative study on the effect of ethanol extract of wild Scrophularia deserti and streptomycin on *Brucellla melitensis*. *Journal of HerbMed Pharmacology*, ***2***, 17-20.

Baradaran, A., Madihi, Y., Merrikhi, A., Rafieian-Kopaei, M., & Nasri, H. (2013). Serum lipoprotein (a) in diabetic patients with various renal function not yet on dialysis. *Pakistan Journal of Medical Sciences*, ***29*****(S),** 354-357.

Baradaran, A., Nasri, H., Nematbakhsh, M., & Rafieian-Kopaei, M. (2014). Antioxidant activity and preventive effect of aqueous leaf extract of Aloe Vera on gentamicin-induced nephrotoxicity in male Wistar rats. *La Clinica Terapeutica*, ***165*****(1),** 7-11.

Barbosa, A.L.T., Del Carlo, R.J., Gomes, H.C., Oliveira, A.C., Monteiro, B.S., and Del Carlo, B.N. (2008). Platelet-rich plasma for the repair of bone defects in dogs. *Rural Science* , ***38*** **(5),** 1335-1340.

Bardens, J. W., & Hardwick, H. (1968). New observations on the diagnosis and cause of hip dysplasia. *Veterinary Medicine, Small Animal Clinician: VM, SAC*, ***63*****(3)**, 238-245.

Barlow, T. G. (1962). Early diagnosis and treatment of congenital dislocation of the hip. *The Journal of Bone and Joint Surgery. British volume*, ***44*****(2)**, 292-301.

Barr, A. R. S., Denny, H. R. and Gibbs, C. (1987). Clinical hip dysplasia in growing dogs: the long-term results of conservative management. Journal of Small Animal Practice, 28: 243–252.

Batista, M. A., Leivas, T. P., Rodrigues, C. J., Arenas, G. C. F., Belitardo, D. R., & Guarniero, R. (2011). Comparison between the effects of platelet-rich plasma and bone marrow concentrate on defect consolidation in the rabbit tibia. *Clinics*, ***66*****(10),** 1787-1792.

Becton-Dickinson. (1998) Unopette WBC determination for manual methods. Rutherford, N.J. Becton, Dickinson, and Company.

Bendinelli, P., Matteucci, E., Dogliotti, G., Corsi, M. M., Banfi, G., Maroni, P., & Desiderio, M. A. (2010). Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: Mechanisms of NF-κB inhibition via HGF. *Journal of Cellular Physiology*, ***225*****(3),** 757-766.

Benzioni, H., Shahar, R., Yudelevitch, S., & Milgram, J. (2008). Bacterial infective arthritis of the coxofemoral joint in dogs with hip dysplasia. *Veterinary and Comparative Orthopaedics and Traumatology*, ***21*****(03)**, 262-266.

Berg, L. C., Koch, T. G., Heerkens, T., Besonov, K., Thomsen, P. D., & Betts, D. H. (2009). Chondrogenic potential of mesenchymal stromal cells derived from equine bone marrow and umbilical cord blood. *Veterinary and Comparative Orthopaedics and Traumatology*, ***22*****(05),** 363-370.

Berg-Foels, W. S. V., Todhunter, R. J., Schwager, S. J., & Reeves, A. P. (2006). Effect of early postnatal body weight on femoral head ossification onset and hip osteoarthritis in a canine model of developmental dysplasia of the hip. *Pediatric Research*, ***60*****(5)**, 549.

Black, L. L., Gaynor, J., Adams, C., Dhupa, S., Sams, A. E., Taylor, R., & Harman, R. (2008). Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. *Veterinary Therapeutics: Research in Applied Veterinary Medicine*, ***9*****(3),** 192-200.

Black, L. L., Gaynor, J., Gahring, D., Adams, C., Aron, D., Harman, S., & Harman, R. (2007). Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter controlled trial. *Veterinary Therapeutics*, ***8*****(4),** 272.

Bockstahler, B., Mueller, M., Skalicky, M., Mlacnik, E., & Lorinson, D. (2007). Effects of radial shockwave therapy on the limb function of dogs with hip osteoarthritis. *Veterinary Record*, ***160*****(22)**, 762-765.

Borena, B. M., Pawde, A. M., Aithal, H. P., Kinjavdekar, P., Singh, R., & Kumar, D. (2009). Evaluation of healing potential of autologous bone marrow-derived nucleated cells on incisional wounds in dogs. *Indian Journal of Veterinary Surgery*, ***30*****(2)**, 85-89.

Boswell, S. G., Cole, B. J., Sundman, E. A., Karas, V., & Fortier, L. A. (2012). Platelet-rich plasma: a milieu of bioactive factors. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, ***28(3),*** 429-439.

Braden, T. D., Olivier, N. B., Blaiset, M. A., Averill, S. M., Bolliger, C., & DeCamp, C. E. (2004). Objective evaluation of total hip replacement in 127 dogs utilizing force plate analysis. *Veterinary and Comparative Orthopaedics and Traumatology*, ***17*****(02)**, 78-81.

Brass, W. (1989). Hip dysplasia in dogs. *Journal of Small Animal Practice*, ***30*****(3),** 166-170.

Braun, D., Lautersack, O., Schimke, E. and Braun, D.G. (2003). Dorsal acetabular denervation of capsule in the dog - a long-term study results. *Small Anim. Vet. Pract.* **4**: 185-244.

Brinker, W.O., Piermattei D.L. and Flo G.L. (1990). Hand Book of Small Animal Orthopaedics and Fracture Management, **(2), W.B**. Saunders Company, Philadelphia. 341-377.

Campbell, K. A., Saltzman, B. M., Mascarenhas, R., Khair, M. M., Verma, N. N., Bach Jr, B. R., & Cole, B. J. (2015). Does intra-articular platelet-rich plasma injection provide clinically superior outcomes compared with other therapies in the treatment of knee osteoarthritis? A systematic review of overlapping meta-analyses. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, ***31*****(11),** 2213-2221.

Canapp, J. S., McLaughlin, J. R., Hoskinson, J. J., Roush, J. K., & Butine, M. D. (1999). Scintigraphic evaluation of dogs with acute synovitis after treatment with glucosamine hydrochloride and chondroitin sulfate. *American Journal of Veterinary Research*, ***60*****(12)**, 1552-1557.

Cardinet, G.H., Kass, P.H. and Wallace, L.J. (1997). Association between pelvic muscle mass and canine hip dysplasia. *J. Am. Vet. Med. Ass.* **210**: 1446.

Carney, S. L., & Muir, H. (1988). The structure and function of cartilage proteoglycans. *Physiological Reviews*, ***68*****(3)**, 858-910.

Carrade, D. D., Owens, S. D., Galuppo, L. D., Vidal, M. A., Ferraro, G. L., Librach, F., & Borjesson, D. L. (2011). Clinicopathologic findings following intra-articular injection

of autologous and allogeneic placentally derived equine mesenchymal stem cells in horses. *Cytotherapy*, ***13*****(4),** 419-430.

Caspi, D., Snel, F. W. J. J., Batt, R. M., Bennett, D., Rutteman, G. R., Hartman, E. G., Baltz, M.L., Gruys, E., and Pepys, M. B. (1987). C-reactive protein in dogs. *Am J Vet Res*, ***48*****(6),** 919-921.

Centeno, C. J., Busse, D., Kisiday, J., Keohan, C., Freeman, M., & Karli, D. (2008). Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells. *Medical hypotheses*, ***71*****(6)**, 900-908.

Ceron JJ, Eckersall PD, Martinez-Subiela S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet Clin Pathol.* 2005;**34**:85–99.

Chalman, J. A., & Butler, H. C. (1985). Coxofemoral joint laxity and the Ortolani sign. *Journal of the American Animal Hospital Association*, ***21*****(5)**, 671-676.

Chapman, A., Frankel, M., and Garfinkel, M., American Association for the Advancement of Science. (1999). *Stem cell research and applications: monitoring the frontiers of biomedical research*.

Chen, J., Wang, C., Lü, S., Wu, J., Guo, X., Duan, C., and Wu, L. (2005). In vivo chondrogenesis of adult bone-marrow-derived autologous mesenchymal stem cells. *Cell and tissue research*, ***319*****(3),** 429-438.

Chen, L. X., Lin, L., Wang, H. J., Wei, X. L., Fu, X., Zhang, J. Y., & Yu, C. L. (2008). Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-κBp65-specific siRNA. *Osteoarthritis and Cartilage*, ***16*****(2)**, 174-184.

Cheng, X., Lei, D., Mao, T., Yang, S., Chen, F., & Wu, W. (2008). Repair of critical bone defects with injectable platelet rich plasma/bone marrow-derived stromal cells composite: experimental study in rabbits. *Turkish Journal of Trauma and Emergency Surgery*, ***14*****(2),** 87-95.

Choi, J., Kim, H., Kim, H., Jang, J., Kim, M., & Yoon, J. (2008). Evaluation for canine hip dysplasia in Golden and Labrador retrievers using PennHIP method in Korea. *Korean Journal of Veterinary Research*, ***48*****(3)**, 375-379.

Christensen, L. N., Mastbergen, S. C., Garssen, J., Hartog, A., Hazewinkel, H. A., & Lafeber, F. P. (2006, September). Effect of glucosamine combined with omega-3 fatty acids on the development of canine experimental osteoarthritis. In *ARTHRITIS AND RHEUMATISM* (Vol. 54, No. 9, pp. S568-S569). DIV JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA: WILEY-LISS.

Citi, S., Vignoli, M., Roni, F. and Morgan, J.P. (2005). A radiological study of the incidence of unilateral canine hip dysplasia. *Schwieiz. Arch. Tierheilkd*. **147**: 173-178.

Collard, F., Maitre, P., Quang, T.L., Fau, D., Carozzo, C., Genevois, J.P. and Viguier, E. Canine hip denervation: comparison between clinical outcome and gait analysis (9 cases). In: Collard, F. (ed.), *Proceedings of the European Society of Veterinary Orthopaedics and Traumatology;* 10^{th} to 14^{th} September, 2008 - ESVOT, Munich, Germany. European Society of Veterinary Orthopaedics and Traumatology. pp. 46-47.

Colleoni, S., Bottani, E., Tessaro, I., Mari, G., Merlo, B., Romagnoli, N., & Lazzari, G. (2009). Isolation, growth and differentiation of equine mesenchymal stem cells: effect of donor, source, amount of tissue and supplementation with basic fibroblast growth factor. *Veterinary Research Communications*, ***33*****(8),** 811.

Comhaire, F. H., & Schoonjans, F. A. (2011). Canine hip dyslasia: the significance of the Norberg angle for healthy breeding. *Journal of Small Animal Practice*, ***52*****(10),** 536-542.

Coopman, F., Verhoeven, G. and Saunders, J. (2008). Prevalance of hip dysplasia, elbow dysplasia and femoral head osteochondrosis in dog breeds in Belgium. *Vet. Rec*. **163**: 654.

Corley, E. A. (1992). Role of the Orthopedic Foundation for Animals in the control of canine hip dysplasia. *Veterinary Clinics of North America: Small Animal Practice*, ***22*****(3),** 579-593.

Corley, E. A., Keller, G. G., Lattimer, J. C., & Ellersieck, M. R. (1997). Reliability of early radiographic evaluations for canine hip dysplasia obtained from the standard ventrodorsal radiographic projection. *Journal of the American Veterinary Medical Association*, ***211*****(9),** 1142-1146.

Corr, S. (2007). Hip dysplasia in dogs: treatment options and decision making. *In Practice*, ***29*****(2)**, 66-75.

Crovace A., Francioso E., Hendry J., & Quarto R., (2004). Use of autogenous bone marrow stromal cells (BMSC) and resorbable bone graft substitute in dogs with orthopaedic lesions. Proceedings of ACVS *Veterinary Symposium*, 199–201.

Crovace, A., Favia, A., Lacitignola, L., Di Comite, M. S., Staffieri, F., & Francioso, E. (2008). Use of autologous bone marrow mononuclear cells and cultured bone marrow stromal cells in dogs with orthopaedic lesions. *Veterinary Research Communications*, ***32*****(S1)**, 39–44.

Crovace, A., Lacitignola, L., De Siena, R., Rossi, G., & Francioso, E. (2007). Cell therapy for tendon repair in horses: an experimental study. *Veterinary Research Communications*, ***31***, 281-283.

Crovace, A., Lacitignola, L., Rossi, G., & Francioso, E. (2010). Histological and immunohistochemical evaluation of autologous cultured bone marrow mesenchymal stem cells and bone marrow mononucleated cells in collagenase-induced tendinitis of equine superficial digital flexor tendon. *Veterinary Medicine International*, *2010*. 250978. 10.4061/2010/250978.

Culp, W. T., Kapatkin, A. S., Gregor, T. P., Powers, M. Y., Mckelvie, P. J., & Smith, G. K. (2006). Evaluation of the Norberg angle threshold: a comparison of Norberg angle and

distraction index as measures of coxofemoral degenerative joint disease susceptibility in seven breeds of dogs. *Veterinary Surgery*, ***35*****(5),** 453-459.

Daheshia, M., & Yao, J. Q. (2008). The interleukin 1β pathway in the pathogenesis of osteoarthritis. *The Journal of rheumatology*, ***35*****(12)**, 2306-2312.

D'Amico, L. L., Xie, L., Abell, L. K., Brown, K. T., & Lopez, M. J. (2011). Relationships of hip joint volume ratios with degrees of joint laxity and degenerative disease from youth to maturity in a canine population predisposed to hip joint osteoarthritis. *American Journal of Veterinary Research*, ***72*****(3),** 376-383.

Dassler, C.L. (2003). Canine hip dysplasia: Diagnosis and non surgical treatment. In: Text Book of Small Animal Surgery, Vol 2, Slatter, D.H. (Eds.). 3rd edn., W.B. Saunders Company, Philadelphia. pp. 2019-2029.

DeCamp, C. E. (2015). *Brinker, Piermattei and Flo's handbook of small animal orthopedics and fracture repair*. Elsevier Health Sciences.

Dennis, R. (2012). Interpretation and use of BVA/KC hip scores in dogs. *In Practice*, ***34*****(4)**, 178-194.

Dezateux, C., & Rosendahl, K. (2007). Developmental dysplasia of the hip. *The Lancet*, ***369*****(9572)**, 1541-1552.

Doig, P. A., Purbrick, K. A., Hare, J. E., & McKeown, D. B. (2000). Clinical efficacy and tolerance of meloxicam in dogs with chronic osteoarthritis. *The Canadian Veterinary Journal*, ***41*****(4)**, 296.

Dyce, J., Wisner, E. R., Wang, Q., & Olmstead, M. L. (2000). Evaluation of risk factors for luxation after total hip replacement in dogs. *Veterinary Surgery*, ***29*****(6),** 524-532.

Evans, H.E. and Lahunta, A.D. (1993). *Miller's Anatomy of the Dog*, **(3),** W.B. Saunders Company, Philadelphia, 850.

Everts, P. A., Brown Mahoney, C., Hoffmann, J. J., Schönberger, J. P., Box, H. A., Van Zundert, A., & Knape, J. T. (2006). Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors*, ***24*****(3)**, 165-171.

Farese, J. P., Lust, G., Williams, A. J., Dykes, N. L., & Todhunter, R. J. (1999). Comparison of measurements of dorsolateral subluxation of the femoral head and maximal passive laxity for evaluation of the coxofemoral joint in dogs. *American Journal of Veterinary Research*, ***60*****(12),** 1571-1576.

Farese, J. P., Todhunter, R. J., Lust, G., Williams, A. J., & Dykes, N. L. (1998). Dorsolateral subluxation of hip joints in dogs measured in a weight-bearing position with radiography and computed tomography. *Veterinary Surgery***, *27*****(5),** 393-405.

Farrell, M., Clements, D. N., Mellor, D., Gemmill, T., Clarke, S. P., Arnott, J. L., & Carmichael, S. (2007). Retrospective evaluation of the long-term outcome of non-surgical management of 74 dogs with clinical hip dysplasia. *Veterinary Record*, ***160*****(15)**, 506-511.

Ferrigno, C.R.A., Schmaedecke, A., Oliveira, L.M., D'Avila, R.S., Yamamoto, E.Y. and Saut, J.P.E. (2007). Cranial and dorsal acetabular denervation technique in treatment of hip dysplasia in dogs: 360 days evaluation of 97 cases. *Brazilian J. Vet. Res.* **27**(8): 333-340.

Flecknell, P. A., & Waterman-Pearson, A. (2000). *Pain management in animals*. WB Saunders.

Flückiger, M. (2007). Scoring radiographs for canine hip dysplasia-The Big three Organizations in the world. *European Journal of Companion Animal Practice*, ***17*****(2)**, 135-140.

Flückiger, M. A., Friedrich, G. A., & Binder, H. (1999). A radiographic stress technique for evaluation of coxofemoral joint laxity in dogs. *Veterinary Surgery*, ***28*****(1)**, 1-9.

Freed, C. R., Greene, P. E., Breeze, R. E., Tsai, W. Y., DuMouchel, W., Kao, R., ... & Eidelberg, D. (2001). Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *New England Journal of Medicine*, ***344*****(10),** 710-719.

Fries, C. L., & Remedios, A. M. (1995). The pathogenesis and diagnosis of canine hip dysplasia: a review. *The Canadian Veterinary Journal***,** ***36*****(8),** 494.

Frost, H. M. (1989). Pathogenesis of congenital hip dysplasia (CDH). A proposal. *Veterinary and Comparative Orthopaedics and Traumatology*, ***2*****(01)**, 1-10.

Fry, T. R., & Clark, D. M. (1992). Canine hip dysplasia: clinical signs and physical diagnosis. *The Veterinary Clinics of North America. Small Animal Practice*, ***22*****(3),** 551-558.

Frykberg, R. G., Driver, V. R., Carman, D., Lucero, B., Borris-Hale, C., Fylling, C. P., & Clausen, P. A. (2010). Chronic wounds treated with a physiologically relevant concentration of platelet-rich plasma gel: a prospective case series. *Ostomy/wound management*, ***56*****(6),** 36.

Gaspar, A. R., Hayes, G., Ginja, C., Ginja, M. M., & Todhunter, R. J. (2016). The Norberg angle is not an accurate predictor of canine hip conformation based on the distraction index and the dorsolateral subluxation score. *Preventive Veterinary Medicine*, **135**, 47-52.

Gatineau, M., Dupuis, J., Beauregard, G., Charette, B., Breton, L., Beauchamp, G., & d'Anjou, M. A. (2012). Palpation and dorsal acetabular rim radiographic projection for early detection of canine hip dysplasia: a prospective study. *Veterinary Surgery***,** ***41*****(1),** 42-53.

Gendreau, C., & Cawley, A. J. (1977). Excision of femoral-head and neck-long-term results of 35 operations. *Journal of the American Animal Hospital Association*, ***13*****(5)**, 605-608.

Genevois, J. P., Fau, D., Brunon, G., Crance, L., & Remy, D. (2000). Hip dysplasia: epidemiologic considerations based on the analysis of 9738 radiographs. *Revue de Médecine Vétérinaire*, ***151*****(8/9),** 829-834.

Genevois, J.P., Remy, D.E., Viguier, E., Carozzo, C., Collard, F., Cachon, T., Maitre, P. and Fau, D. (2008). Prevalence of hip dysplasia according to official radiographic screening, among 31 breeds of dogs in France. *Veterinary Comparative Orthopaedics and Traumatol*. **21**: 21-24.

Georgieva, N. V. (2005). Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems—a review. *Bulgarian Journal of Veterinary Medicine*, *8*(1), 1-11.

Gerwin, N., Hops, C., and Lucke, A., (2006). Intra-articular drug delivery in osteoarthritis. *Adv. Drug. Delivery. Rev.,* ***58***: 226-242.

Gibbs, C. (1997). The BVA/KC scoring scheme for control of hip dysplasia: interpretation of criteria. *The Veterinary Record*, ***141*****(11)**, 275.

Ginja, M. M. D., Gonzalo-Orden, J. M., Melo-Pinto, P., Bulas-Cruz, J., Orden, M. A., San Roman, F., & Ferreira, A. J. A. (2008b). Early hip laxity examination in predicting moderate and severe hip dysplasia in Estrela mountain dog. *Journal of Small Animal Practice*, ***49*****(12),** 641-646.

Ginja, M. M. D., Silvestre, A. M., Colaço, J., Gonzalo-Orden, J. M., Melo-Pinto, P., Orden, M. A. & Ferreira, A. J. (2008a). Hip dysplasia in Estrela mountain dogs: Prevalence and genetic trends 1991–2005. *The Veterinary Journal*, ***182*****(2)**, 275-282.

Ginja, M. M. D., Silvestre, A. M., Gonzalo-Orden, J. M., & Ferreira, A. J. A. (2010). Diagnosis, genetic control and preventive management of canine hip dysplasia: a review. *The Veterinary Journal*, ***184*****(3),** 269-276.

Ginja, M. M., Ferreira, A. J., Jesus, S. S., Melo-pinto, P., Bulas-cruz, J., Orden, M. A., ... & Gonzalo-orden, J. M. (2009). Comparison of clinical, radiographic, computed tomographic, and magnetic resonance imaging methods for early prediction of canine hip laxity and dysplasia. *Veterinary radiology & ultrasound*, ***50*****(2),** 135-143.

Ginja, M., Gaspar, A.R. and Ginja, C. (2015). Emerging insights into the genetic basis of canine hip dysplasia. *Vet. Med. Res. Rep.* **6**: 193-202.

Ginja, M., Llorens-Pena, M., Gonzalo-Orden, J., & Ferreira, A. (2007). Mechanical devices to help in PennHIP examination. *Acta Veterinaria Hungarica*, ***55*****(2),** 199-205.

Gladstein, B. (2012). A case for prolotherapy and its place in veterinary medicine. *J of Prolotherapy*, *4*, e870-e885.

Gladstein, B. 2010. Canine hip dysplasia. J. Prolotherapy. **2**: 387-390.

Goldring, S. R., and Goldring, M. B. (2006). Clinical aspects, pathology and pathophysiology of osteoarthritis. Journal of Musculoskeletal and Neuronal Interactions, ***6(4)***, 376.

Gossec, L., and Dougados, M. (2004). Intra-articular treatments in osteoarthritis: from the symptomatic to the structure modifying. Annals of the rheumatic diseases, ***63(5)***, 478-482.

Gotterbarm, T., Richter, W., Jung, M., Vilei, S. B., Mainil-Varlet, P., Yamashita, T., & Breusch, S. J. (2006). An in vivo study of a growth-factor enhanced, cell free, two-layered collagen–tricalcium phosphate in deep osteochondral defects. *Biomaterials*, **27(18),** 3387-3395.

Greene, L. M., Little, D. J. M. and Lascelles, B. D. X. 2013. Associations among exercise duration, lameness severity, and hip joint range of motion in Labrador Retrievers with hip dysplasia.Journal of American Veterinary Medical Association, 242: 1528–1533.

Gronthos, S., & Zannettino, A. C. (2008). A method to isolate and purify human bone marrow stromal stem cells. In *Mesenchymal Stem Cells* (pp. 45-57). Humana Press.

Guest, D. J., Smith, M. R. W., & Allen, W. R. (2008). Monitoring the fate of autologous and allogeneic mesenchymal progenitor cells injected into the superficial digital flexor tendon of horses: preliminary study. *Equine veterinary journal*, ***40*****(2),** 178-181.

Gunetti, M., Castiglia, S., Rustichelli, D., Mareschi, K., Sanavio, F., Muraro, M., Signorino, E., Castello, L., Ferrero, I. & Fagioli, F. (2012). Validation of analytical methods in GMP: the disposable Fast Read 102® device, an alternative practical approach for cell counting. *Journal of translational medicine*, ***10*****(1)**, 112.

Guo, G., Zhang, Z., Zhou, Z., Wang, Y., Zhao, K., Zhu, L., Lust, G., Hunter, L., Friedenberg, S., Li, J., Zhang, Y., Harris, S., Jones, P., Sandler, J., Krostscheck, U., Todhunter, R. & Zhang, Z. (2011). Canine hip dysplasia is predictable by genotyping. Osteoarthritis and Cartilage, ***19(4)***, 420–429.

Guo, G., Zhang, Z., Zhou, Z., Wang, Y., Zhao, K., Zhu, L., Lust, G., Hunter, L., Friedenberg, S., Li, J., Zhang, Y., Harris, S., Jones, P., Sandler, J., Krostscheck, U., Todhunter, R. & Zhang, Z. (2011). Canine hip dysplasia is predictable by genotyping. *Osteoarthritis and Cartilage, **19(4),*** 420–429.

Hagen, M.A.V, Ducro, B.J. and Broek, V.D.J. (2005). Incidence, risk factors and heritability estimates of hind limb lameness caused by hip dysplasia in a birth cohort of Boxers. *Am. J. Vet. Res.* **66**: 307-312.

Halpern, B., Chaudhury, S., Rodeo, S. A., Hayter, C., Bogner, E., Potter, H. G., & Nguyen, J. (2013). Clinical and MRI outcomes after platelet-rich plasma treatment for knee osteoarthritis. *Clinical Journal of Sport Medicine*, ***23*****(3)**, 238-239.

Ham, O., Song, B. W., Lee, S. Y., Choi, E., Cha, M. J., Lee, C. Y., & Lee, C. H. (2012). The role of microRNA-23b in the differentiation of MSC into chondrocyte by targeting protein kinase A signaling. *Biomaterials***, *33*****(18),*** 4500-4507.

Harris, M. T., Butler, D. L., Boivin, G. P., Florer, J. B., Schantz, E. J., and Wenstrup, R. J. (2004). Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. *Journal of orthopaedic research*, ***22*****(5),*** 998-1003.

Hedhammar, A., Olsson, S. E., Andersson, S. A., Persson, L., Pettersson, L., Olausson, A., & Sundgren, P. E. (1979). Canine hip dysplasia: study of heritability in 401 litters of German Shepherd dogs. *Journal of the American Veterinary Medical Association*, ***174*****(9)**, 1012-1016.

Henrigson, B., Norberg, I., & Olssons, S. E. (1966). On the etiology and pathogenesis of hip dysplasia: a comparative review. *Journal of Small Animal Practice*, ***7*****(11),*** 673-688.

Herrero-beaumont, G., Guerrero, T., Sanchez-Pernaute, O., Acebes, C., Palacios, I., Mas, S., Rodriguez, I., Egido, J. and Vivanco, F., (2001). Cartilage and bone biological markers in the synovial fluid of osteoarthritic patients after hyaluronan injections in the knee. *Clin. Chim. Act.,* ***308*****:** 107-115.

Hosseini-Asl, K., & Rafieian-Kopaei, M. (2002). Can patients with active duodenal ulcer fast Ramadan?. *The American journal of gastroenterology*, ***97*****(9),** 2471.

Hou, Y., Wang, Y., Lu, X., Zhang, X., Zhao, Q., Todhunter, R. J., & Zhang, Z. (2013). Monitoring hip and elbow dysplasia achieved modest genetic improvement of 74 dog breeds over 40 years in USA. *PloS One*, ***8*****(10),** 76390.

Hou, Y., Wang, Y., Lust, G., Zhu, L., Zhang, Z., & Todhunter, R. J. (2010). Retrospective analysis for genetic improvement of hip joints of cohort labrador retrievers in the United States: 1970–2007. *PloS One*, ***5*****(2),** 9410.

Houlton, J.E.F., (1994). Ancillary aids to the diagnosis of joint disease, in Collinson RW, Houlton JEF (Eds): *Manual of Small Animal Arthrology*. Cheltenham, UK, British Small Animal Veterinary Association. 22-38.

Huang, C. H., Hou, S. M., & Yeh, L. S. (2013). The innervation of canine hip joint capsule: an anatomic study. *Anatomia, Histologia, Embryologia*, **42(6),** 425-431.

Hulse, D. (1998). Treatment methods for pain in the osteoarthritic patient. *Veterinary Clinics: Small Animal Practice*, ***28*****(2)**, 361-375.

Hulse, D.A. and A.L. Johnson. 2007. Management of joint disease. In: Small Animal Surgery, Fossum, T.W., 3rd edn. Mosby, St. Louis. Pp. 883-998.

Hulton, N. R., Johnson, D. J., & Wilmore, D. W. (1985). Limited effects of prostaglandin inhibitors in Escherichia coli sepsis. *Surgery*, ***98*****(2),** 291-297.

Hummel, D.W., Lanz, I.O. and Were, S.R., (2010). Complications of cementless total replacement - a retrospective study of 163 cases. *Vet. Comp. Ortho. Traumatol*. **23**: 424-232.

Hunziker, E. B. (1999). Articular cartilage repair: are the intrinsic biological constraints undermining this process insuperable?. *Osteoarthritis and Cartilage*, ***7*****(1)**, 15-28.

Hunziker, E. B., & Rosenberg, L. C. (1996). Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. *JBJS*, ***78*****(5)**, 721-33.

Iamaguti, P., Iamaguti, L.S. and Sartor, R. 2009. Acetabular deepening in the treatment of severe canine hip dysplasia. *Brazilian J. Vet. Res.* **29**(2):163-166.

Impellizeri, J. A., Tetrick, M. A., & Muir, P. (2000). Effect of weight reduction on clinical signs of lameness in dogs with hip osteoarthritis. *Journal of the American Veterinary Medical Association*, ***216*****(7)**, 1089-1091.

Ishida, K., Kuroda, R., Miwa, M., Tabata, Y., Hokugo, A., Kawamoto, T., & Kurosaka, M. (2007). The regenerative effects of platelet-rich plasma on meniscal cells in vitro and its in vivo application with biodegradable gelatin hydrogel. *Tissue engineering*, **13(5),** 1103-1112.

Jacques, D., Cauzinille. L., Bouvy, B. and Dupre, G., (2002). A Retrospective Study of 40 Dogs with Polyarthritis. *Vet. Surg., **31***: 428-434.

Jaegger, G., Marcellin-Little, D. J., & Levine, D. (2002). Reliability of goniometry in Labrador Retrievers. American journal of veterinary research, ***63(7)***, 979-986.

Jaiswal, N., Haynesworth, S. E., Caplan, A. I., & Bruder, S. P. (1997). Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *Journal of Cellular Biochemistry*, ***64*****(2)**, 295-312.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine***, 9(6),** 515-540.

Janssens, L., De Ridder, M., Verhoeven, G., Gielen, I., & van Bree, H. (2014). Comparing Norberg angle, linear femoral overlap and surface femoral overlap in radiographic assessment of the canine hip joint. *Journal of Small Animal Practice*, ***55*****(3),** 135-138.

Janutta, V., & Distl, O. (2006). Inheritance of canine hip dysplasia: review of estimation methods and of heritability estimates and prospects on further developments. *DTW. Deutsche tierarztliche Wochenschrift*, ***113*****(1)**, 6-12.

Janutta, V., Hamann, H., & Distl, O. (2005). Complex segregation analysis of canine hip dysplasia in German shepherd dogs. *Journal of Heredity*, ***97*****(1),** 13-20.

Johnson, A. L., & Hulse, D. A. (2007). Management of specific fractures. *Small animal surgery*, *3*, 1015-1142.

Johnston, S. A., McLaughlin, R. M., & Budsberg, S. C. (2008). Nonsurgical management of osteoarthritis in dogs. *Veterinary Clinics of North America: Small Animal Practice*, ***38*****(6)**, 1449-1470.

Jung, D. I., Ha, J., Kang, B. T., Kim, J. W., Quan, F. S., Lee, J. H., Woo, E.J. & Park, H. M. (2009). A comparison of autologous and allogenic bone marrow-derived mesenchymal stem cell transplantation in canine spinal cord injury. *Journal of the Neurological Sciences*, ***285*****(1-2)**, 67-77.

Kaderly, R. E., Anderson, B. G., & Anderson, W. D. (1983). Intracapsular and intraosseous vascular supply to the mature dog's coxofemoral joint. *American Journal of Veterinary Research*, ***44*****(10),** 1805-1812.

Kafash-Farkhad, N., Asadi-Samani, M., & Rafieian-Kopaei, M. (2013). A review on phytochemistry and pharmacological effects of Prangos ferulacea (L.) Lindl. *Life Science Journal*, ***10*****(S),** 360-367.

Kalra, K., & Tomar, P. (2014). Stem cell: basics, classification and applications. *American Journal of Phytomedicine and Clinical Therapeutics*, ***2*****(7)**, 919-930.

Kapatkin, A. S., Fordyce, H. H., Mayhew, P. D., & Smith, G. K. (2002). Canine hip dysplasia: the disease and its diagnosis. *Compendium*, ***24*****(7),** 526-36.

Kapatkin, A. S., Gregor, T. P., Hearon, K., Richardson, R. W., McKelvie, P. J., Fordyce, H. H., & Smith, G. K. (2004). Comparison of two radiographic techniques for evaluation of

hip joint laxity in 10 breeds of dogs. *Journal of the American Veterinary Medical Association*, ***224*****(4),** 542-546.

Karimi, A., Moradi, M. T., Saeedi, M., Asgari, S., & Rafieian-Kopaei, M. (2013). Antiviral activity of Quercus persica L.: high efficacy and low toxicity. *Advanced Biomedical Research*, ***2*****.** 36.

Kasstrom, H. (1975). Nutrition, weight gain and development of hip dysplasia: an experimental investigation in growing dogs with special reference to the effect of feeding intensity. *Acta Radiol. Suppl*. **344**: 135-179.

Kate, L.T. and Keith, E.M. (2006). Clinical and genetic assessments of hip joint laxity in the Boykin Spaniels. *Can. J. Vet. Res*. **70**(2): 148-150.

Kealy, R. D., Olsson, S. E., Monti, K. L., Lawler, D. F., Biery, D. N., Helms, R. W. & Smith, G. K. (1992). Effects of limited food consumption on the incidence of hip dysplasia in growing dogs. *Journal-American Veterinary Medical Association*, ***201***, 857-857.

Kafash-Farkhad, N., Asadi-Samani, M., & Rafieian-Kopaei, M. (2013). A review on phytochemistry and pharmacological effects of Prangos ferulacea (L.) Lindl. *Life Science Journal*, ***10*****(8)**, 360-367.

Khosravi-Boroujeni, H., Mohammadifard, N., Sarrafzadegan, N., Sajjadi, F., Maghroun, M., Khosravi, A., & Azadbakht, L. (2012). Potato consumption and cardiovascular disease risk factors among Iranian population. *International Journal of Food Sciences and Nutrition*, ***63*****(8)**, 913-920.

Khosravi-Boroujeni, H., Sarrafzadegan, N., Mohammadifard, N., Sajjadi, F., Maghroun, M., Asgari, S., & Azadbakht, L. (2013). White rice consumption and CVD risk factors among Iranian population. *Journal of Health, Population, and Nutrition*, ***31*****(2),** 252.

Kiani, M. A., Khodadad, A., Mohammadi, S., Ghayour Mobarhan, M., Saeidi, M., Jafari, S. A., & Ahanchian, H. (2013). Effect of peppermint on pediatrics' pain under endoscopic examination of the large bowel. *Journal of HerbMed Pharmacology*, ***2*****(2)**, 41-44.

Kim, D., Kim, C. H., Moon, J. I., Chung, Y. G., Chang, M. Y., Han, B. S., Ko, S., Yang, E., Cha, K.Y., Lanza, R. & Kim, K. S. (2009). Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell*, ***4*****(6),** 472.

Kim, Y. J., Bonassar, L. J., and Grodzinsky, A. J. (1995). The role of cartilage streaming potential, fluid flow and pressure in the stimulation of chondrocyte biosynthesis during dynamic compression. *Journal of biomechanics*, ***28*****(9),** 1055-1066.

Kim, Y. S., Lew, D. H., Tark, K. C., Rah, D. K., & Hong, J. P. (2010). Effect of recombinant human epidermal growth factor against cutaneous scar formation in murine full-thickness wound healing. *Journal of Korean Medical Science*, ***25*****(4)**, 589-596.

Kimeli, P., Mbugua, S. W., Cap, R. M., Kirui, G., Abuom, T. O., Mwangi, W. E. & Mande, J. D. (2015). A retrospective study on findings of canine hip dysplasia screening in Kenya. *Veterinary World*, ***8*****(11)**, 1326.

Kinzel, S., Fasselt, R., Prescher, A., Selzer, C., von Keyserlingk Graf, D., & Küpper, W. (1998). Sensory innervation of the hip joint capsule in dogs. *Tierarztliche Praxis. Ausgabe K, Kleintiere/Heimtiere*, ***26*****(5),** 330-335.

Kinzel, S., Scheven, C.V., Buecker, A., Stopinski, T. and Kupper. W. 2002. Clinical evaluation of denervation of the canine hip joint capsule: a retrospective study of 117 dogs. *Vet. Comp. Ortho. Traumatol*. **15**: 51-56.

Kirkby, K. A., & Lewis, D. D. (2012). Canine hip dysplasia: reviewing the evidence for nonsurgical management. *Veterinary Surgery*, ***41*****(1)**, 2-9.

Kisiday, J. D., Kopesky, P. W., Evans, C. H., Grodzinsky, A. J., McIlwraith, C. W., & Frisbie, D. D. (2008). Evaluation of adult equine bone marrow-and adipose-derived progenitor cell chondrogenesis in hydrogel cultures. *Journal of Orthopaedic Research*, ***26*****(3),** 322-331.

Kjelgaard-Hansen, M., & Jacobsen, S. (2011). Assay validation and diagnostic applications of major acute-phase protein testing in companion animals. *Clinics in Laboratory Medicine*, ***31*****(1),** 51-70.

Kraus, K. H., & Kirker-Head, C. A. R. L. (2006). Mesenchymal stem cells and bone regeneration. *Veterinary Surgery*, ***35*****(3)**, 232-242.

Kretlow, J. D., Spicer, P. P., Jansen, J. A., Vacanti, C. A., Kasper, F. K., & Mikos, A. G. (2010). Uncultured marrow mononuclear cells delivered within fibrin glue hydrogels to porous scaffolds enhance bone regeneration within critical-sized rat cranial defects. *Tissue Engineering Part A*, ***16*****(12),** 3555-3568.

Krontveit, R. I., Trangerud, C., Nødtvedt, A., Dohoo, I., Moe, L., & Sævik, B. K. (2012a). The effect of radiological hip dysplasia and breed on survival in a prospective cohort study of four large dog breeds followed over a 10 year period. *The veterinary journal*, ***193*****(1),** 206-211.

Krontveit, R.I., Nodtvedt, A., Bente, K.S., Erik R. and Cathrine, T. (2012b). Housing and exercise related risk factors associated with the development of hip dysplasia as determined by radiographic evaluation in a prospective cohort of Newfoundlands, Labrador Retrievers, Leonbergers, and Irish Wolfhounds in Norway. *Am. J. Vet. Res*. **73**:838-846.

Kumar, M. and Agarwal, K.P.B., 2001. Biochemical alterations of synovial fluid in induced traumatic arthritis in buffalo calves. *Indian. J. Vet. Surg.,* **22**: 87-91.

Lacitignola, L., Crovace, A., Rossi, G., & Francioso, E. (2008). Cell therapy for tendinitis, experimental and clinical report. *Veterinary Research Communications*, ***32*****(1),** 33-38.

LaFond, E., Breur, G. J., & Austin, C. C. (2002). Breed susceptibility for developmental orthopedic diseases in dogs. *Journal of the American Animal Hospital Association***,** ***38*****(5),** 467-477.

Leighton, E. A. (1997). Genetics of canine hip dysplasia. *J. Am. Vet. Med. Ass.* **210**: 1474-1479.

Lettry, V., Hosoya, K., Takagi, S., & Okumura, M. (2010). Coculture of equine mesenchymal stem cells and mature equine articular chondrocytes results in improved chondrogenic

differentiation of the stem cells. *Japanese Journal of Veterinary Research*, ***58*****(1)**, 5-15.

Lewis, T. W., Blott, S. C., & Woolliams, J. A. (2013). Comparative analyses of genetic trends and prospects for selection against hip and elbow dysplasia in 15 UK dog breeds. *BMC genetics*, ***14*****(1)**, 16.

Lipowitz, A.J. and Newton, C.D., (1985). Degenerative joint disease and traumatic arthritis. In: *Text Book of Small Animal Orthopedics* (Eds.), NEWTON, C.D. and NUNAMAKER, D.M., J.B. Lippincott company, Philadelphia. *pp*. 1029-1046.

Luna, S. P., Basílio, A. C., Steagall, P. V., Machado, L. P., Moutinho, F. Q., Takahira, R. K., & Brandão, C. V. (2007). Evaluation of adverse effects of long-term oral administration of carprofen, etodolac, flunixin meglumine, ketoprofen, and meloxicam in dogs. *American Journal of Veterinary Research*, ***68*****(3)**, 258-264.

Lust, G. (1997). An overview of the pathogenesis of canine hip dysplasia. *Journal of the American Veterinary Medical Association*, ***210*****(10)**, 1443-1445.

Lust, G., & Farrell, P. W. (1977). Hip dysplasia in dogs: the interplay of genotype and environment. *The Cornell Veterinarian*, ***67*****(4)**, 447.

Lust, G., & Summers, B. A. (1981). Early, asymptomatic stage of degenerative joint disease in canine hip joints. *American Journal of Veterinary Research*, ***42*****(11),** 1849-1855.

Lust, G., Geary, J. C., & Sheffy, B. E. (1973). Development of hip dysplasia in dogs. *American Journal of Veterinary Research*, ***34*****(1),** 87-91.

Lust, G., Todhunter, R. J., Erb, H. N., Dykes, N. L., Williams, A. J., Burton-Wurster, N. I., & Farese, J. P. (2001). Comparison of three radiographic methods for diagnosis of hip dysplasia in eight-month-old dogs. *Journal of the American Veterinary Medical Association*, ***219*****(9),** 1242-1246.

Mackenzie, S. A., Oltenacu, E. A., & Leighton, E. (1985). Heritability estimate for temperament scores in German shepherd dogs and its genetic correlation with hip dysplasia. *Behavior genetics*, ***15*****(5)**, 475-482.

MacPhail, C. M. (2000). Treatment of canine osteoarthritis. *Waltham Focus*, ***10*****(2)**, 25-31.

Madesh, M., and Balasubramanian, K. A. (1998). Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian journal of biochemistry & biophysics*, ***35*****(3)**, 184-188.

Madihi, Y., Merrikhi, A., Baradaran, A., Rafieian-Kopaei, M., Fard, S., Ansari Samani, R., & Mesripour, A. (2013). Impact of sumac on postprandial high-fat oxidative stress. *Pakistan Journal of Medical Sciences*, ***29*****(S),** 340-345.

Mäki, K., Janss, L. L. G., Groen, A. F., Liinamo, A. E., & Ojala, M. (2004). An indication of major genes affecting hip and elbow dysplasia in four Finnish dog populations. *Heredity*, ***92*****(5)**: 402.

Mala, S., (2006). Clinical evaluation of affections of the canine shoulder, elbow and stifle joints by arthroscopy. *Ph.D. thesis,* Tamil nadu Veterinary and Animal Sciences University, Chennai, India.

Manley, P. A., Adams, W. M., Danielson, K. C., Dueland, R. T., & Linn, K. A. (2007). Long-term outcome of juvenile pubic symphysiodesis and triple pelvic osteotomy in dogs with hip dysplasia. *Journal of the American Veterinary Medical Association*, ***230*****(2)**, 206-210.

Marcacci, M., Kon, E., Moukhachev, V., Lavroukov, A., Kutepov, S., Quarto, R., & Cancedda, R. (2007). Stem cells associated with macroporous bioceramics for long bone repair: 6-to 7-year outcome of a pilot clinical study. *Tissue Engineering***, *13*****(5),** 947-955.

Marx, C., Silveira, M. D., Selbach, I., da Silva, A. S., Braga, L. M. G. D. M., Camassola, M., & Nardi, N. B. (2014). Acupoint injection of autologous stromal vascular fraction and allogeneic adipose-derived stem cells to treat hip dysplasia in dogs. *Stem cells international*,

Marx, R. E. (2001). Platelet-rich plasma (PRP): what is PRP and what is not PRP?. *Implant Dentistry*, **10(4),** 225-228.

Mastrogiacomo, M., Corsi, A., Francioso, E., Comite, M. D., Monetti, F., Scaglione, S., ... & Cancedda, R. (2006). Reconstruction of extensive long bone defects in sheep using resorbable bioceramics based on silicon stabilized tricalcium phosphate. *Tissue Engineering*, ***12*****(5)**, 1261-1273.

Mayhew, P. D., McKelvie, P. J., Biery, D. N., Shofer, F. S., & Smith, G. K. (2002). Evaluation of a radiographic caudolateral curvilinear osteophyte on the femoral neck and its relationship to degenerative joint disease and distraction index in dogs. *Journal of the American Veterinary Medical Association*, ***220*****(4),** 472-476.

Mazzocca, A. D., McCarthy, M. B. R., Chowaniec, D. M., Cote, M. P., Romeo, A. A., Bradley, J. P. & Beitzel, K. (2012). Platelet-rich plasma differs according to preparation method and human variability. *JBJS*, ***94*****(4)**, 308-316.

McCarthy, G., O'Donovan, J., Jones, B., McAllister, H., Seed, M., & Mooney, C. (2007). Randomised double-blind, positive-controlled trial to assess the efficacy of glucosamine/chondroitin sulfate for the treatment of dogs with osteoarthritis. *The Veterinary Journal*, ***174*****(1)**, 54-61.

McNamara, P. S., Barr, S. C., & Erb, H. N. (1996). Hematologic, hemostatic, and biochemical effects in dogs receiving an oral chondroprotective agent for thirty days. *American journal of veterinary research*, ***57*****(9)**, 1390-1394.

Mehta, S. K., Breitbart, E. A., Berberian, W. S., Liporace, F. A., & Lin, S. S. (2010). Bone and wound healing in the diabetic patient. *Foot and Ankle Clinics*, ***15*****(3),** 411-437.

Minto, B. W., Monteiro, B. P., Brandao, C. V.S., Padovani, C. R., Mamprim, M. J. and Filgueira, F. G. F. 2013. Retrospective study of 180 dysplastic dogs admitted at the veterinary teaching hospital, *Unesp-Botucatu.Veterinária e Zootecnia, **20 (4)***: 624-631.

Miranda, F. G., Rezende, C. M. F., Silva, E. F., Rocha, B. D., Melo, M. M., & Tôrres, R. C. S. (2016). Morgan line and its relationship with distraction index, angle of inclination

and degenerative joint disease in the diagnosis of canine hip dysplasia. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, ***68*****(4),** 894-900.

Mirhoseini, M., Baradaran, A., & Rafieian-Kopaei, M. (2013). Medicinal plants, diabetes mellitus and urgent needs. *Journal of HerbMed Pharmacology*, ***2*****(2).**

Montavon, P. M., Hohn, R. B., Olmstead, M. L., & Rudy, R. L. (1985). Inclination and anteversion angles of the femoral head and neck in the dog evaluation of a standard method of measurement. *Veterinary Surgery*, ***14*****(4),** 277-282.

Morgan, J. P. (1987). Canine hip dysplasia: significance of early bony spurring. *Veterinary Radiology*, ***28*****(1),** 2-5.

Morgan, J. P., & Stephens, M. (1985). *Radiographic diagnosis and control of canine hip dysplasia*. Iowa State University Press, ***8*****(4),** 4-9.

Morgan, J.P. (1997). Pathological alteration in canine hip dysplasia*. J. Am. Anim. Med. Assoc.,* **210**: 1446-1450.

Moussa, M., Lajeunesse, D., Hilal, G., El Atat, O., Haykal, G., Serhal, R., Chalhoub A., Khalil C. & Alaaeddine, N. (2017). Platelet rich plasma (PRP) induces chondroprotection via increasing autophagy, anti-inflammatory markers, and decreasing apoptosis in human osteoarthritic cartilage. *Experimental cell research*, ***352*****(1)**, 146-156.

Muraglia, A., Cancedda, R., & Quarto, R. (2000). Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. *J Cell Sci*, ***113*****(7)**, 1161-1166.

Murphy, J. M., Fink, D. J., Hunziker, E. B., & Barry, F. P. (2003). Stem cell therapy in a caprine model of osteoarthritis. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, ***48*****(12),** 3464-3474.

Nap, R. C., & Hazewinkel, H. A. W. (1994). Growth and skeletal development in the dog in relation to nutrition; a review. *Veterinary Quarterly*, ***16*****(1),** 50-59.

Nasri H, Baradaran A, Shirzad H, Rafieian-Kopaei M. (2014a) New concepts in nutraceuticals as alternative for pharmaceuticals. *International Journal Preventive Medine*, **5(12),** 1487-99.

Nasri, H. (2013). Impact of diabetes mellitus on parathyroid hormone in hemodialysis patients. *Journal of Parathyroid Diseases*, ***1*(1),** 9-11.

Nasri, H., Tavakoli, M., Ahmadi, A., Baradaran, A., Nematbakhsh, M., & Rafieian-Kopaei, M. (2014b). Ameliorative effect of melatonin against contrast media induced renal tubular cell injury. *Pakistan journal of medical sciences*, ***30*(2),** 261.

Nauta, A., Seidel, C., Deveza, L., Montoro, D., Grova, M., Ko, S. H. & Yang, F. (2013). Adipose-derived stromal cells overexpressing vascular endothelial growth factor accelerate mouse excisional wound healing. *Molecular Therapy*, ***21*(2)**, 445-455.

Newton, C. D. & Nunamaker, D. M. (1985). *Textbook of small animal orthopaedics* (No. SF991 N55).

Nganvongpanit, K., Boonsri, B., Sripratak, T. & Markmee, P. (2013). Effects of one-time and two-time intra-articular injection of hyaluronic acid sodium salt after joint surgery in dogs. *Journal of Veterinary Science*, ***14*(2),** 215-222.

Nielsen, C., & Pluhar, G. E. (2005). Diagnosis and treatment of hind limb muscle strain injuries in 22 dogs. *Veterinary and Comparative Orthopaedics and Traumatology*, ***18*(04)**, 247-253.

Noordin, S., Umer, M., Hafeez, K., & Nawaz, H. (2010). Developmental dysplasia of the hip. *Orthopedic Reviews*, ***2*(2).**

Nouh, S. R., Abo-Ahmad, H. M., Farghali, H. A. and Saleh, M. M. 2014. A Retrospective Study on Canine Hip Dysplasia in Different Breeds in Egypt. *Global Veterinaria, **13***(4): 503-510.

O'Brien, R.T., R.T. Dueland, W.C. Adams and J.M. Einen. (1997). Dynamic ultrasonographic measurement of passive coxofemoral joint laxity in puppies. *J. Am. Anim. Hosp. Assoc., **33**:* 275-281.

Ohlerth, S., Busato, A., Rauch, M., Weber, U., & Lang, J. (2003). Comparison of three distraction methods and conventional radiography for early diagnosis of canine hip dysplasia. *Journal of Small Animal Practice*, ***44*****(12),** 524-529.

Ohlerth, S., Lang, J., Busato, A. and Gaillard, C. (2001). Estimation of genetic population variables for six radiographic criteria of hip dysplasia in a colony of Labrador retrievers. *American Journal of Veterinary Research, **62*****:** 846– 852.

Olmstead, M.L. (1995). Canine cemented total hip replacement: State of art. J. *Small Anim. Prac., **36***: 395-399.

Olmstead, M.L. (1998). Total Hip Replacement. In: Bojrab, M.J. (ed.), *Current Techniques in Small Animal Surgery.* (4th Ed.). Williams and Wilkins, Philadelphia, pp. 1173-1178.

Olsewski, J. M., Lust, G., Rendano, V. T., & Summers, B. A. (1983). Degenerative joint disease: multiple joint involvement in young and mature dogs. *American Journal of Veterinary Research*, ***44*****(7)**, 1300-1308.

Ortolani, M. (1976). Congenital hip dysplasia in the light of early and very early diagnosis. *Clinical Orthopaedics and Related Research*, ***(119)***, 6-10.

Ouyang, H. W., Goh, J. C., & Lee, E. H. (2004). Use of bone marrow stromal cells for tendon graft-to-bone healing: histological and immunohistochemical studies in a rabbit model. *The American journal of Sports Medicine*, ***32*****(2)**, 321-327.

Owens, J. M., & Biery, D. N. (1999). *Radiographic interpetation for the small animal clinician*. 2nd ed. Baltimore (Md.): Williams & Wilkins.

Panigrahi, P.N. (2014). Conservative Treatment of Hip Dysplasia in a Labrador Dog. *Veterinary Research International, **2***(4):105-107.

Park, K. H., & Na, K. (2008). Effect of growth factors on chondrogenic differentiation of rabbit mesenchymal cells embedded in injectable hydrogels. *Journal of bioscience and bioengineering*, ***106*****(1)**, 74-79.

Park, S. A., Reilly, C. M., Wood, J. A., Carrade, D. D., Deremer, S. L., Seraphin, R. L., Clark, C. K., Zwingenberger, A. L., Borjesson, D. L.,& Russell, P. (2013). Safety and immunomodulatory effects of allogeneic canine adipose-derived mesenchymal stromal cells transplanted into the region of the lacrimal gland, the gland of the third eyelid and the knee joint. *Cytotherapy*, **15(12),** 1498-1510.

Parry, B.W., 1999. Synovial fluid, in Cowell RL, Tyler RD, Meinkoth JH (Eds). *Diagnostic Cytology and Hematology of the Dog and Cat (Eds 2).* St Louis, MO, Mosby, 104-119.

Parsaei, P., Karimi, M., Asadi, S. Y., & Rafieian-Kopaei, M. (2013). Bioactive components and preventive effect of green tea (Camellia sinensis) extract on post-laparotomy intra-abdominal adhesion in rats. *International Journal of Surgery*, ***11***(9), 811-815.

Penwick, R. C. (1992). The variables that influence the success of femoral head and neck excision in dogs. Veterinary medicine (USA).

Piermattei, D.L. & Flo, G.L. & DeCamp, C.E. (2006). Brinker, Piermattei and Flo's Handbook of Small Animal Orthopedics and Fracture Repair. Brinker, Piermattei and Flo's Handbook of Small Animal Orthopedics and Fracture Repair. 4th edtion. St Louis (MO): Saunders Elsevier; pp. 416-511.

Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., and Marshak, D. R. (1999). Multilineage potential of adult human mesenchymal stem cells. *science*, ***284*****(5411)**, 143-147.

Popa, C., Netea, M. G., Van Riel, P. L., Van Der Meer, J. W., & Stalenhoef, A. F. (2007). The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. *Journal of Lipid Research*, ***48*****(4)**, 751-762.

Popovitch, C. A., Smith, G. K., Gregor, T. P. and Shofer, F. S. (1995). Comparison of susceptibility for hip dysplasia between Rottweilers and German Shepherd dogs. *Journal of the American Veterinary Medical Association, **206***: 648-650

Powers, M. Y., Biery, D. N., Lawler, D. E., Evans, R. H., Shofer, F. S., Mayhew, P., Gregor, T. P., Kealy, R. D. and Smith, G. K. (2004). Use of the caudolateral curvilinear osteophyte as an early marker for future development of osteoarthritis associated with hip dysplasia in dogs. *Journal of American Veterinary Medical Association,* ***225*****(2)**: 233-237.

Puerto, D. A., Smith, G. K., Gregor, T. P., LaFond, E., Conzemius, M. G., Cabell, L. W., & McKelvie, P. J. (1999). Relationships between results of the Ortolani method of hip joint palpation and distraction index, Norberg angle, and hip score in dogs. *Journal of the American Veterinary Medical Association*, ***214*****(4),** 497-501.

Quinn, M. M., Keuler, N. S., Lu, Y. A. N., Faria, M. L., Muir, P., & Markel, M. D. (2007). Evaluation of agreement between numerical rating scales, visual analogue scoring scales, and force plate gait analysis in dogs. *Veterinary Surgery*, ***36*****(4)**, 360-367.

Rafieian-Kopaei, M. (2013). Metformin and renal injury protection. *Journal of Renal Injury Prevention*, ***2*****(3),** 91-92.

Rafieian-Kopaei, M., Baradaran, A., & Rafieian, M. (2013). Plants antioxidants: From laboratory to clinic. *Journal of Nephropathology*, ***2*****(2).**

Rafieian-Kopaei, M., Baradaran, A., Merrikhi, A., Nematbakhsh, M., Madihi, Y., & Nasri, H. (2013). Efficacy of co-administration of garlic extract and metformin for prevention of gentamicin–renal toxicity in wistar rats: A biochemical study. *International Journal of Preventive Medicine***, *4*****(3),** 258.

Raghuvir, H.B., Shivrajsinh, K.J., Dipak, N.S., Harit, D.B., Chirag, A.B. and Naresh, H.K. (2013). Treatment of canine hip dysplasia: a review. *J. Anim. Sci. Adv.* **3**(12): 589-597.

Ranganath, L. and Subin, K. (2006). Hip scoring in dogs. *Indian J. Vet. Surg*. **27**(2): 116-118.

Rettenmaier, J.L., Keller, G.G., Lattimer, J.C., Corley, E.A. and Ellersieck, M.R. (2002). Prevalence of canine hip dysplasia in veterinary teaching hospital population. *Vet. Radiol. Ultrasound.* **43**: 313-318.

Richardson DC, Nap RC, Zentek J, Toll PW, Hazewinkel HAW, Zicker SC, Towell TL. Musculoskeletal disorders. In: Hand MS, Thatcher CD, Remillard RL, Roudebush P, Novotny BJ, eds. Small animal clinical nutrition. 5th ed. Topeka, Kan: Mark Morris Institute, cop. 2010, 666-713.

Riley, S. M., Miller, C. W., Dobson, H., & Binnington, A. G. (1996). Surgical procedure simulation via three dimensional computer aided reconstruction of dysplastic canine hips. *Veterinary and Comparative Orthopaedics and Traumatology*, ***9*****(04),** 152-157.

Rischer, W.H. (1975). The dog as a model for study of hip dysplasia. Growth, form, and development of the normal and dysplastic hip joint. *Veterinary Pathology,* **(12),** 229-234.

Riser WH, Rhodes WH, Newton CD. Hip dysplasia. In: Newton CD, Nunamaker (1985). DM, editors. *Textbook of small animal orthopedics.* Philadelphia: JB Lippincott; 953–80.

Riser, W. H. (1973). The dysplastic hip joint: its radiographic and histological development. *Journal of American Veterinary Radiology Society*, **14**: 35–50.

Riser, W. H. (1975a). The dysplastic hip joint: Radiologic and histologic development. *Veterinary Pathology, **12***: 279–305.

Riser, W. H. (1975b). The dog as a model for the study of hip dysplasia: Growth, form, and development of the normal and dysplastic hip joint. *Veterinary Pathology, **12***: 235.

Riser, W. H. and Shirer, J. F. (1966). Hip dysplasia: Coxofemoral abnormalities in neonatal German Shepherd dogs. *Journal of Small Animal Practice, **7***: 7-12.

Roberts, T. and Paul, D.M. (2009). Selection for breed-specific long-bodied phenotypes is associated with increased expression of canine hip dysplasia. *Vet. J.* **183**: 266-272.

Rocha, B.D. and R.C.S. Torres. (2007). Ultrasonic and radiographic study of laxity in hip joint of young dogs. *Arq. Bras. Med. Vet. Zootec., **59***: 90-96.

Rocha, L.B., Tudury, E.A., Roehsig, C., Barauna, D., Chioratto, R., Araujo, F.P. and Kemper, B. (2013). Hip joint denervation in dogs with degenerative joint disease secondary to dysplasia. *Brazilian Anim. Sci.* ***14***(1): 120-134.

Roohafza, H., Sarrafzadegan, N., Sadeghi, M., Rafieian-Kopaei, M., Sajjadi, F., & Khosravi-Boroujeni, H. (2013). The association between stress levels and food consumption among Iranian population. *Archives of Iranian Medicine*, ***16*****(3),** 145-148.

Runge, J. J., Kelly, S. P., Gregor, T. P., Kotwal, S., & Smith, G. K. (2010). Distraction index as a risk factor for osteoarthritis associated with hip dysplasia in four large dog breeds. *Journal of Small Animal Practice*, ***51*****(5),** 264-269.

Rungea, A. S., Kelly, S., Gregor, T. P., Kotwal, S. and Smith, G. K.2010. Distraction index as a risk factor for osteoarthritis associated with hip dysplasia in four large dog breeds. *Journal of Small Animal Practice,* ***51***:264–269.

Saad Setta, H., Elshahat, A., Elsherbiny, K., Massoud, K., & Safe, I. (2011). Platelet-rich plasma versus platelet-poor plasma in the management of chronic diabetic foot ulcers: a comparative study. *International Wound Journal*, ***8*****(3),** 307-312.

Salgia, G., Kulkarni, D. G., & Shetty, L. (2015). C-reactive protein estimation: A quantitative analysis for three nonsteroidal anti-inflammatory drugs: A randomized control trial. *Indian Journal of Dental Research*, ***26*****(1),** 43.

Samdani, A. F., Paul, C., Betz, R. R., Fischer, I., & Neuhuber, B. (2009). Transplantation of human marrow stromal cells and mono-nuclear bone marrow cells into the injured spinal cord: a comparative study. *Spine*, ***34*****(24),** 2605-2612.

Samir, R.N, Hoyda, M.A.A., Haithem, A.F. and Mohamed, M.S. (2014). A retrospective study on canine hip dysplasia in different breeds in Egypt. *Global Vet.* ***13***(4): 503-510.

Sánchez-Molano, E., Pong-Wong, R., Clements, D. N., Blott, S. C., Wiener, P., & Woolliams, J. A. (2015). Genomic prediction of traits related to canine hip dysplasia. *Frontiers in Genetics*, ***6***, 97.

Sánchez-Molano, E., Woolliams, J. A., Blott, S. C., & Wiener, P. (2014). Assessing the impact of genomic selection against hip dysplasia in the L abrador R etriever dog. *Journal of Animal Breeding and Genetics*, ***131*****(2),** 134-145.

Sauvé, F., Paradis, M., Refsal, K. R., Moreau, M., Beauchamp, G., & Dupuis, J. (2003). Effects of oral administration of meloxicam, carprofen, and a nutraceutical on thyroid function in dogs with osteoarthritis. *The Canadian Veterinary Journal*, ***44*****(6)**, 474.

Schmaedecke, A., Saut, J. P. E., & Ferrigno, C. R. A. (2008). A quantitative analysis of the nerve fibres of the acetabular periosteum of dogs. *Veterinary and Comparative Orthopaedics and Traumatology*, ***21*****(05),** 413-417.

Schnelle, G. B. (1935). Some new diseases in the dog. *American Kennel Gazette*, ***52***, 25-26.

Schnelle, G. B. (1937). Congenital subluxation of the coxofemoral joint in a dog. *Univ Pa Bull*, ***65***, 15-16.

Schulz, K.S. and Dejardin, L.M. (2003). Surgical treatment of canine hip dysplasia. In: Slatter D. (ed.), *Text Book of Small Animal Surgery*, **(3),** W.B. Saunders Company, Philadelphia, 2029-2059.

Setorki, M., Rafieian-Kopaei, M., Merikhi, A., Heidarian, E., Shahinfard, N., Ansari, R., & Baradaran, A. (2013). Suppressive impact of anethum graveolens consumption on biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. *International Journal of Preventive Medicine*, ***4*****(8),** 889.

Shepherd, J. (1986). Canine Hip-Dysplasia-Etiology, Pathogenesis and Eradication. *Australian Veterinary Practitioner*, ***16*****(2)**, 71-78.

Shiju Simon, M., Ganesh, R., Ayyappan, S., Rao, G. D., Suresh Kumar, R., Manonmani, M., & Das, B. C. (2010). Incidence of Canine Hip Dysplasia: A Survey of 272 Cases. *Veterinary World*, ***3*****(5).**

Shirzad, H., Burton, R. C., Smart, Y. C., Rafieian-kopaei, M., & Shirzad, M. (2011). Natural Cytotoxicity of NC-2+ Cells against the Growth and Metastasis of WEHI-164 Fibrosarcoma. *Scandinavian Journal of Immunology*, ***73*****(2),** 85-90.

Shirzad, M., Kordyazdi, R., Shahinfard, N., & Nikokar, M. (2013). Does Royal jelly affect tumor cells. *Journal of HerbMed Pharmacology*, ***2*****(2).**

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology: Translation and Integration*, ***82*****(2),** 291-295.

Silva, G.F.D., Carvalho, A.V., Penteado, B.M., Lins, B.T. and Selmi. A.L. (2012). Percutaneous versus open hip denervation in the treatment of canine hip dysplasia. *Sci. Rural.* **42**(4): 685-690.

Slocum, B., & Slocum, T. D. (1998). Hip. *Current Techniques in Small Animal Surgery*, *2*, 1127-1185.

Smith GK, McKelvie PJ: Current concepts in the diagnosis of canine hip dysplasia, in Bonagura, (1995). J *Kirk's Current Veterinary Therapy XII*, *Small Animal Practice*, ed 12. Philadelphia, WB Saunders Co, 1188–1190.

Smith, G. K., Biery, D. N., & Gregor, T. P. (1990). New concepts of coxofemoral joint stability and the development of a clinical stress-radiographic method for quantitating hip joint laxity in the dog. *Journal of the American Veterinary Medical Association*, ***196*****(1),** 59-70.

Smith, G. K., Gregor, T. P., Rhodes, W. H., & Biery, D. N. (1993). Coxofemoral joint laxity from distraction radiography and its contemporaneous and prospective correlation with laxity, subjective score, and evidence of degenerative joint disease from conventional hip-extended radiography in dogs. *American journal of veterinary research*, ***54*****(7),** 1021-1042.

Smith, G. K., Karbe, G. T., Agnello, K. A., & McDonald-Lynch, M. B. (2012). Pathogenesis, diagnosis, and control of canine hip dysplasia. *Veterinary Surgery Small Animal*, ***1***, 824-48.

Smith, G. K., Kealy, R. D., Lawler, D. R., Biery, D. N., Olsson, S., & Lust, G. (2002). Influence of diet and age on subjective hip score and hip OA: A life-long study in Labrador Retrievers. In *Veterinary Orthopedic Society Annual Conference* **(41).**

Smith, G. K., Mayhew, P. D., Kapatkin, A. S., McKelvie, P. J., Shofer, F. S., & Gregor, T. P. (2001). Evaluation of risk factors for degenerative joint disease associated with hip dysplasia in German Shepherd Dogs, Golden Retrievers, Labrador Retrievers, and Rottweilers. *Journal of the American Veterinary Medical Association*, ***219*****(12)**, 1719-1724.

Smith, G. K., Popovitch, C. A., Gregor, T. P., & Shofer, F. S. (1995). Evaluation of risk factors for degenerative joint disease associated with hip dysplasia in dogs. *Journal of the American Veterinary Medical Association*, ***206*****(5),** 642-647.

Smith, R. G., Gassmann, C. J., & Campbell, M. S. (2007). Platelet-rich plasma: properties and clinical applications. *The Journal of Lancaster General Hospital*, ***2*****(2)**, 73-77.

Smith, R. K. (2008). Mesenchymal stem cell therapy for equine tendinopathy. *Disability and Rehabilitation*, ***30*****(20-22)**, 1752-1758.

Smith, R. K. W., Korda, M., Blunn, G. W., & Goodship, A. E. (2003). Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. *Equine Veterinary Journal*, ***35*****(1)**, 99-102.

Snedecor, G. W., and Cochran, W. G. (1989). Statistical Methods, eight edition. *Iowa state University press, Ames, Iowa*.

Solorio, L. D., Dhami, C. D., Dang, P. N., Vieregge, E. L., & Alsberg, E. (2012). Spatiotemporal regulation of chondrogenic differentiation with controlled delivery of transforming

growth factor-β1 from gelatin microspheres in mesenchymal stem cell aggregates. *Stem Cells Translational Medicine*, ***1*****(8)**, 632-639.

Souza, A. N. A., Pinto, A. C. B. F., Marvulle, V. and Matera, J. M. (2015). Vertical forces assessment according to radiographic hip grade in German shepherd dogs. *Journal of Small Animal Practice,* ***56*** (2):108-111.

Spreafico, A., Chellini, F., Frediani, B., Bernardini, G., Niccolini, S., Serchi, T., & Marcolongo, R. (2009). Biochemical investigation of the effects of human platelet releasates on human articular chondrocytes. *Journal of cellular biochemistry*, **108(5),** 1153-1165.

Stocks, J., and Dormandy, T. L. (1971). The autoxidation of human red cell lipids induced by hydrogen peroxide. *British journal of haematology*, ***20*****(1)**, 95-111.

Strober, W. (2001). Trypan blue test of cell viability. *Curr. Protoc. Immunol*, *21*. Appendix 3, Appendix 3B.

Szabo, S. D., Biery, D. N., Lawler, D. F., Shofer, F. S., Powers, M. Y., Kealy, R. D., & Smith, G. K. (2007). Evaluation of a circumferential femoral head osteophyte as an early indicator of osteoarthritis characteristic of canine hip dysplasia in dogs. *Journal of the American Veterinary Medical Association*, ***231*****(6)**, 889-892.

Thomas, T. M., Marcellin-Little, D. J., Roe, S. C., Lascelles, B. D. X., & Brosey, B. P. (2006). Comparison of measurements obtained by use of an electrogoniometer and a universal plastic goniometer for the assessment of joint motion in dogs. *American journal of Veterinary Research*, ***67*****(12)**, 1974-1979.

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., & Jones, J. M. (1998). Embryonic stem cell lines derived from human blastocysts. *science*, ***282*****(5391),** 1145-1147.

Tiwary, R., Amarpal, Aithal, H. P., Kinjavdekar, P., Pawde, A. M., and Singh, R. (2014). Effect of IGF-1 and uncultured autologous bone-marrow-derived mononuclear cells on repair of osteochondral defect in rabbits. *Cartilage*, ***5*****(1)**, 43-54.

Todhunter, R. J., Acland, G. M., Olivier, M., Williams, A. J., Vernier-Singer, M., Burton-Wurster, N., & Lust, G. (1999). An outcrossed canine pedigree for linkage analysis of hip dysplasia. *Journal of Heredity*, ***90*****(1),** 83-92.

Todhunter, R.J. and Lust, G. (2003). Hip dysplasia: Pathogenesis. In: Text book of Small Animal Surgery, Vol. 2, Slatter, D.H. (eds.), 3rd edn: W.B. Saunders Company, Philadelphia, pp: 2009-2029.

Tomlinson, J. L., & Johnson, J. C. (2000). Quantification of measurement of femoral head coverage and Norberg angle within and among four breeds of dogs. *American Journal of Veterinary Research*, ***61*****(12)**, 1492-1500.

Udehiya, R. K., Aithal, H. P., Kinjavdekar, P., Pawde, A. M., Singh, R., & Sharma, G. T. (2013). Comparison of autogenic and allogenic bone marrow derived mesenchymal stem cells for repair of segmental bone defects in rabbits. *Research in Veterinary Science*, ***94*****(3),** 743-752.

Van Hagen, M. A., Ducro, B. J., Broek, J. V. D., & Knol, B. W. (2005). Incidence, risk factors, and heritability estimates of hind limb lameness caused by hip dysplasia in a birth cohort of boxers. *American Journal of Veterinary Research*, ***66*****(2),** 307-312.

Van Pham, P., Bui, K. H. T., Ngo, D. Q., Vu, N. B., Truong, N. H., Phan, N. L. C., ... & Phan, N. K. (2013). Activated platelet-rich plasma improves adipose-derived stem cell transplantation efficiency in injured articular cartilage. *Stem Cell Research & Therapy*, ***4*****(4)**, 91.

Van Pham, P., Bui, K. H. T., Ngo, D. Q., Vu, N. B., Truong, N. H., Phan, N. L. C., and Phan, N. K. (2013). Activated platelet-rich plasma improves adipose-derived stem cell transplantation efficiency in injured articular cartilage. *Stem cell research & therapy*, ***4*****(4)**, 91.

Vasseur, P. B. (2008). Femoral head and neck ostectomy. In Bojrab MJ (ed): *Current Techniques in Small Animal Surgery* 4th ed. William & Wilkins, pp: 1170-1173.

Vasseur, P.B.: Femoral head and neck ostectomy. In Bojrab MJ (ed): *Current Techniques in Small Animal Surgery* 4th ed. William & Wilkins, 2008, pp 1170-1173.

Vezzoni, A. (2004). Is early evaluation reliable for diagnosis of canine hip dysplasia. In *Proceedings of the 12th Orthopaedic Veterinary Congress ESVOT-VOS* (145-151).

Vezzoni, A., Dravelli, G., Corbari, A., De Lorenzi, M., Cirla, A., Tranquillo, V., (2005). Early diagnosis of canine hip dysplasia. *European Journal of Companion Animal Practice*, ***15***, 173-184.

Vidal, M. A., Kilroy, G. E., Johnson, J. R., Lopez, M. J., Moore, R. M., & Gimble, J. M. (2006). Cell growth characteristics and differentiation frequency of adherent equine bone marrow–derived mesenchymal stromal cells: adipogenic and osteogenic capacity. *Veterinary Surgery*, ***35*****(7),** 601-610.

Vidal, M. A., Walker, N. J., Napoli, E., & Borjesson, D. L. (2011). Evaluation of senescence in mesenchymal stem cells isolated from equine bone marrow, adipose tissue, and umbilical cord tissue. *Stem cells and development*, ***21*****(2),** 273-283.

Vishal B.N., (2011). Arthroscopic study of elbow and stifle joint in dogs. *MVSc. Thesis.* Karnataka Veterinary Animal and Fisheries Sciences University, Bidar.

Wagner, W., Wein, F., Seckinger, A., Frankhauser, M., Wirkner, U., Krause, U., & Ho, A. D. (2005). Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Experimental Hematology*, ***33*****(11),** 1402-1416.

Wallace L.J., & Olmstead M.L. (1995). Disabling conditions of canine coxofemoral joint. In: Olmstead M.L. (ed.): Small Animal Orthopaedics. Mosby, Philadelphia. 361–393.

Wallace L.J., & Olmstead M.L. (1995). Disabling conditions of canine coxofemoral joint. In: Olmstead M.L. (ed.): Small Animal Orthopaedics. Mosby, Philadelphia. 361–393.

Weeren, P.R.V. and Degrauw J.C., (2010). Pain in osteoarthritis. *Vet. Clin. Equine*., **26**: 619-642.

Westminster, C. O., Westminister, C. O., Vail, C. O., & Busse, D. (2008). Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician*, ***11*****(3),** 343-353.

Whiteside, D.P., Remedios, A.M., Black, S.R., Path, D. and Bodner, S.T.F. (2006). Meloxicam and surgical denervation of the coxofemoral joint for the treatment of degenerative osteoarthritis in a Bengal Tiger (*Panthera tigris tigris*). *J. Zoo Wildlife Med.* **37**(3): 416-419.

Whittington, K., Banks, W. C. and Carlson, W. D. 1961. Report of panel on canine hip dysplasia. *Journal of American Veterinary Medical Association,* ***139***: 791– 806.

Wilke, M. M., Nydam, D. V., & Nixon, A. J. (2007). Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *Journal of Orthopaedic Research*, ***25*****(7),** 913-925.

Willis, M. B. (1997). A review of the progress in canine hip dysplasia control in Britain. *Journal of the American Veterinary Medical Association*, ***210*****(10),** 1480-1482.

Wilson, B. J., Nicholas, F. W., James, J. W., Wade, C. M., Tammen, I., Raadsma, H. W. & Thomson, P. C. (2012). Heritability and phenotypic variation of canine hip dysplasia radiographic traits in a cohort of Australian German shepherd dogs. *PloS One*, ***7*****(6)**, 39620.

Wood, J. L. N., & Lakhani, K. H. (2003). Hip dysplasia in Labrador retrievers: the effects of age at scoring. *Veterinary Record*, ***152*****(2)**, 37-40.

Wood, J.L.N., Lakhani, K.H. and Rogers, K. (2002). Heritability and epidemiology of canine hip dysplasia score and its components in Labrador Retrievers in the United Kingdom. *Vet. Med.* ***55***: 95-108.

Xu, Q.R., Dong, Y.H., Chen, S.L., Bao, C.D. and Du, H., (2009). Degeneration of normal articular cartilage induced by late phase osteoarthritic synovial fluid in beagle dogs. *Tissue and Cell.* ***41***: 13-22.

Yamada, Y., Ueda, M., Naiki, T., Takahashi, M., Hata, K. I., & Nagasaka, T. (2004). Autogenous injectable bone for regeneration with mesenchymal stem cells and platelet-rich plasma: tissue-engineered bone regeneration. *Tissue Engineering*, ***10*****(5-6),** 955-964.

Yoo, J. U., Barthel, T. S., Nishimura, K., Solchaga, L., Caplan, A. I., Goldberg, V. M., & Johnstone, B. (1998). The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. *JBJS*, *80*(12), 1745-57.

Yun, S., Ku, S. K., & Kwon, Y. S. (2016). Adipose-derived mesenchymal stem cells and platelet-rich plasma synergistically ameliorate the surgical-induced osteoarthritis in Beagle dogs. *Journal of Orthopaedic Surgery and Research,* ***11***(1), 9.

Zhang, B. Y., Wang, B. Y., Li, S. C., Luo, D. Z., Zhan, X., Chen, S. F., & Li, D. S. (2018). Evaluation of the curative effect of umbilical cord mesenchymal stem cell therapy for knee arthritis in dogs using imaging technology. *Stem Cells International*, ***10*****(5-6),** 955-964.

Zhou, Z., Sheng, X., Zhang, Z., Zhao, K., Zhu, L., Guo, G. & Krotscheck, U. (2010). Differential genetic regulation of canine hip dysplasia and osteoarthritis. *PloS One*, ***5*****(10)**, 13219.

Zhu, L., Chen, S., Jiang, Z., Zhang, Z., Ku, H. C., Li, X. & Todhunter, R. (2012). Identification of quantitative trait loci for canine hip dysplasia by two sequential multipoint linkage analyses. *Journal of Applied Statistics*, ***39*****(8)**, 1719-1731.

Zhu, S., Makosa, D., Miller, B., & Griffin, T. M. (2019). Glutathione as a mediator of cartilage oxidative stress resistance and resilience during aging and osteoarthritis. *Connective tissue research*, 1-14.

Zscharnack, M., Hepp, P., Richter, R., Aigner, T., Schulz, R., Somerson, J. & Marquass, B. (2010). Repair of chronic osteochondral defects using predifferentiated mesenchymal stem cells in an ovine model. *The American Journal of Sports Medicine*, ***38*****(9),** 1857-1869.

7 APPENDIX (CES)

I. Estimation of total leukocyte count (TLC):

Reagents and Equipment

1. Two leukocyte Unopette reservoirs; each containing 0.475 mL of diluent: Glacial acetic acid 28.6 mL
   QS with distilled water to 1 liter
2. Two Unopette capillary pipets, 25 µL
3. Hemocytometer with cover glass
4. Petri dish with filter paper
5. Hand counter
6. Microscope

Calculations

1. The calculation formula for hemocytometer cell counts determines the number of cells within 1 µL (1 mm^3) of blood. To make this determination, the total number of cells counted must be corrected for the initial dilution of blood and the volume of diluted blood used. The standard dilution of blood for leukocyte counts is 1:20; therefore the dilution factor is 20. The volume of diluted blood used is based on the area and depth of the counting area. The area counted is 4 mm^2 and the depth is 0.1 mm; therefore the volume factor is 0.4 mm^3.

   Total number of cells counted x dilution factor x 1/volume factor = cells/mm^3
2. Average leukocyte counts from the duplicate pipets and report result (x 10^9/L or /mm^3).

II. Estimation of C-reactive protein (CRP):

Reagents:

1. TURBILYTE*-CRP Activation buffer (R1): ready to use.
2. TURBILYTE*-CRP latex reagent (R2): ready to use uniform suspension of polystyrene latex particulars coated with anti- CRP antibody.
3. TURBILYTE*-CRP calibrator; A lyophilized preparation of serum equivalent to the stated amount of CRP on mg/dl basis, when hydrated appropriately.
   The TURBILYTE*-CRP calibrator is traceable to the W.H.O. to the W.H.O.
   International Reference Standard (85/506) for Human C-reactive. Protein.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity. Sensitivity, and performance

Calculations:

1. Calculate ΔA

ΔA= (A2-A1)

2. Concentration of CRP in sample = $\frac{\Delta A\ Sample}{\Delta A\ Calibrator} \times Conc.(S)\ of\ calibrator$

III. Estimation of Lipid peroxidation (LPO):

Reagents:

1) **Trichloroacetic Acid (TCA) 10% solution:** TCA (10 g) was dissolved in distilled water and the volume was made up to 100 ml with distilled water.
2) **Thiobarbituric acid (TBA) (0.67%) solution:** Prepared by taking 0.67 g of TBA in 100 ml of distilled water and warmed up for dissolving TBA (prepared freshly).

Calculation:

i. Calculation was done using the molar extinction coefficient (EC) of MDA-TBA complex at 535 nm, i.e., 1.56 x 10^8 /M/cm. The amount of LPO was expressed as n M of MDA formed per ml of serum homogenates employing following formula**:**

$$\text{MDA}= \frac{OD\ of\ test}{EC} \times \frac{\text{Total volume of reaction mixture}}{Volume\ of\ sample\ taken} \times DF\ \times Time\ of\ incubation \times 10^9$$

ii. Where**:** - DF=Dilution factor, 10^9= nM

IV. Estimation of Superoxide dismutase (SOD):

Reagents:

i. **100 µM Pyrogallol:** 6.3 mg of Pyrogallol was dissolved in 5 ml of distilled water. One ml from this solution was added to 100 ml of distilled water (prepared freshly).

ii. **1.25 ml MTT:** 2.58 mg MTT was dissolved in 5 ml of distilled water (prepared freshly).

iii. **Phosphate buffer saline (PBS):** PBS was prepared as described elsewhere for separation of erythrocytes.

Calculation:

Activity was expressed as U/g of serum [one unit of SOD is the amount of serum required to inhibit the MTT reduction by 50 %.] and calculated by using the following formula**:**

$$\text{SOD (U)} = \frac{mg\ of\ homogenate\ in\ 0.01\ ml}{Y\%} \times 50 \times DF$$

Where **Y** % is inhibition of MTT reduction by SOD protein.

$$\text{Y\%} = \frac{OD\ of\ test}{OD\ of\ Control} \times 100$$

DF = Dilution factor

V. Estimation of Catalase (CAT):

Reagents:

1) Phosphate buffer (50 mM; pH 7.0)

(a) 50 mM KH_2PO_4 - 1.37 g/200 ml.

(b) 50 mM Na_2HPO_4 - 1.42 g/200 ml.

The solutions (a) and (b) were mixed in 1: 1.5 (v/v) and the pH adjusted to 7.

2) H_2O_2 (10mM): 0.1 ml of 30% H_2O_2 was diluted to 100 ml in water. The solution was checked at 230 nm and the concentration was adjusted using the molar extinction coefficient of H_2O_2 (0.081/mM/cm) (prepared freshly).

Calculations:

$$\frac{\Delta \text{ OD/ time}}{0.067} \times \frac{\text{Total Volume of reaction mixture}}{\text{amount of sample taken}} \times \frac{1}{\text{mg of Protein}}$$

Where

OD= Mean of difference between ODs at 10 sec intervals

VI. Estimation of Glutathione peroxidase (GPx):

Reagents:

Glutathione Reductase Assay Buffer, 100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA (Catalog Number G8789)	125 ml
Glutathione Reductase Dilution Buffer, 100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA and 1 mg/ml bovine serum albumin (Catalog Number G0790)	100 ml

Glutathione Reductase Positive Control, Lyophilized powder containing yeast glutathione reductase, potassium phosphate buffer, pH 7.5, with EDTA and trehalose as a stabilizer (Catalog Number G0665)	1 vial
β-Nicotinamide Adenine Dinucleotide, Phosphate, Reduced (NADPH, Catalog Number N6505)	25 mg
Glutathione, Oxidized, Disodium Salt, (Catalog Number G4626)	100 mg
5,5'-Dithiobis(2-nitrobenzoic acid), (Catalog Number D8130)	50 mg

Preparation instructions:

Solutions were prepared in the volume required for the number of assays to be performed, according to the detection method to be used.

1. Glutathione Reductase Positive Control Solution - Reconstitute the vial with 1 ml of water to obtain a glutathione reductase activity of > 1 unit per ml in a solution containing 100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA and 38 mg/ml trehalose. For long term storage, divide the solution into aliquots and freeze at –20 °C. The solution will be stable for at least 6 months at –20°C.
2. 2 mM NADPH Solution - Dissolve a portion of the β-Nicotinamide adenine dinucleotide phosphate, reduced (NADPH) at 1.85 mg/ml in Assay Buffer to prepare a working solution of 2 mM. Store at 4°C. Prepare the NADPH solution fresh every day.

3. 2 mM Oxidized Glutathione Solution - Dissolve the Glutathione, Oxidized, Disodium salt (GSSG) at 1.42 mg/ml in Assay Buffer to prepare a working solution of 2 mM. Store at 25 °C while performing the test. The solution may be kept up to 7 days at 2–8 °C for temporary storage.
4. 3 mM DNTB Solution - Dissolve the 5, 5'-Dithiobis (2-nitrobenzoic acid) (DNTB) at 1.19 mg/ml in Assay Buffer to prepare a working solution of 3 mM. This solution is very unstable; prepare the solution fresh every 4 hours and store at 4 °C.
5. Sample preparation. Dilute the samples to be assayed in Dilution Buffer as needed immediately before assaying. The concentration dependent enzymatic reaction is linear from 0.003–0.03 units per ml of reaction mixture for the colorimetric assay and from 0.003–0.012 units per ml of reaction mixture for the UV assay.

Calculations:

$$\text{Units/ml} = \frac{(\Delta A_{sample} - \Delta A_{blank}) \times (\text{dilution factor})}{\varepsilon^{mM} \times (\text{volume of sample in ml})}$$

For NADPH $\varepsilon^{mM} = 6.22\ mM^{-1}cm^{-1}$

For TNB^6 $\varepsilon^{mM} = 14.15\ mM^{-1}cm^{-1}$

8

BRIEF BIO DATA

Name	Sukhjinder Singh
Father's Name	Sh. Satwant Singh
Mother's Name	Smt. Sukhwinder Kaur
Date of Birth	4th September, 1992

Permanent Address: Sukhjinder Singh S/O Sh. Satwant Singh, V.P.O. Rajpur bhaian distt. Hoshiarpur, Punjab Pin code: 146112

Academic	**Qualification**			
	Month, Year	**School/College**	**Board/University**	**Marks (%)**
10th class	March, 2009	Mount Carmel School, Mehtiana	ICSE	73.28
12th class	May, 2011	Mount Carmel Senior Secondary School, Kakkon	ICSE	63.16
B.V.Sc. & AH	July, 2017	Khalsa college of veterinary and animal sciences, Amritsar	GADVASU, Ludhiana	6.68 (OGPA)

Title of M.V.Sc. Thesis: "*Clinical studies on nutraceuticals versus autologous uncultured bone marrow derived mono-nucleated cells (BMNCs) for treatment of hip dysplasia in canine*"

Rewards:

1. Second prize in poster presentation during 32nd Annual Convention of Indian Association of Veterinary Microbiologists, Immunologist and Specialist in Infectious Diseases and National Conference held in Bihar Veterinary College, Patna.
2. 3rd prize in poster presentation during 27th Annual Convention of ISSGPU, held in Bihar Veterinary College, Patna.

DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY
BIHAR ANIMAL SCIENCES UNIVERSITY,
PATNA, BIHAR

Title of thesis:	Clinical Studies on Nutraceuticals versus Autologous Uncultured Bone Marrow Derived Mono-Nucleated Cells (BMNCs) for treatment of Hip Dysplasia in Canine
Name of student:	Sukhjinder Singh
Major discipline:	Veterinary Surgery and Radiology
Minor discipline:	Veterinary Medicine
Date of thesis submission:	27/09/2019
Total pages of thesis:	122
Major advisor:	Dr. Ramesh Tiwary

Abstract:

Eighteen dogs suffering from either sex were used in this study. The animal were randomly divided into three groups A, B, & C consisting of six animals each to study the effect of biomaterials i.e. once in affected hip joint with uncultured autologous bone marrow derived mono-nuclear cells (BMNCs) (4.35 ($\pm 0.07 \times 10^6$) (group B), BMNCs (4.35 ($\pm 0.07 \times 10^6$) and activated platelets (2.54 ($\pm 0.12 \times 10^8$) (group C) and nutraceuticals orally for three months and 1 ml RPMI intra- articularly once (group A-control). Evaluation of treatment protocols were done at four weeks intervals for twelve weeks on the basis of clinical examination like Ortolani test, scores for pain, lameness, ability to jump and ability to climb stairs. Radiographic evaluation based on Norberg Angle (NA), Percent Femoral Head Coverage (PFHC) and Distraction Index (DI), haematobiochemical test for CBC and C reactive protein (CRP). The oxidative stress parameters were also observed for malondialdehyde (MDA), superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx). No significant result were observed in physiological as well as Haematobiochemical parameters whereas, significant improvement in the scores for pain, lameness, ability to jump, ability to climb stairs and norberg angles were observed with better response in group C followed by group B and A at various interval of time. The mean value of C reactive protein on days 90 were found significantly different among the groups that indicates minimal inflammatory reaction in hip joint with lowest value in the animals of groups C (4.02 ± 0.14) followed by groups B (4.60 ± 0.13) and A (8.50 ± 0.20). There were significant differences in the mean value of Percent Femoral Head Coverage (PFHC) and Distraction Index (DI) and oxidative stress parameters observed with better response group C followed by group B and A. in osteoarthritis (OA) glutathione provides resistance and resilience to damage of cartilage from oxidative stress. On day 90^{th} the mean value of glutathione peroxidase was also significant and highest in group C (0.148 ± 0.001) followed by group B (0.124 ± 0.000) and A (0.0850 ± 0.001) that further support better improvement in clinical signs of animals suffering from hip dysplasia in group C followed by group B and A. At the end of study it was concluded that the Nutraceuticals can ameliorate the clinical signs associated with canine hip dysplasia whereas, the implantation of uncultured autologous BMNCs in hip joint can improve the clinical signs and augment the healing of cartilage combination of BMNCs and activated platelets further improve the clinical signs and healing of cartilage in canine hip dysplasia.

Student	Major Advisor	HOD
(Sukhjinder Singh)	(Dr. Ramesh Tiwary)	(Dr. Mithilesh Kumar)