

**ROLE OF *SYMPHYTUM OFFICINALE* AS AN OSTEOINDUCER  
IN LONG BONE FRACTURE REPAIR IN CANINE**

**THESIS**

**Submitted**

*in partial fulfillment of the requirements for the Degree of*

**MASTER OF VETERINARY SCIENCE  
IN  
VETERINARY SURGERY & RADIOLOGY**

**BY**

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**(INDIA)**

**2014**

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I hereby declare that the experimental research work and interpretation of the thesis entitled "**ROLE OF SYMPHYTUM OFFICINALE AS AN OSTEOINDUCER IN LONG BONE FRACTURE REPAIR IN CANINE**" or part thereof has not been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any university or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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## TABLE OF CONTENTS

CHAPTER	PAGE
I INTRODUCTION	1 - 4
II REVIEW OF LITERATURE	5 - 30
III MATERIAL AND METHODS	31 - 38
IV RESULTS AND DISCUSSION	39 - 63
V SUMMARY AND CONCLUSIONS	64 - 69
A) BIBLIOGRAPHY	i – xii
B) APPENDICES	xiii - xvii
C) VITA	xviii
D) THESIS ABSTRACT	xix - xxi
E) MARATHI ABSTRACT	xxii - xxiv

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## LIST OF TABLES

Table No.	Particulars	Page No.
1.	Treatment methodology	32
2.	Age-wise distribution of long bone fracture in dogs	40
3.	Breed-wise distribution of long bone fracture in dogs	41
4.	Sex-wise distribution of long bone fracture in dogs	41
5.	Location-wise distribution of long bone fracture in dogs	42
6.	Types of fracture in long bone in dogs	42
7.	Limbs involved in long bone fracture	43
8.	Types of bones involved in long bone fracture	43
9.	Type of immobilization technique	44
10.	Mean value ( $\pm$ S.E.) of days recorded for weight bearing in two treatment groups	51
11.	Mean value ( $\pm$ S.E.) of Total Erythrocyte Count (million/cu.mm), in two treatment groups	56
12.	Mean value ( $\pm$ S.E.) of Total Leucocyte Count (thousand/cu.mm), in two treatment groups	56
13.	Mean value ( $\pm$ S.E.) of Hemoglobin (gm %) in two treatment groups	57
14.	Mean value ( $\pm$ S.E.) of Lymphocyte Count (per cent), in two treatment groups	58
15.	Mean value ( $\pm$ S.E.) of Neutrophil Count (per cent), in two treatment groups	58
16.	Mean value ( $\pm$ S.E.) of Monocyte Count (per cent), in two treatment groups	59
17.	Mean value ( $\pm$ S.E.) of Eosinophil Count (per cent), in two treatment groups	59
18.	Mean value ( $\pm$ S.E.) of Basophil Count (per cent), in two treatment groups	60
19.	Mean value ( $\pm$ S.E.) of Serum Alkaline phosphatase (in IU/L), in different groups	62

## LIST OF FIGURES

<b>Fig. No.</b>	<b>Particulars</b>	<b>After page</b>
1.	Mean $\pm$ S.E of total erythrocyte count (million/ cumm) in two treatment groups	56
2.	Mean $\pm$ S.E of total leucocyte count (thousands/cumm) in two treatment groups	56
3.	Mean $\pm$ S.E of Hemoglobin (gm %) in two treatment groups	57
4.	Mean $\pm$ S.E of Lymphocyte (%) in two treatment groups	57
5.	Mean $\pm$ S.E of Neutrophil (%) in two treatment groups	58
6.	Mean $\pm$ S.E of Monocyte (%) in two treatment groups	58
7.	Mean $\pm$ S.E of Eosinophil (%) in two treatment groups	59
8.	Mean $\pm$ S.E of Basophil (%) in two treatment groups	59
9.	Mean $\pm$ S.E of serum alkaline phosphatase (U/L) in two treatment groups	62

## LIST OF PLATES

Plate No.	Particulars	After page
1.	Surgical site prepared for femur fracture repair	33
2.	Exteriorization of proximal fractured fragment	33
3.	Drilling through femoral proximal shaft	33
4.	Retrograde insertion of V nail in femur	33
5.	Alignment of fracture fragments after intramedullary pinning	33
6.	Surgical site prepared for Humerus fracture repair	47
7.	Exteriorization of proximal fractured fragment	47
8.	Cerclage wiring for additional support to the fractured fragments	47
9.	Cerclage wiring for additional support to the fractured fragments	47
10.	Closure of surgical wound after retrograde pin insertion	47
11.	Surgical site prepared for Radius fracture repair	48
12.	Fixation of Shermanns plate with screws for Radius fracture repair	48
13.	Partial Weight Bearing in Group II ( Dog B2, day 3)	50
14.	Partial Weight Bearing and knuckling in Group II (Dog B1, day 3)	50
15.	Complete weight bearing in Group II (Dog B1, day 28)	50
16.	Radiograph of fractured humerus ( Group I, Dog A3, day 0)	52
17.	Radiograph of fractured humerus (Group I, Dog A3, day 0 postop.)	52
18.	Radiograph of fractured humerus (Group I, Dog A3, 15th day postop.)	52
19.	Radiograph of fractured humerus (Group I, Dog A3, 30th day postop.)	52
20.	Radiograph of fractured humerus (Group I, Dog A3, 45th day postop.)	52
21.	Radiograph of fractured femur (Group I, Dog A1, 0 <sup>th</sup> day postop.)	52
22.	Radiograph of fractured femur (Group I, Dog A1, 15 <sup>th</sup> day postop.)	52
23.	Radiograph of fractured femur (Group I, Dog A1, 30 <sup>th</sup> day postop.)	52
24.	Radiograph of fractured femur (Group I, Dog A1, 45 <sup>th</sup> day postop.)	52
25.	Preoperative radiograph of femur in Group II (Dog B1, day 0)	52
26.	Radiograph of femur (Group II, dog B1, day 0 postop.)	53
27.	Radiograph of femur (Group II, dog B1, 15 <sup>th</sup> day postop.)	53
28.	Radiograph of femur (Group II, dog B1, 30 <sup>th</sup> day postop.)	53
29.	Radiograph of femur fracture (Group II, dog B1, 45 <sup>th</sup> day postop.)	53
30.	Radiograph of a humerus (Group II, Dog B2, day 0)	53
31.	Radiograph of a humerus (Group II, Dog B2, day 0)	53
32.	Radiograph of fractured humerus (Group II, Dog B2, day 15 postop.)	53
33.	Radiograph of fractured humerus (Group II, Dog B2, day 30 postop.)	54
34.	Radiograph of fractured humerus (Group II, Dog B2, day 45 postop.)	54

### LIST OF ABBREVIATIONS

&	: And
@	: At the rate of
°C	: Degree centigrade
C	: Cent
ALP	: Alkaline phosphatase
b. wt.	: Body weight
Cm	: Centimeter
cumm	: Cubic mili meter
DLC	: Differential Leucocyte Count
EDTA	: Ethylene Diamine Tetra Acetic Acid
<i>et al.</i>	: et alii /alia, And others
etc.	: Etcetera
Fig.	: Figure
Gr	: Group
Hb	: Hemoglobin
HCL	: Hydrochloride
i.e.	: id est/that is
inj.	: Injection
U/L	: Unit Per Liter
i/m.	: Intra muscular
i/v.	: Intra venous
Kg	: Kilogram
Mg	: Milligram
mg/dl	: Milligram per deciliter
ml	: Milliliter
viz.	: for example
No.	: Number
%	: Percent
±	: Plus Minus
®	: Registered
No.	: Number
K-nail	: Kuntscher nail
S.D.	: Standard Deviation

S.E. : Standard Error  
TVCC : Teaching Veterinary Clinical Complex  
TEC : Total Erythrocyte Count  
TLC : Total Leucocyte Count  
TA : Tannic acid  
P.O. : Post operative  
RBC Red Blood Cell  
WBC White Blood Cells

# INTRODUCTION

## INTRODUCTION

Dog is the first domesticated animal and became best companion animal of human because of its unique features like controlling flock of sheep and goats, herds of animals and loyalty towards his master, guarding of estate, detection of theft cases and explosives and proven physical, psychological and health benefits for the dog owners. Modern lifestyle creates loneliness, isolation and sense of vulnerability to man. Dog ownership provides companionship, gives immense pleasure and reduces stress level, without the demand of human relationships.

Due to fast growing urbanization the dogs have been observed to suffer with various cases of long bone fractures such as fracture of femur, tibia, radius, and ulna due to accidents and injuries of various types and pathological fracture in young age such as rickets and geriatric problems like osteoporosis. The fracture in animals invariably causes pain and suffering to them, apart from loss of functions of their affected limb. To reduce pain and time of fracture healing, efforts have been made continuously by the researchers, so that the period of suffering in animals could be reduced.

Among the long bone fractures, higher incidence of fracture of femur were recorded in canines due to more rotational and bending forces acting on the proximity of femur.

According to Simon *et al.* (1995) highest incidence of pelvic limb fracture limb fracture in canines is more common than that of fore limb. The incidence was highest in young animals less than six months of age. Majority of the fractures were recorded in non-descript dogs. Male dogs were affected more than female dogs of all the age groups. Among the various bones of the pelvic limb the incidence was highest in femur.

Patil *et al.* (1991) recorded high incidence (35%) of femoral fractures in canines.

A fracture may cause extreme pain and tenderness in the injured area, swelling, a protruded bone or blood under skin, numbness and tingling or paralysis below the fracture. Most of the fractures are caused by accidents, but some occur because of tumors, osteoporosis or deficiency of vitamin D or calcium. Fracture symptoms include limb deformities, limited limb functioning,

shortening of limb in the fracture of long bones, pain, grating sensation, swelling and discoloration of skin overlying the fracture area.

The incidence of femur fractures in small animals was about 20-25% which is higher than for any other long bone in the body. Fractures involving the distal metaphysis and epiphysis of femur are frequently found in immature dogs. Proper reduction of such fractures is difficult due to distal fragment, proximity of fracture to the joint and caudal bow of the distal femur. Majority of femur fractures were located at the mid shaft in dogs (Ganesh *et al.*, 2004).

Compound fracture of radius, ulna and tibia are among the most common orthopedic injuries in dog due to less soft tissues coverage of these bones. Approximately 18% of fractures that occur in dogs involves radius and ulna. With higher incidence of long bone fractures there is a great need of surgical management of these fractures. Because of limited soft tissue covering, a large percent of these fractures are of upper extremities (Dwivedi *et al.*, 2010).

The incidence of humerus fracture ranges from 5.4 per cent to 7.7 per cent in dogs. The most common cause of fractures of humerus is automobile accidents and falls. Bandaging and splinting of humeral fracture is not recommended before surgery because of standard configurations of splint does not reach for enough proximity to stabilize the bone. In fact, splints and bandages tend to make fracture more unstable than if no bandage is present. The humerus has a complex shape that often makes fracture repair challenging. The head of humerus is oval and has an area approximately twice that of the glenoid (Slatter, 2002).

The treatment of fracture is comprised of reduction, retention and immobilization of fractured part and supportive therapy to promote healing and to reduce pain experienced by animal. During the treatment of fracture, several complications are often recorded which delays the healing. However, some drugs are available which increase osteogenic activity at the fracture site and there by enhance healing mechanism. In the recent past many researchers have worked to assess the efficacy of osteoinducers like anabolic synthetic hormones, magnet therapy and herbal plant extracts. Several medicinal plants have been used by rural people in the remote areas of Indian subcontinent to treat fractures in domestic animals, these plant possibly possess the activities which may be helpful in enhancing healing process and alleviating pain and swelling. A few

plants have also been used to immobilize the fracture fragments (Kumar *et al.*, 1997).

These herbs being cheaper and safer than allopathic drugs may be very useful in veterinary practice, especially in India where these are found in plenty. When the research on herbal preparations has achieved universal acceptance, role of these herbs and bio-stimulators in tissue healing has been well proved scientifically and they form the basis of different herbal preparations that have flooded the market (Jaiswal *et al.*, 2004)

Fracture healing phenomenon is a complex process which involves cell and tissue differentiation and proliferation. Herbs influence rate of fracture healing by influencing the regeneration of the connective tissue of mesenchymal origin viz chondroblast, osteoblast, fibroblast that are involved in healing and quicker mineralization of callus (Chhavi *et al.*, 2011).

Comfrey (*Symphytum officinale*), also known as knit bone, has been advocated in folk medicine for the treatment of wounds, sores, sprains, and bone fractures. In Germany, comfrey has been used in medicine since 1920 for the treatment of musculoskeletal conditions. It has been suggested that the efficacy of comfrey is primarily due to its anti-inflammatory, analgesic, granulation promoting, and antiexudative properties. Comfrey has pharmacological components that include rosmarinic acid and tannin. Rosmarinic acid is a natural polyphenol antioxidant, and both rosmarinic acid and tannin are considered anti-inflammatory agents. Tannic acid (TA), an antioxidant, contains antimutagenic and anticarcinogenic properties that exhibit oxygen free radical trapping activity. Levanon and Stein suggested that the ability of TA to augment glycosaminoglycan binding to collagen most possibly contributes to the structural reinforcement of synovial articulating surfaces (Smith *et al.*, 2011).

Chhavi *et al.* (2011) reported that *Symphytum officinale* removes the inflammation surrounding the fracture. It induced union of affected bones, and contains allantoin, a crystallizable substance used in orthodox medicine to encourage epithelial formation in ulcer and wound.

The WHO has also recommended to all the members of the countries to actively promote native medicines and also to initiate steps to conserve and cultivate medicinal plants. In recent times focus on plant research has increased all over the world and a large body of evidence has been collected to show

immense potential medicinal plants used in various traditional systems. Medicinal herbs are lightened due to their wide use and less side effects (Mate *et al.*, 2008).

Comfrey (*Symphytum officinale*) preparations made from comfrey root were externally applied in case of injuries of the musculoskeletal system, skin affections, and sores and mastitis. Roots were either used freshly crushed, or as extracts prepared with alcohol, oil or fat. Leaves were applied directly onto skin to treat injuries of the musculoskeletal system. Phytoveterinarian literature recommends topical use of comfrey preparations for treatment of contusions, sprains, and pulled muscles (Disler *et al.*, 2014).

*Symphytum officinale* also known as comfrey, knitbone and bone-set in herbal medicine, a homeopathic first aid remedy the later names precisely describe its sphere of genius, the mending of broken bones, and the treatment of bone diseases, ranging from inflammation of the bones, or osteitis to cancers of the bones or sarcomas. *Symphytum officinale* is found to be widely used for treatment of injuries of bones, cartilage, tendons and periosteum. It helps in non-union of fractures, with mending bones that are slow to heal, and where the wound has penetrated into the bones. It helps in alleviating the pain remaining in the periosteum or lining of the bones after the wound has healed. It is recommended in wounds resulted with blows, falls and bruises, and even helps with the tendency to have such mishaps similar to Arnica. The remedy can help with bruises to the tendons and bone healing. *Symphytum* helps healing of sprains and injuries from straining of muscles, bones and tendons similar to Ruta. *Symphytum* helps in treatment of fractures due to osteoporosis. *Symphytum* can help with diseases of the bones, such as inflammation of the bones, inflammation of the inferior maxillary bone and cancer of the bones or sarcomas (Olenev 2014).

Considering the complex phenomenon of bone healing, the present study was designed to compare the healing process of bone using open reduction and internal fixation techniques alone and with osteoinducer homoeopathy drug *Symphytum officinale*. Keeping in view of the above facts, clinical study on 'Role of *Symphytum officinale* as an osteoinducer in long bone fracture repair in canine' was undertaken with the following objective of studying the role of homoeopathic drug *Symphytum officinale* as a stimulating agent in the process of bone healing.

**Objectives** :

- 1) To study the efficacy of *symphytum officinale* as a osteoinducer in long bone fracture repair in canine.
- 2) To study the hematological and biochemical changes during the process of bone healing.
- 3) To evaluate bone healing by radiological observations.
- 4) To study the complication during the study, if any.

# REVIEW OF LITERATURE

## REVIEW OF LITERATURE

The long bone fracture treatment is basically comprised of reduction, retention and immobilization of fractured part but meanwhile supportive therapy is also essential to supplement healing and to reduce discomfort to the animal. However, during the treatment of fracture, several complications are often recorded which delays the bone healing (Kumar *et al.*, 1997).

*Symphytum officinale* L., also known as comfrey, (Boraginaceae), has been considered in traditional medicine for its anti-inflammatory (Predel *et al.*, 2005), analgesic (Goldman *et al.*, 1985; Kucera *et al.*, 2004 and Grube *et al.*, 2007), anti-edematous (Kucera *et al.*, 2004) and astringent properties (Staiger, 2007). For over 2000 years, it has been widely used by the population to treat a variety of ailments and to favor the growth of new tissues in wounds and bone fractures (Staiger, 2007). Additionally, numerous compounds obtained from comfrey like mucilage, allantoin, alkaloids, tannins and sugars, have had their biological activities documented (Youngken, 1950). The wound healing action of comfrey has been attributed to the presence of allantoin (Saito and Oliveira, 1986; Martindale, 2002; Cunha *et al.*, 2003 ), Anti-irritating, hydrating and anti-inflammatory properties are attributed to mucilage (Saito and Oliveira, 1986; and Cunha *et al.*, 2003) and tannins, which are astringent and haemostatic (Cunha *et al.*, 2003).

Extensive research has been undertaken in the area of fractures and its treatment in canines which has been reviewed in short under appropriate sub-headings here under.

### **2.1 Incidence of fracture**

In case of canines the incidence of fractures has been more frequently reported as compare to any other species. The following literature on incidence of fractures has been reviewed with respect to etiology, type, location, sex, age, breed and bone with pathological causes in canine.

Aithal *et al.* (1996) conducted a study and recorded the history of fracture and in most of the cases it was either automobile accident or fall/ jump. Non-descript indigenous dogs were mostly affected. The number of males (63.16%) affected was significantly higher than females (36.84%). The incidence of fracture was highest in less than a year old dogs (56.65%). Femur was affected more than any other bones. They also recorded that oblique or spiral

fractures (56.86%) were significantly more than comminuted (16.57%), transverse (14.86%), incomplete (6.57%) and multiple (5.14%) fractures.

Singh *et al.* (1999) reported incidence of fractures in the age group of six months to eight years. They further reported that fractures involved were proximal, distal or mid-shaft of the bone, and the fractures were oblique, transverse and over-riding in nature. They also observed that the number of males affected was higher than females and fractures of hind limb were more than that of the forelimb., and in hind limb, the fractures of femur were the highest followed by tibia.

Gahlod *et al.* (2002) reported retrospective study on 109 cases of canine fractures. Majority of fractures were seen in young dogs of 0-6 months of age (44.95%) while incidence of fracture was lowest (11%) in dogs above 5 years of age. Small breeds of dogs such as Pomeranian, Lhasa and Cocker spaniel had a higher incidence of fractures (58.71%) than the larger breeds (41.28%). Males were more commonly affected than females. Among the long bones, the incidence of fractures was higher in femur (45.87%), followed by radius and ulna (18.34%), tibia /fibula (12.44%) and humerus (7.33%). The occurrence of oblique fractures was maximum (41.28%) followed by transverse fractures (25.46%), incompletes (2.93%) and comminuted (10.9%) fractures.

Roy *et al.* (2005) reviewed 150 clinical cases of canine fractures. Majority of cases were recorded in Pomeranian (48.70%) followed by German shepherd (18.70%), mongrel (13.40%), Labrador retriever (8.4%) and other breeds accounted for 10.8 percent. Higher incidence of fracture was seen in the age group of 3-6 months (43.33%) followed by 6 months-1 year (23.33%), 2 years and above (20%) and 2 years (13.33%) and fracture of hind limbs more than forelimbs (26.6%). Fracture was found more in females (58.67%) than the males (41.33%). Fractures of femur (42.66%) were highest followed by tibia and fibula (17.33%), radius and ulna (14.67%) and humerus (10%).

Simon *et al.* (2010) analysed 478 cases of pelvic limb fracture reported to radiology unit of Madras Veterinary College, and stated that the incidence was highest in young animals (46.02 %) less than six months of age. Majority of fractures were recorded in non-descript dogs (47.48 %). Male dogs were affected more (61.5 %) than female dogs of all age groups. Among the bones of various pelvic limb the incidence was highest in femur (47.48 %) followed by tibia and

fibula (42.67 %). The occurrence of oblique/transverse fractures were more (44.8 percent) than comminuted (26.8 %) and avulsion fractures (7.53 %).

Kushwaha *et al.* (2011) studied the incidence of fracture and outcome of their management in different animals. The data were analysed with respect to the species, breed, sex, and age of the animal, duration and cause of fracture, limb and bone involved, type of fracture and the technique of fixation. The results showed higher occurrence of fractures in young, male dogs and bovines. Intramedullary pinning was found satisfactory for fixation of long bone fractures in most of the dogs, and in large animals plaster of paris cast with splint was satisfactory in closed fractures below the stifle and elbow joints.

### **2.1.1 Etiology**

Ness and Armstrong (1995) reported fractures of radius and ulna to be the most common in dogs and were usually due to road traffic accidents although kicks, bites and crushing injuries were also responsible.

Aithal *et al.* (1999) reported that the major cause for fracture of radius and ulna was falling from a height (53.1 %) and automobile accidents (34.69 %).

Rao *et al.* (1999) recorded the cause for long bone fracture in canines, which included road traffic accidents (22.66 %), fall from height (43.06 %), animal interaction (11.73 %) and crush injury (22.54 %).

Rani *et al.* (2004) observed that fractures in dogs occurred due to automobile accidents (68.24 %) and falling from a height (31.76 %).

### **2.1.2 Type of fracture**

Aithal *et al.* (1999) reported 50 per cent oblique fractures, 20.61 per cent comminuted fractures, 33.33 per cent transverse fractures, incomplete fractures of 4.55 per cent and 1.52 per cent others, among the 402 cases of fractures of radius and ulna.

Ozsoy and Altunatmaz (2003) reported that out of six cases of radius fracture, three were fractured at the mid-diaphysis and other three were fractured at distal diaphysis.

Milovancev and Ralphs (2004) reported that fractures of radius and ulna were commonly seen in the small animal population and diaphysis as the most common site in the bone.

Rani *et al.* (2004) recorded 45.88 per cent of transverse fractures followed by 42.35 per cent of oblique fractures among the fractures of radius and ulna. Diaphyseal fractures were common in the distal shaft (40.0 %) of radius and ulna.

Rovesti *et al.* (2007) studied 49 cases of radial fracture and observed that 20 were open and 29 were of closed type. Seventeen cases had comminuted type of fracture.

## **2.2 Fracture biology and biomechanics**

Nunamaker (1985) explained that bone as an osseous structure, could be loaded with tension, compression, bending, shear, torsion or a combination of these forces. If the magnitude of the applied load slightly exceeded beyond the elastic limit, catastrophic failure take place.

Johnson and Hulse (2002) reported that bending caused a transverse fracture on the tension side and slight oblique fracture on the compression side of the bone. Axial compression caused an oblique fracture. Combination of axial compression and bending caused oblique comminuted fracture. Torsion caused a spiral fracture, whereas high energy force caused a comminuted, non-reducible fracture.

Hulse and Hyman (2003) observed that bones get fractured when extrinsic or intrinsic forces were applied and both elastic (reversible) and plastic (irreversible) deformation occurred before breakage. In an oblique fracture, shear and compressive forces predominated whereas, in transverse fracture, rotational or torsion forces predominated.

## **2.3 Long bone fracture management**

Singh *et al.* (1999) reviewed 30 clinical cases of fractures in canine with respect to their breed, age, sex, anatomical location, limb involved and its management. Nineteen cases of simple fractures were immobilized by application of Plaster of Paris supported by Thomas splint. In remaining 11 cases, fractures were immobilized by using internal fixation devices viz. intramedullary pins and Kuntscher nails. Fracture union was complete within 8 to 12 weeks following intramedullary pinning and nailing.

Tyagi *et al.* (2002) evaluated different internal fixation techniques for the management of olecranon fracture in 18 adult mongrel dogs. After creating short oblique fracture in olecranon under general anesthesia, the fracture was fixed with stainless steel plate (group I), tension band wiring (group II) and single cancellous screw (group III). The comparison, on the basis of intraoperative, clinical and radiological observations showed that the technique of cancellous screw application was easiest, less traumatic, less time consuming and allowed

early return of full weight bearing and faster healing and hence it was considered best among various techniques evaluated.

Kumar *et al.* (2007) studied occurrence and pattern of long bone fractures, and the efficacy of Intramedullary (IM) Steinmann pin fixation in growing dogs. All the records of growing dogs during a 10 year period were screened to record the cause of trauma, age and sex of the animal, bone involved, type and location of fracture, status of fixation, alignment, maintenance of fixation and fracture healing. The results were analysed and comparisons were made between growing dogs with normal and osteopenic bones. Among the 310 cases of fractures recorded, the bones were osteopenic in 91 cases (29%). Minor trauma was the principal cause of fracture in dogs with osteopenia (25%), and indigenous breeds were most commonly affected (38%). Fractures in dogs with osteopenic bones were most commonly recorded in the age group of 2-4 months (53%), whereas fractures in normal dogs were almost equally distributed between 2 and 8 months of age. Male dogs were affected significantly more often in both groups. In osteopenic bones, most fractures were recorded in the femur (56%), and they were distributed equally along the length of bone. Whereas in normal bones, fractures were almost equally distributed in radius/ulna, femur and tibia, and were more recorded at the middle and distal third of long bones. Oblique fractures were most common in both groups; however, comminuted fractures were more frequent in normal bones, whereas incomplete fractures were more common in osteopenic bones. Ninety-nine fracture cases treated with IM pinning (66 normal, 33 osteopenic) were evaluated for the status of fracture reduction and healing. In a majority of the cases (61%) with the osteopenic bones, the diameter of the pin was relatively smaller than the diameter of the medullary cavity (<70-75%), whereas in 68% of the cases in normal bones the pin diameter was optimum. The status of fracture fixing was satisfactory to good in significantly more osteonormal (59%) than osteopenic dogs (42%). Fracture healing, however, was satisfactory in significantly more cases with osteopenic than normal bones. The appearances of callus were relatively early and the amount of bridging callus was relatively large in greater number of osteopenic bone fractures. Mal-union and non-union were recorded more often in osteopenic cases than in normal cases. However, the incidence of bone shortening and osteomyelitis was significantly higher in normal bones than in osteopenic bones.

Uma Rani *et al.* (2007) recorded the fracture pattern of femur in canine surgically managed by open reduction and internal fixation either with Steinmann pin or Kuntscher nail (K nail) or dynamic compression plate depending on type of fracture. Fracture healing was evaluated based on clinical and radiological examinations. The dogs with K nail showed early functional usage by 1<sup>st</sup> week and full weight bearing by 6<sup>th</sup> week. Complete fracture healing and clinical union of fracture was evident in all the animals with 8 to 12 hours postoperative week.

Dwiwedi *et al.* (2009) conducted a study on six clinical cases of compound fracture in dogs of either sex, weighing 10 to 20 kg. The fracture was reduced and stabilized by transfixation using Ilizarov's ring fixator. The Ilizarov's ring fixator was designed with two full and one 5/8 aluminium rings of 90 mm and 100 mm diameter for radius and tibia respectively, six Kirschner-wires (K-wires) of 1.6 mm diameter each and six connecting rods each of 100 mm length were found suitable. The parameters studied during the postoperative period were weight bearing on the operated limb, pain, apparatus stability, K- wire site discharge, K- wire loosening, K-wire breakage/bending, osteolysis, periosteal reaction, callus formation, haematology and serum biochemistry. Transfixation using Ilizarov's technique provided anatomical reduction, axial stiffness of the fractured fragments, joint mobility, functional weight bearing and prevented muscle atrophy.

Julie *et al.* (2010) conducted a study in twelve clinical cases of complete fracture of long bone in dogs subjected to treatment with acrylic external fixation of fractured bone and evaluated by radiographical observations. In animals of less than 15kg body weight, Type I A fixator or type II fixator with acrylic connecting bars were used. In case of heavy animals of more than 15 kg body weight, type II fixator (bilateral configuration with acrylic connecting bar on one side and stainless steel bar on another side) were used. They stated that acrylic external fixation was clinically successful and reliable alternative for stainless steel external fixator for immobilization of fractures of radius and ulna and tibia-fibula in animals less than 15 kg body weight and it provided early return of functional limb usage. In heavier animals, usage of acrylic bar on one side of type II fixator gave adequate stability in case of radial fractures but not for tibial fractures.

Ayyapan *et al.* (2011) reported a case of that a two year old non-descript male dog with the history of non weight bearing lameness of right forelimb. Palpation of the right shoulder indicated pain, swelling and crepitus. Confirmative

diagnosis of an unstable humeral diaphyseal fracture was made by radiographic evaluation of lateral and craniocaudal views. Haematological parameters were within the normal range. Open reduction and internal fixation was accomplished using a 3.5 mm dynamic compression plate. The plate was applied on the tension surface of the bone based on ASIF technique. Additional stability was provided by insertion of 3 mm intramedullary Steinmann pin. Normal weight bearing was noticed on immediate post operative day.

Raghunath *et al.* (2012) studied and evaluated the suitability of static intramedullary interlocking nailing for stabilization of segmental tibial and femoral fractures in dogs. In the study period between 2002 to 2012, a total of 216 cases of long bone fractures were stabilized with static intramedullary interlocking nailing of which 12 fractures with 8 tibial and 4 femoral were segmental type with two level fracture lines having intact / split intercalary segment. In 8 fractures the intercalary segment was intact tubular, in one there was incomplete longitudinal split in the intercalary segment and in 3 cases the intercalary segment was having complete longitudinal split. Along with static intramedullary interlocking nailing, in eight cases adjunctive stabilization with full cerclage wiring was done. A rapid return of function was noted in all cases by 10<sup>th</sup> post operative day with complete fracture union in 11 cases with variable healing times for proximal and distal fracture lines. Out of three open fractures, one resulted in non-union.

#### **2.4 Bone healing agents**

Gauri *et al.* (1992) conducted experiments on different plants used for tissue healing of animals. They stated that tissue healing is an important process, which is the basis of various surgical manipulations. It can be enhanced by using several drugs having antiseptic, anti-inflammatory, antimicrobial and biostimulator properties. They reported certain plants, which are proved helpful in wound healing, fracture healing and healing of nervous tissue in animals. They also mentioned that direct application of root paste of *Symphytum officinale* Linn. (Comfrey, healing herb) aids in union of fractured bone.

Asgar *et al.* (1999) studied the osteogenesis effect of *Bambusia arundinacea* and *Cissus quadrangularis*, *Uraria picta* *Lepidium sativum* on radius-ulna fracture healing in eighteen dogs which were divided equally in control and treatment groups. In all animals, close fracture of radius ulna was induced experimentally and immobilized with plaster of paris cast. Animals of treatment group were fed with 6 ml of *Cissus quadrangularis- Uraria picaliptidium*

*sativum* suspension (Caldhun, Dabur ayurved Ltd.) twice a day for a period of 20 days post fracture while control group animals were not given any supplements. Clinical observations revealed early functional restoration of limbs in treated group than the control group and concluded that *Cissus quadrangularis- Uraria pictalepidium sativum* if given per oral for twenty days increases the rate of fracture healing and calcification.

Draghaia *et al.* (2003) conducted study focused on the evaluation of the pharmacological and toxicological effects of plasmid-mediated GHRH supplementation with electroporation in normal adult dogs over a 180-d period. Twenty-eight dogs (< 2 yr of age) were randomized to four groups. Three groups (four dogs/sex for each group) were treated with ascending doses of GHRH-expressing plasmid: 0.2, 0.6, and 1 mg. One group (two dogs of each sex) served as the control. Clinical observations and body weights were recorded and hematological, serum biochemical, and urine analyses were performed. Serum IGF-I, ACTH, and insulin were also determined. Necropsies were performed on day 93 and 180; organs were weighed and tissues were fixed and processed for light microscopy. Selected tissues were used to assess plasmid biodistribution on day 93. At all doses, plasmid GHRH caused increased weight gain ( $P < 0.001$ ), without organomegaly. Serum glucose and insulin in fasted dogs remained within normal ranges at all time points. Adrenocorticotrophic hormone was normal in all groups. Significant increase in number of red blood cells, hematocrit, and hemoglobin ( $P < 0.01$ ) were observed. In conclusion, their study showed that plasmid-mediated GHRH supplementation was safe in electroporated doses up to 1.0 mg in young healthy dogs.

Mahendra *et al.* (2006) conducted study in 12 clinical cases of femur diaphyseal fractures in dogs. The animals were randomly divided into two groups of six animals each (group A and group B). In dogs of group A, the femur diaphyseal fractures were repaired by retrograde intramedullary pinning technique with cerclage wiring. In dogs of group B, femur diaphyseal fractures were repaired by intramedullary pinning, and gentamicin impregnated polymethylmethacrylate was used as a luter in the form of small splints, placed on the medial and lateral aspect of the fracture site and secured with cerclage wire. The radiographic studies undertaken on days 0, 1, 15, 30, 45 and 60 revealed better healing of femoral fracture treated with gentamicin impregnated polymethyl-

methacrylate splints than with conventional intramedullary pinning and cerclage wiring.

Simpson *et al.* (2006) studied that bone morphogenetic proteins (BMPs), transforming growth factor (TGF) beta, insulin-like growth factor (IGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) were all present during normal healing of fractures. They were all naturally-occurring agents, each of which has the range of molecular variants which have been identified as having greater osteogenic potency *in vivo*. The BMPs were members of the larger TGF-beta super family. To date, over 20 different BMPs have been isolated, but only some had the potential to induce formation of new bone. TGF-beta, another of the TGF-beta super family, had five isoforms and was found in many types of tissue. Animal studies have demonstrated varying results in its influence on the healing of fractures—The IGF family consists of two polypeptide members; IGF-II was the most abundant in bone, but IGF-I had the greater osteogenic potency. *In vitro*, IGF-I had been shown to stimulate chemotaxis and activity of osteoblasts, whilst *in vivo*, increased bone formation had been observed in bone-repair models in animals. IGF-I was of greatest effect when used in combination with TGF-beta. To date, nine members of the FGF family have been identified, the most abundant in human tissue being FGF-1 (alpha) and FGF-2 (beta). The FGF has been shown to promote an osteogenic response in progenitor cells by providing cytotoxic resistance to inflammatory oxidants, accelerate fracture healing directly. VEGF was stored and secreted by osteoblasts and endothelium and had an important role in angiogenesis during repair of fractures. The role of PDGF in the healing of fractures has not been clarified. It was released from platelets during the formation of haematoma and has been shown to stimulate the migration of osteoblasts and mesenchymal progenitor cells. In addition to these agents, various hormones such as growth hormone and parathyroid hormone (PTH) have an effect on the repair of fractures.

Alecu *et al.* (2007) evaluated the effect of the homeopathic remedies Arnica Montana and Staphisagria dynamizations 7CH and 30CH, on the speed of the cicatrization of surgical incisions in experimental animals. The decrease of the number of days required for the complete cicatrization was statistically significant for both remedies, by comparison to a placebo. There was no significant difference between both remedies nor between both dynamizations.

Amanat *et al.* (2007) hypothesized that zoledronic acid treatment would bolster fracture repair. In a rat model for closed fracture healing, a single dose of ZA at 0, 1, or 2 wk after fracture significantly increased BMC and strength of the healed fracture. Delaying the dose (1 or 2 wk after fracture) displayed superior results compared with dosing at the time of fracture. Bisphosphonates are known to increase bone strength and thus the resistance to fracture by decreasing osteoclastic bone resorption. These properties may enable bisphosphonates to also increase the strength of fracture repair. Zoledronic acid (ZA) is a potent bisphosphonate with a high affinity for bone mineral, allowing bolus intravenous dosing in a range of indications. In this study, they examined the application of bolus dose ZA in endochondral fracture repair.

Carbon-14 labeled ZA was used in a closed rat fracture model. Rats were divided into five treatment groups ( $n=25$  per group): saline control, local ZA (0.01 mg/kg), and three systemic bolus ZA groups (0.1 mg/kg) with different administration times: at fracture, 1 wk after fracture, and 2 wk after fracture. Rats were killed 6 wk postoperatively. Postmortem analyses included radiography, QCT, CT, biomechanical testing, scintillation counting, autoradiography, and histology.

Single-dose systemic ZA administration significantly increased callus volume, callus BMC, and mechanical strength. Perioperative treatment increased mechanical strength by 30% compared with controls ( $p<0.05$ ). Administering the systemic dose at 1 or 2 wk after fracture further increased mechanical strength compared with controls by 44% and 50%, respectively ( $p<0.05$ ). Autoradiographic analysis indicated that ZA binds significantly to bone that is present at the time of administration. ZA quantification indicated that delayed administration significantly increased the uptake efficiency in the callus. Histological and CT analysis showed that ZA treated calluses had a distinctive internal structure consisting of an intricate network of retained trabecular bone.

Rao *et al.* (2007) studied the bone healing property of *Cissus quadrangularis* which is a shrub, with thick and fleshy quadrangular stem and the roots and stem are supposed to be most useful for healing of fracture of the bones evaluating the effect of alcoholic extract of *Cissus quadrangularis* on the intrauterine growth of trabeculae in the long bones in rats. Pregnant rats were administered with Ethanol extract of *Cissus quadrangularis* (CQ), orally, at the dose of 750 mg/kg body weight from 9<sup>th</sup> day of gestation till delivery. Femur bone

of the newborn pups were collected, decalcified and processed for paraffin sectioning. Thickness of cortical bone at mid shaft level, thickness of individual trabeculae were measured using a calibrated ocular micrometer. Results showed a significantly increase in thickness of the cortical bone at mid shaft level compared to control rats. Pregnant rats were administered with Ethanol extract of *Cissus quadrangularis* (CQ), orally, at the dose of 750 mg/kg body weight from 9<sup>th</sup> day of gestation till delivery. Femur bone of the newborn pups were collected, decalcified and processed for paraffin sectioning. Thickness of cortical bone at mid shaft level, thickness of individual trabeculae were measured using a calibrated ocular micrometer. Results showed a significantly increase in thickness of the cortical bone at mid shaft level compared to control rats. The thickness of individual trabecula was increased significantly. The present study demonstrates enhanced bone formation during fetal growth by CQ, which may be related to rich content of calcium, phosphorus and phytoestrogenic property of the plant.

Wang *et al.* (2007) studied the effect of Simvastatin solution which was injected subcutaneously to the site of fractured tibiae of ovariectomized rats. Afterwards healing quality was evaluated by morphologic, radiographic, biomechanical, histological and histomorpho-metric methods at 1, 2 and 4 weeks after fracture. Results showed that locally applied simvastatin improved fracture healing. Statins are used clinically for reduction of cholesterol synthesis to prevent cardiovascular disease. This study aimed to prevent this degradation by developing a locally administered formulation of simvastatin that is encapsulated in poly(lactic-co-glycolic acid)/hydroxyapatite (SIM/PLGA/HAp) microspheres with controlled-release properties. They suggested that SIM/PLGA/HAp should be developed as an osteoinductive agent to treat osteonecrosis or in combination with an osteoconductive scaffold to treat severe bone defects.

Haque *et al.* (2007) established surgical technique i.e. distraction osteogenesis (DO) that generates new bone by gradual distraction of two bony segments. In this study, they investigated the temporal and spatial profile of FGF 1, 2 and 18, IGF 1 and 2, and TGF $\beta$ -1 during distraction osteogenesis using immunohistochemistry. An osteotomy was performed on the right tibia of 13 white male New Zealand rabbits. After a delay of 7 days, distraction was started at a rate of 0.25mm/12hrs for 3 weeks which was followed by a 3 week period of consolidation. Immunohistochemical analysis was performed on a weekly interval to determine the expression of the growth factors. Staining of all growth factors

was apparent at various levels in the centre and callus region in fibroblasts and chondrocyte cells. FGF2 however, showed continued high expression in osteoblasts. Within two weeks after the end of distraction all growth factors showed a reduction in expression except for FGF18 which maintained high levels of expression (up to 100% staining) throughout the distraction and consolidation phases. The study suggested that in comparison to the other investigated growth factors, FGF18 may play an important role throughout the entire process of distraction osteogenesis.

Ghodadra and Singh (2008). stated that instead of improvements in surgical technique, internal fixation, and understanding of biologics, fracture healing is delayed or impaired in up to 4% of all fractures in U.S.A. Complications due to impaired fracture healing present therapeutic challenges to the orthopedic surgeon and often lead to chronic functional and psychological disability for the patient. As a result, it has become clinically desirable to augment mechanical fixation with biologic strategies in order to accelerate osteogenesis and promote successful arthrodesis. The discovery of bone morphogenic protein (BMP) has been pivotal in understanding the biology of fracture healing and has been a source of intense clinical research as an adjunct to fracture treatment. Multiple *in vitro* and *in vivo* studies in animals have elucidated the complex biologic interactions between BMPs and cellular receptors and have convincingly demonstrated rhBMP-2 to be a safe, effective treatment option to enhance bone healing. Multiple clinical trials in trauma surgery have provided level 1 evidence for the use of rhBMP-2 as a safe and effective treatment of fractures. Human clinical trials have provided further insight into BMP-2 dosage, time course, carriers, and efficacy in fracture healing of tibial defects. These promising results have provided hope that a new biologic field of technology has emerged as a useful adjunct in the treatment of skeletal injuries and conditions.

Herbenick *et al.* (2008) reported that the NSAIDs have been mainstays of treatment for pain after surgery and in patients with fractures or soft tissue injuries. They *studied the* effects of a Cyclooxygenase 2 Inhibitor on Fracture Healing in a Rat Model. The study was designed to examine the effects of COX-2 inhibitors on biomechanical strength and direct callus measurement in fracture healing. They observed a statistically significant difference in the biomechanical strength of fracture in the pooled data from their treatment rats and control rats, but they did not find any statistically significant difference in biomechanical

strength at 4 sacrifice intervals (2, 4, 8 and 12 weeks). At the 8-week interval, more callus was noted in treatment group relative to the control group. However, no statistically significant difference was found in mean elliptical area of callus formation at any interval. The data suggested that COX-2 enzyme function is important for fracture healing and that caution should be used when considering use of COX-2 inhibitors in patients with fractures.

Simman *et al.* (2008) studied that Platelet-rich plasma (PRP) is a common therapy for acceleration of maxillofacial and spinal fusion bone-graft healing. This study analyzed the therapeutic role of PRP during long-bone fracture healing evaluated Lewis rats. Following creation of unilateral open femur fractures, either 500 L thrombin-activated PRP (PRP treated group) or 500 L saline (control group) were applied once to the fracture site. Fracture healing was analyzed after 1 and 4 weeks. Following 4 weeks of fracture healing, radiographic analysis demonstrated higher callus to cortex width ratio (P0.05) in the PRP group (PRP: 1.650.06; control: 1.480.05). Three-point load bearing showed increased bone strength following PRP treatment (PRP: 60.85 6.06 Newton, control: 47.66 5.49 Newton). Fracture histology showed enhanced bone formation in the PRP group. Immunohistochemistry and Western-blotting demonstrated healing-associated changes in transforming growth factor (TGF) - 1 and bone morphogenetic protein (BMP)-2. Their results suggest that PRP accelerates bone fracture healing of rat femurs via modulation of TGF- 1 and BMP-2 growth factor expression.

Greiner *et al.* (2008) investigated that local application of zoledronic acid (ZOL) accelerates fracture healing in rats. Moreover local and systemic application of bisphosphonates has been shown to enhance screw fixation (Tengvall *et al.*, 2004) and even prevent prosthetic loosening (Hilding *et al.*, 2007 and Friedl *et al.*, 2009). Since implant ingrowth is not a desirable effect in fracture healing, especially regarding intramedullary implants, this remained a concern in local application of ZOL in the fracture situation. Whether, intramedullary application of a ZOL coated implant leads to enhanced ingrowth was therefore investigated. Systemic application of ZOL was also analyzed for comparison in the present setting.

Tarantino *et al.* (2009) studied that osteoporosis the most common alteration of bone metabolism. It derives from an increase in bone resorption with respect to bone formation and is characterized by microarchitectural alterations, decreased bone mass and increased risk of fracture. The coupling between bone

formation and resorption is a fundamental concept in skeletal metabolism, and it explains how a certain amount of removed tissue can be replaced by the same amount of new bone. Various substances used to treat osteoporosis may also be used for orthopaedic conditions such as fracture healing, implant fixation, bone grafts and osteonecrosis. Fracture healing consists in the replacement of the lost bone by a tissue that has the same biomechanical properties as those preceding the fracture. The repair process is triggered by the local response to the tissue injury that damaged the continuity of bone.

Neto *et al.* (2010) evaluated the effect of *Symphytum Officinale*, homeopathic drug of potency (6cH), on the removal torque and radiographic bone density around titanium implants, inserted in rats tibiae. Implants were placed in male rat tibiae, and the animals randomized to two groups (Control and *S. officinale* 6cH treated), which were evaluated at 7, 14, 28 and 56 days post-implantation. Radiographic bone density was measured at 6 points around the implant, using digital radiographic images, when implants were inserted and at sacrifice. Removal torque of the implants was also evaluated. Both removal torque and radiographic bone density evaluation showed that *S. officinale* 6cH treatment enhanced bone formation around the micro-implants, mainly at 14 days. At 56 days, the radiographic bone density was higher in the treated group. Results concluded that *S. officinale* 6cH enhances, principally at the early stages of osseointegration, bone formation around titanium implants in rats' tibia, based on radiographic and mechanical analysis.

Nandi *et al.* (2010) stated that the treatment of delayed union, malunion, and nonunion is a challenge to the orthopaedic surgeons in veterinary field. Apart from restoration of alignment and stable fixation, in many cases adjunctive measures such as bone-grafting or use of bone-graft substitutes are of paramount importance. Bone-graft materials usually have one or more components: an osteoconductive matrix, which acts as scaffold to new bone growth; osteoinductive proteins, which support mitogenesis of undifferentiated cells; and osteogenic cells, which are capable of forming bone in the appropriate environment. Autologous bone remains the "gold standard" for stimulating bone repair and regeneration, but its availability may be limited and the procedure to harvest the material is associated with complications. Bone-graft substitutes can either substitute autologous bone graft or expand an existing amount of autologous bone graft.

Araujo *et al.* (2011) evaluated wound healing activity of leaves extracts of *Symphytum officinale* L. (comfrey) incorporated in three pharmaceutical formulations. Wound healing activity of comfrey was determined by qualitative and quantitative histological analysis of open wound in rat model, using allantoin as positive control. Three topical formulations, carbomer gel, glycerol-alcoholic solution and O/W emulsion (soft lotion) were compared. The histological analysis of the healing process showed significant differences in treatment, particularly on its intensity and rate. The results indicated that emulsion containing both extracts, commercial and prepared, induced the largest and furthest repair of damaged tissue. This could be evidenced from day 3 to 28 by increase in collagen deposition from 40% to 240% and reduction on cellular inflammatory infiltrate from 3 percent to 46 percent. However, 8 percent prepared extract in emulsion presented the best efficacy. The work clearly demonstrated that comfrey leaves have a wound healing activity. The O/W emulsion showed to be the vehicle most effective to induce healing activity, particularly with extracts obtained from comfrey leaves collected in Minas Gerais state in Brazil. It showed the best efficacy to control the inflammatory process and to induce collagen deposition at 8 percent concentration.

Ahmad *et al.* (2011) conducted study on herb *Berberis lycium* Royle (family: *Berberidaceae*), a native to Pakistan, India and whole region to Himalayas widely used like food and in folk medicine. A wide range of medicinally and nutritionally important phytochemical constituents have been isolated from plant such as alkaloids, cardioactive glycosides, saponins, tannins, anthocyanins, vitamins, carbohydrates, proteins, lipids, fiber content,  $\beta$  carotene, cellulose, phytic acid and phytate phosphorous. Plant possesses minerals such as Sodium, Calcium, Sulphur, Iron, Zinc, Copper, Lead, Manganese, Potassium and Phosphorus, which contribute to broad variety of biological processes and are valuable in the treatment of various disorders including bone fractures.

Chhavi *et al.* (2011) stated that fracture healing is a complex process that involves cell and tissue differentiation and proliferation. Herbs like *Symphytum officinale* Linn. (knit bone) influence the rate of fracture healing by influencing the regeneration of connective tissue of mesenchymal origin viz. chondroblast, fibroblast and osteoblast that are involved in healing and earlier mineralization of callus. *Symphytum officinale* Linn. (*boraginaceae*) removes the inflammation surrounding the fracture. It induced union of affected parts and contain allantoin,

a crystallizable substance used in orthodox medicine to encourage epithelial formation in ulcer and wound.

Smith and Jacobsan (2011) conducted an experiment to determine the effect of 2 concentrations of topical, comfrey-based botanical creams containing a blend of tannic acid and with primary osteoarthritis of the knee. Forty-three male and female subjects (45-83 years old) with diagnosed primary osteoarthritis of the knee who met the inclusion criteria were entered into the study. The subjects were randomly assigned to 1 of 3 treatment groups: 10% or 20% comfrey root extract (*Symphytum officinale* L.) or a placebo cream. Outcomes of pain, stiffness, and functioning were evaluated on the Western Ontario and MacMaster Universities Osteoarthritis Index. Participants applied the cream 3 times a day for 6 weeks and were evaluated every 2 weeks during the treatment. Repeated-measures analyses of variance yielded significant differences in all of the Western Ontario and MacMaster Universities Osteoarthritis Index categories (pain  $P<0.01$ , stiffness  $P<0.01$ , daily function  $P<0.01$ ), confirming that the 10% and 20% comfrey-based creams were superior to the reference cream. The active groups each had 2 participants who had temporary and minor adverse reactions of skin rash and itching, which were rapidly resolved by modifying applications. Both active topical comfrey formulations were effective in relieving pain and stiffness and in improving physical functioning and were superior to placebo in those with primary osteoarthritis of the knee without serious adverse effects.

Wang (2012) conducted research on shockwave generation which include electrohydraulic, electromagnetic and piezoelectric principles. Electrohydraulic shockwaves are high-energy acoustic waves generated under water explosion with high voltage electrode. Shockwave in urology (lithotripsy) is primarily used to disintegrate urolithiasis, whereas shockwave in orthopedics (orthotripsy) is not used to disintegrate tissues, rather to induce tissue repair and regeneration. ESWT was utilized in the treatment of non-union of long bone fracture, avascular necrosis of femoral head, chronic diabetic and non-diabetic ulcers and ischemic heart disease.

Olenev (2014) reported that *Symphytum officinale* a homeopathic first aid remedy is also known as comfrey, knitbone and bone-set in herbal medicine. The later names precisely describe its sphere of genius, the mending of broken bones, and the treatment of bone diseases, ranging from inflammation of the

bones, or osteitis to cancers of the bones or sarcomas. *Symphytum* 30C or 200C strength helps with the non-union of fractures, with mending bones that are slow to heal, and where the wound has penetrated into the bones. It helps with pain remaining in the periosteum or lining of the bones after the wound has healed. The remedy can help with bruises to the tendons and bones. Like *Ruta*, *Symphytum* helps with sprains, and injuries from straining of muscles, bones and tendons. *Symphytum* can help with diseases of the bones, such as inflammation of the bones, inflammation of the inferior maxillary bone and cancer of the bones or sarcomas. It can also help with necrosis of the bone.

Staiger (2008) reported that comfrey has been used over many centuries as a medicinal plant. It is known as various names such as Ass-ear, Blackwort, Bruisewort, Consormol, knitbone and Knitback, belongs to the Boraginaceae family. It is a perennial herb having black roots resembling turnip and bears broad leaves with bristles. The herb is indigenous to Europe and grows in soggy and lush green areas, especially along the river side. The flowers of the plant are bell-shaped and vary in hue from white to pink or purple. It is widely used in treating a number of cases. The remedial characteristics of comfrey owe to allantoin, an active element of the herb. Allantoin is basically a mediator that endorses propagation of cell. The herb also has some amount of tannin and mucilage. The parts of plant under the ground, especially the root, has around 0.6 to 0.7 per cent of allantoin and approximately 4.0 to 6.5 per cent tannin. Topical application of comfrey over a fractured bone results in dissemination of its prime element allantoin into the tissues below and helps in speeding up the healing and closing process. Allantoin is known to encourage cell growth and thereby, mend damaged cells. In addition, the herb also has rosmarinic acid and additional phenolic acids that enable comfrey to function as an anti-inflammatory agent. The mucilage is analgesic by nature and facilitates in calming down the exasperating conditions. The extracts of comfrey has been used for the treatment of painful muscle and joint disorders and it is clinically proven to relieve pain, inflammation and swelling of muscles and joints in the case of degenerative arthritis, acute myalgia in the back, sprain, contusions and strain after injuries and accidents.

Disler *et al.* (2014) stated that preparations made from *Symphytum officinale* root were extensively applied in case of injuries of the musculoskeletal system, skin affections, and sores and mastitis. Roots were either used freshly

crushed, or as extracts prepared with alcohol, oil or fat. Leaves were applied directly onto skin to treat injuries of the musculoskeletal system. Phytoveterinarian literature also recommended topical use of comfrey preparations for treatment of contusions, sprains, pulled muscles.

Comfrey (*Symphytum officinale*) has been shown in human studies to aid wound healing (Barna *et al.*, 2007) and positive results have been shown in reducing symptoms of back pain (Giannetti *et al.*, 2010; Kucera *et al.*, 2005), arthritis of the knee (Grube *et al.*, 2007) and it was superior to conventional antiinflammatory agent diclofenac gel in reducing symptoms in ankle sprain (D'anchise *et al.*, 2007; Predel *et al.*, 2005).

For centuries, comfrey has been used as a traditional medicinal plant for the treatment of painful muscle and joint complaints (Kothmann, 2003; Englert *et al.*, 2005). Comfrey has also been used in veterinary medicine (Rabinovich, 1981). The German Commission E has assessed preparations containing *Symphytum officinale* L. positively for the treatment of blunt injuries (Kommission E, 1990a, 1990b). Comfrey also stimulates granulation and tissue regeneration, and supports callus formation (Kommission, 1990a, 1990b). The constituents of comfrey root include 0.6–4.7% allantoin (Dennis *et al.*, 1987); abundant mucilage polysaccharides (about 29%) composed of fructose and glucose units (Franz, 1969); phenolic acids such as rosmarinic acid (up to 0.2%), chlorogenic acid (0.012%) as well as caffeic acid (0.004%) and *o*-hydroxy caffeic acid (Andres, 1991; Grabias and Swiatek, 1998; Teuscher *et al.*, 2009); glycopeptides and amino acids (Hiermann and Writzel, 1998); and triterpene saponins in the form of monodesmosidic and bidesmosidic glycosides based on the aglycones hederagenin (e.g. symphytoxin A), oleanolic acid (Aftab *et al.*, 1996) and lithospermic acid (Wagner *et al.*, 1970).

## **2.5 Clinical evaluation**

Egger *et al.* (1995) described the clinical assessment of limb function which included, rating of the animal as excellent with continuous normal leg use whereas an animal which use leg with occasional mild lameness as good, while animal with consistent severe or non weight bearing lameness was rated as poor. They reported that, clinical examination revealed premature pin loosening and complications of external skeletal fixation.

Butterworth (1993) opined that the frame had to be checked on weekly basis to ensure that the clamps and/or pins were not loosened and to rule out the pin tract infection.

Fox *et al.* (1995) classified limb use after external skeletal fixator application as Excellent, when the leg was functionally normal; Good, when a slight lameness was observed only after an extensive exercise; Fair, when a slight to moderate lameness was seen but having consistent weight bearing and Poor, when there was non weight bearing lameness.

Chaudhary (1997) observed a non significant variation in the serum calcium and phosphorus values in dogs during the fracture healing. It was also observed that, the serum alkaline phosphatase values increased till the 30th to 45th day during the healing of tibial fracture.

Piermattei and Flo (1997) concluded that, young animals showed faster clinical union of fractured bones following external fixation than the older animals.

Aithal *et al.* (1998) observed the elevated heart rate and respiratory rate postoperatively in dogs undergoing supracondylar femur fracture treatment.

Chandy (2000) reported that, the treatment of femur fractures with external skeletal fixators resulted in increased rectal temperature along with local warmth of the affected region of the limb. The author opined that, the rise in temperature might be due to osteomyelitis.

Srinivasamurthy (2000) reported that, there was a rise in temperature, heart rate and respiratory rate up to third post-operative day in dogs treated for femur fracture. The author opined that, these changes might be due to reparative inflammatory process occurring at the fracture site of surgical wound.

Riaz (2004) found that, there was no statistically significant variation in the serum alkaline aminotransferase and aspartate aminotransferase levels in dogs surgically treated for vertebral fractures. The author also reported that, there was no variation in the enzyme levels. However, The author observed a significant increase in serum alkaline phosphatase levels till the end of the study period (six weeks) in dogs which underwent surgical correction for vertebral fracture by stabilization of vertebral column.

Julie (2005) reported that, there was no significant variation in the physiological parameters on using external skeletal fixation and opined that, incidence of fracture and the immobilization procedure employed did not produce any untoward systemic effects.

Chandy (2006) observed a non significant variation in serum calcium, phosphorus, ALT, AST and ALP values in dogs treated for vertebral fractures by stabilization of vertebral column.

Julie *et al.* (2007) used the Ceftriaxone sodium at the dose rate of 20 mg / kg body weight intravenously to prevent post-operative infection. They also advised the owner to restrict the movement of animal for two weeks after surgery and then allowing less walk.

Pardeshi (2008) reported in the study of external skeletal fixation for tibial fracture repair, there was a significant elevation of temperature, heart rate, pulse rate and respiratory rate between first and third post-operative days which subsequently returned to normal.

## **2.6 Radiological evaluation**

Ackerman and Silverman (1978) stated callus was first visible radiographically seven to fourteen days after fixation of fracture of femur. They reported that minimal callus was formed in case of rigid immobilization and mobility of fractured fragments resulted in large callus formation.

Saravanan *et al.* (1999) evaluated radiographically different techniques of internal fixation in fifteen matured mongrel dogs divided in to three groups for management of comminuted femoral fracture. Immobilization with intramedullary pinning with cerclage wiring in f group A, dynamic compression bone plating (DCP) in group B and with cerclage wiring in group C respectively. Little evidence of periosteal bridging callus was formed at the fracture site in all affected dogs. However , this periosteal reaction was extensive in subsequent intervals, in group A than group B and C. Excellent primary fracture healing with periosteal continuity was observed in dogs of group B and C, whereas healing was delayed in group A.

Asgar *et al.* (1999) used 6 ml of suspension of *Cissus quadrangularis* for twenty days in case of experimentally induced close fracture of radius and ulna and it was immobilized with plaster of paris cast. Radiographic picture after 60 day revealed well organized, dense, periosteal callus around the fracture site in the dogs fed with suspension of *Cissus quadrangularis*.

Greiner *et al.* (2008) conducted study in twelve clinical cases of complete fracture of long bones which were subjected to treatment with acrylic external skeletal fixation of the fractured bone. The fractured bone was immobilized either by close method or open internal fixation. In all cases plain radiographs in lateral

view were taken at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> week, postoperatively. They finally concluded that there was good apposition between fracture fragments in most of the cases. The fracture gap in all cases was found progressively filled with callus.

## **2.7 Haematological and biochemical observations**

Komnenou *et al.* (2005) expressed that Bone healing is monitored mainly by physical and serial radiologic examinations of the fracture site. However, it is sometimes difficult to distinguish a delayed union from a nonunion, and advanced imaging techniques may not be available. Serum biochemical markers of bone formation, such as alkaline phosphatase (ALP) activity, may be clinically useful in evaluating the progress of healing. The purpose of this study was to correlate serial values of serum ALP activity with the process of fracture healing in dogs and to assess its potential as a postsurgical prognostic indicator. Changes in serum ALP activity were studied in 83 dogs with closed long bone diaphyseal fractures treated surgically. Physical and radiologic examinations of the fracture site and determination of serum ALP activity and calcium (Ca) and phosphate (P) concentrations were performed on admission (day 0); postoperatively on days 10, 20, and 30; and subsequently on a monthly basis until bone union was completed or signs of nonunion were evident. The dogs were allocated into 3 groups with respect to the fracture healing progress as documented by physical and serial radiologic examination. Group A dogs (n=35) developed a medium-sized callus that led to bone union within 2 months. Group B dogs (n=36) had a hypertrophic callus and delayed union, within 3-5 months. Group C dogs (n=12) had slow progress in fracture healing, with minimal callus formation during a 2-month period. Changes in mean serum ALP activity followed the same pattern in groups A and B, reaching a maximum level on day 10. Group A values returned to normal within 2 months, at which point bone union was complete, whereas group B values remained increased and returned to normal within 3-5 months, thus correlating with delayed union. In Group C, mean serum ALP activities showed no significant changes during the 2-month follow-up period, consistent with failure of bone union (nonunion). Serum P and Ca changes followed a proportional and inverse pattern to ALP changes, respectively. They opined that serial determination of serum ALP activity during fracture healing could be an additional tool in predicting fractures at risk of developing a nonunion, helping the clinician to choose the appropriate intervention.

Paskalev *et al.* (2005) found in their experiment, changes in serum concentrations of some bone markers in experimentally-induced normal healing femoral fracture in dogs. In six dogs, a diaphyseal osteotomy of one femur and then, an intramedullary osteosynthesis with a Kuntcher nail were performed. Prior to the operation and at post operation weeks 1, 2, 3 and 4, blood samples were obtained for analysis of serum concentrations of total and ionized calcium, inorganic phosphate, total and bone alkaline phosphatase, osteocalcin and carboxyterminal telopeptide of collagen type I (ICTP). Significant alterations in the levels of these bone markers occurred as follows: for total calcium – by week 1, for ionized calcium – by weeks 1 and 3, for inorganic phosphate – by week 3, for ICTP – by weeks 1 and 2. In the other parameters, the changes were not significant. In conclusion, they stated that within a period of one month, the markers of bone resorption were altered whereas the markers of bone formation showed only a tendency towards decrease.

Mahendra *et al.* (2007) reported no significant change in serum calcium and serum phosphorus levels post-operatively after treating femur fracture in dogs using intramedullary pinning and cerclage wiring. They observed significant increase in serum alkaline phosphatase levels throughout the study period, which might be due to the process of increased osteogenic activity and deposition of calcium salts at the site of fracture.

Yudaniayanti *et al.* (2014) Studied to find out correlation of serum ALP activity with the process of fracture healing in rat with therapy *Cissus quadrangularis* extract. This study used 18 male white rats (*Rattus Norvegicus*) age of 12 weeks. After 1 week of adaptation, all rats were operated for femoral dexter osteotomy under aseptic conditions. Then, reposition of femoral dexter fractures with intramedullary pin fixation. The rats model were evenly randomly divided into three groups at 2nd weeks after surgery: group I treated with normal diet, group II with normal diet + CaCo<sub>3</sub> supplement 150 mg/day, group III with normal diet + *Cissus qudrangularis* extract 500 mg / day per oral. Furthermore, the treatment was carried out for 4 weeks. At the end of the experiment (6th weeks after surgery), the blood samples were taken directly from canthus orbitalis dexter for alkaline phosphatase analysis. The data were analyzed by One Way Anova using SPSS for Windows program. The results indicated that ALP activity was significantly decreased ( $p < 0,05$ ). The more complete the fracture healing process, ALP activity the closer to normal value. They concluded that the serum

total ALP activity have a positive correlation with the process of fracture healing and could be used to determine the progress of fracture healing of the surgically treated fractures.

## **2.8 Complications**

Al-harbay *et al.* (1996) conducted study on denervation of the limbs on fracture healing in dog. Experimental humeral mid-shaft osteotomy and immobilisation by plates were performed in the right forelimbs of two groups of dogs. At the same time, total brachial plexus neurectomy was undertaken in animals of the first group. The second group was control. Clinical and radiological findings were recorded for a period of three months. Animals of the neurectomised group had complete paralysis of the right forelimbs and did not show complete radiological union after 90 days while the control group healed at 45 days. Denervation significantly ( $P < 0.05$ ) delayed bone union and retarded callus formation in experimental fracture. They concluded that further studies were required to understand the exact role of normal innervation of bone in fracture healing, and the effect of trauma on bone repair in paralytic limbs.

Dvorak *et al.* (2000) studied the incidence of both minor complications such as slight malalignment (which did not require repositioning) and hypertrophic callus and major complications such as delayed union and non-union, severe malalignment (requiring positioning), osteomyelitis and implant failure of long bone fracture healing in dogs and also established radiologically apparent complications which can be functionally tolerable to the patients. Fracture management was performed in 156 dogs with 164 fractures. These fractures were treated by open internal fixation (86.59%) or closed (13.41%) reductions and internal fixations (70.73%), external skeletal fixation (25.61%) and external coaptation (3.66%). Two groups of clinical patients were created. The result of treatment in group I were excellent in 70.3% cases, very good in 21.88 % cases and poor in 6 % cases. In group II, the complications were found mainly in cases with an interval between injury and fracture fixation longer than four days. Clinically healthy dogs were able to functionally tolerate radiographic abnormalities in consequence of fracture treatment in 44.45 % cases 36 of 81 fractures with excellent outcome of therapy.

Szczepaniak (2009) reported a rare case of complications of atrophic nonunion following the forearm fracture in a female Italian greyhound aged 8 months. The radiographic examination showed a fracture in the distal one-fifth of

the radial bone diaphysis, and a double fracture of the ulna at the distal one-fifth and one-fourth of the diaphysis. Subsequent radiographs were taken after 3, 5, 6, and 18 months after the first examination. Within the 18-month follow-up, no bone union was recorded. The last follow-up examination revealed advanced bone atrophy. The radial bone was severely narrowed and shortened by 50% as compared to the primary length. The ulna bone had nearly atrophied completely.

# **MATERIAL AND METHODS**

## **MATERIAL AND METHODS**

The study was carried out at the Teaching Veterinary Clinical Complex (TVCC), Nagpur Veterinary College, Nagpur and Department of Veterinary Surgery and Radiology, Nagpur Veterinary College, Nagpur.

During the period of February 2014 to July 2014, fifty six cases of various types of fracture were reported at TVCC Nagpur, out of which 12 surgical cases of long bone fracture in dogs were included in the present study. The history regarding age, sex, breed and etiology of fracture was recorded from the dog owners. Clinical examination of each patient helped in tentative diagnosis and radiographic examination revealed confirmatory diagnosis of long bone fracture. In case of complete fracture of long bones, various internal fixation techniques were adopted depending on the site, type of fracture and bone involved. Proper advice was given to the owner to restrict the movement of the dog as far as possible till the dog gets operated for internal fixation and immobilization.

### **3.1 Incidence of long bone fracture**

A total of 12 clinical cases of either sex of dogs were included in present study and were randomly divided in two groups of six dogs each. All cases were presented with history of trauma and non weight bearing lameness on the affected limb. The incidence of long bone fracture in dogs were recorded based on the basis of age, breed, sex, etiology, type of fracture, location of fracture site and limb involved, and cause of fracture.

A tentative diagnosis was made by palpation of the bone involved and confirmation was made by radiography. Accordingly these dogs were subjected to following treatment regimen depending on diagnosis.

**Table 1: Treatment methodology**

<b>Groups</b>	<b>Number of canine (n)</b>	<b>Treatment regimen</b>
Group I	6	Reduction, retention and immobilization of fractured bone by appropriate internal fixation techniques.
Group II	6	Reduction, retention and immobilization of fractured bone by appropriate internal fixation techniques and post operative administration of <i>Symphytum Officinale</i> (30 C), a homoeopathic osteo-inducer drug, @ four globules twice a day for three weeks orally.

### **3.2 Treatment of patient**

The clinical and radiological observation such as gait, weight bearing, type of fracture and stage of healing were recorded. The preoperative and post operative pain management was done by administration of meloxicam @ 0.2 mg/kg body weight intramuscularly.

In group-I, immobilization of fractured bone was achieved by internal fixation techniques such as pinning or nailing alone, whereas in group II, immobilization was achieved with the combination of internal fixation techniques and oral administration of *Symphytum officinale* 30C at the dose rate of four globules twice a day for twenty one days post operatively.

#### **3.2.1 Surgical Treatment**

A total of 12 clinical cases of either sex of dogs were included in present study and were randomly divided in two groups of six dogs each. All cases were presented with the history of trauma and non weight bearing lameness on the affected limb. All dogs were clinically examined and depending on the type of fracture, they were subjected to open reduction and internal fixation under general anesthesia.

##### **3.2.1.1 Preoperative preparation of the dog**

All the animals were prepared for orthopedic surgery by withholding food for 12 hours and water for 6 hours, prior to surgery. The selected site of operation was prepared aseptically with clipping, shaving, washing and application of antiseptic solution. The affected paw was wrapped with sterile bandages to prevent contamination during surgery.

##### **3.2.1.2 Premedication**

The dogs were premedicated with atropine sulphate @ 0.04 mg /kg b.wt S/C followed by xylazine hydrochloride @ 1mg/kg b.wt. I/M, and dexamethasone

sodium phosphate @ 0.2 mg/kg b.wt I/M. Broad spectrum antibiotic ceftriaxone @ 20 mg/kg b.wt. I/M and anti-inflammatory drug meloxicam @ 0.2 mg/kg b.wt. I/M was administered before operative procedure.

### **3.2.1.3 Anaesthesia**

Dissociative anaesthetic ketamine hydrochloride @ 5 mg/kg in combination of diazepam 0.5 mg/kg b.wt. were infused by intravenous route through the ringers lactate infusion for induction and incremental dose of same mixture was used for maintenance of anaesthesia.

### **3.2.1.4 Surgical Technique**

#### **3.2.1.4(1) Internal fixation technique of femur fracture**

A total eight dogs with femur fracture were reported in this present study. Out of these four (A1, A2, A4 and A5) were subjected to treatment under group I and other four (B1, B3, B4 and B5) under group II.

The affected dog after proper sedation was placed in lateral recumbency with affected limb facing towards surgeon.

A longitudinal skin incision was made along the cranio lateral aspect of the femur from the level of greater trochanter to the lateral surface of patella over the mid shaft femur to expose the fascia lata. After incising fascia lata and retracting the vastus lateralis muscle cranially and the biceps femoris muscle caudally the fractured end of femur shaft were exposed, then drill bit of appropriate diameter was passed through the proximal fractured end retrogradely, a hole was drilled at the trochanteric fossa, followed by introducing the pin/ nail of proper diameter through the intramedullary cavity of the proximal fracture fragment by retrograde method and the pin/nail was taken outside by making a small skin incision medial to major trochanter. The same pin was then directed down into intramedullary cavity of distal fractured fragment after correct reduction and alignment of fractured fragment until the pin was judged to be tucked in the cancellous bone of distal fragment (Plate 1-5).

The muscle layers were properly repositioned and sutured with chromic catgut No.1 by simple interrupted sutures and finally skin was sutured with nylon by advocating horizontal mattress sutures pattern. The surgical wound was cleaned with povidone iodine and sealed with compound benzoin tincture and affected limb was covered with soft thin layer cotton and bandaged and plastered in some cases of femur fracture

Similarly, in group-II the fractures of femoral fragment was immobilized with intramedullary pin/nail of appropriate size by retrograde method after proper reduction and alignment of the fractured fragments as described above and post-operatively supplementation of homoeopathic drug *Symphytum officinale* 30C @ four globules twice a day for twenty one days.

#### **3.2.1.4 (2)Humerus fracture**

In this present study 2 cases (A3 and B2) of humerus fracture were reported and were included one in each group.

The dogs were premedicated with xylazine @ 1 mg/kg body weight and positioned in lateral recumbency with affected forearm upwards. Under dissociative anaesthesia a skin incision was made from the cranial border of the tubercle of the humerus to the lateral epicondyle distally. Subcutaneous fat and brachial fascia were incised to expose the brachiocephalicus muscle and lateral head of triceps. Cephalic vein was isolated and protected. Fascia was incised along the cranial border of triceps overlying the brachialis muscle until the radial nerve is visualized. Once the nerve was isolated, brachiocephalicus and superficial pectoral muscle was reflected cranially and brachialis muscle caudally to expose the humeral mid shaft. The surgical methods for repair of humeral fractures included intramedullary pin and cerclage wire. With open treatment, IM pins can be introduced in normograde or retrograde fashion. Normograde insertion started on the proximal aspect of the crest cranial to greater tubercle, without entering the shoulder joint. The pin was directed distally and medially, the fracture is reduced, and the pin was seated in the medial aspect of the humeral condyle, avoiding the elbow joint. The pin position was evaluated on a craniocaudal radiograph immediately after surgery. Since IM pins did not provide rotational stability to the surgical repair, most IM pin fixations were complemented with cerclage wires used in addition to IM pin fixation for the repair of humeral fractures.

#### **3.2.1.4 (3) Radius ulna Fracture**

In this present study one case (B6) of radius ulna fracture were reported.

The Internal fixation device, plate and screw were used in small breed dogs for correction of radius and ulna fracture. The craniomedial surface of the radius and the caudal lateral surface of the ulna are not covered by muscle and could be easily palpated to serve as landmarks for location of the incision. Extensor muscles are located cranial to and flexor muscles caudal to the radius

and can be retracted to expose the bone. The cephalic vein crosses the medial portion of distal radius. The lateral radial head was palpated beneath the extensor muscles of the forearm. The limb hair were clipped and prepared for aseptic surgery from the shoulder to carpus. The animal was positioned in dorsal recumbency and limb draped out and pulled laterally to expose the cranio-medial aspect of radius. Radius was palpated directly under the skin and subcutaneous tissue on the craniomedial surface of the limb. An incision was made through skin and subcutaneous tissue to expose the radial diaphysis. Incision was extended distally and extensor tendons were evaluated to expose the cranial surface of the distal metaphysis of the radius. The plate was applied to the cranial surface of the radius, and no attempt was made to stabilize the ulna since the ulnar bone fragments were properly apposed and rigid fixation of radius provided adequate stability.

#### **3.2.1.4 (4) Tibia fracture**

In this present study one case (A6) of tibia fracture were reported

The patient was placed in dorsal recumbency with stifle flexed at right angle for better Intramedullary (IM) pinning technique .The pin was then inserted through the skin along the medial border of the patellar ligament, entering the proximal end of the tibia approximately one third to half the distance from the cranial surface of tibial tubercle to medial condyle of the tibia. Entering the pin too far caudally will not allow full extension of the stifle joint because of interference of the pin with the femoral condyle. The pin was started close to medial border of the tibial plateau and angled slightly medially and caudally. After entering the marrow cavity, increased resistance and a coarse grating was felt as the pin struck the medial cortex. Drilling motion was stopped and the pin was simply pushed until it bended slightly and glided along the medial cortical surface. Because of its bending a pin with a slightly smaller than normal diameter is necessary, typically about 50% of the medullary canal diameter. This bending caused the Steinmann pin to function more as a rush pin than as a static IM, gave increased stability.

#### **3.2.2 Homoeopathy drug, *Symphytum Officinale***

*Symphytum Officianale* of 30 C potency globules was procured from chemist shop in ready made form preparation.

### **3.3 Postoperative Management**

All the dogs subjected to open reduction were given inj. ceftriaxone @ 20 mg/kg b. wt. I/M daily for seven days and inj. Meloxicam @ 0.2 mg/kg b. wt. I/M for three postoperative days. Dressing of surgical wound was undertaken with povidone iodine and bandaging was done on alternate day till healing of wound. Sutures of skin were removed on 10<sup>th</sup> to 12<sup>th</sup> postoperative day depending on the healing.

### **3.4 Evaluation of fracture healing**

The process of fracture healing in all the treated dogs was evaluated on the basis of following observations. The following parameters were studied at scheduled intervals.

- Clinical observations
- Radiological observations
- Hematological observations
- Biochemical observations

The plain radiograph was taken immediately after repair i.e. on 0<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day to judge the bone fragment position and process of healing. All the treated dogs were given physical activity until union of fracture gap was observed on plain radiograph.

#### **3.4.1 Clinical observations**

The clinical observations viz. wound condition, number of days required for the dog to touch limb on ground and extent of weight bearing while walking as well as standing were also recorded.

##### **3.4.1 (1) Wound condition**

In operative cases, the surgical wound was observed for contamination, swelling etc. on alternate day at the time of dressing.

##### **3.4.1 (2) Weight bearing**

Weight bearing was evaluated in dogs by their ability to bear weight on the affected limb and classification of weight bearing was done as partial i.e touching the paw on ground and complete weight bearing i.e ability to put complete weight on the affected limb. Dog owners were advised to come at scheduled interval for follow up, evaluation and radiological examination to access healing process at scheduled intervals.

#### **3.4.2 Radiological observations**

The radiological observations of all fractured long bones were taken in cranio-caudal, medio-lateral, view on 0<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup> day to evaluate the healing process viz. periosteal proliferation, callus formation, alignment of fragments, organization and mineralization of callus, apposition of bone fragments and osteo-inducer effect of *Symphytum officinale* to the fractured bone, if any.

### **3.4.3 Haematological Observations**

For haematological observations viz. TEC, TLC and Hb, 2 ml of fresh blood was collected in sterilized glass vials containing EDTA (Ethylene Diamine Tetra Acetic Acid, 2gm/ml blood) on 0<sup>th</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, & 14<sup>th</sup> day post-operative and freshly prepared blood smears were used for differential leucocyte count.

#### **3.4.3 (1) Total Erythrocyte Count (TEC)**

As per the method described by Kerr (2002), estimation of total erythrocyte count was carried out by using Neubar slide method and values obtained were expressed in millions per cubic millimeter.

#### **3.4.3 (2) Total Leukocyte Count (TLC)**

TLC estimation was carried out by using Neubar slide method and values obtained were expressed in thousand per cubic millimeter.

#### **3.4.3 (3) Haemoglobin( Hb)**

The Hb concentration was determined by Sahli's haemoglobinometer and values were expressed in gram percent or gm/dl of blood.

#### **3.4.3 (4) Differential Leukocyte Count (DLC)**

As per the method described by Schalm (1965) freshly prepared blood smears were stained with leishman stain and enumeration of lymphocyte, neutrophill, monocyte, eosinophill and basophill was carried out and values obtained were expressed in percentage.

### **3.4.4 Biochemical studies**

For collection of serum fresh blood was taken in sterilized vials and was allowed to settle for serum separation. Then sample was centrifuged for 5 minutes. The collected serum samples were estimated for serum alkaline phosphatase on 0<sup>th</sup>, 7<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day of observations and estimation was carried out with the help reagent kits using semi auto analyzer.

### **3.5 Complications**

Complications like surgical wound dehiscence/ infection, pin/nail migration, atrophy of muscle, mal union or delayed union, abscess at area of trochaneric fossa due to pin migration etc, if any, were also recorded during the study period.

### **3.6 Statistical analysis**

As per the method of statistical analysis described by Snedecor and Cochran (1994), the data obtained during the study period for different parameters were subjected to statistical analysis.

# RESULTS AND DISSUASION

## RESULTS AND DISCUSSION

The present study was conducted on Twelve clinical cases of long bone fracture in dogs at the Teaching Veterinary Clinical Complex (TVCC), Nagpur Veterinary College, Nagpur.

In order to study the incidence of long bone fracture in dogs ,all clinical cases of fracture in canines presented at TVCC, were screened during the study period February 2014 to July 2014. According to data available at TVCC, total number of 56 clinical cases of complete fracture were reported during the study period. Total 14 clinical cases of long bone fracture were subjected to surgical intervention i.e. open reduction and internal fixation, out of which, 12 surgical cases were included in the present study.

Twelve dogs were randomly divided in two equal groups, and subjected to treatment as per the schedule.

### 4.1 Incidence

During the present study, Incidence of long bone fracture in dogs were analyzed as per the age, breed, sex, location of fracture, type of fracture, limbs involved, type of internal fixation technique used during the study was recorded .

#### 4.1.1 Age wise distribution of long bone fracture

During the study, the incidence of long bone fracture was highest among younger dogs of 0 to 6 months age group (41.66 %) followed by 6 to 12 months age (25%) and 1 to 2 years (16.66%) and 2 yrs and above (16.66%). Incidence was higher (41.66 %) in the young dogs below 6 months of age (Table 2).

It was revealed that the younger dogs below six months of age group were more prone to long bone fractures. This was in consequence with the findings recorded by Roy *et al.*,(2005) and Gahlod *et al.*,(2002) in dog. However, Aithal *et al.*, (1999) documented more incidence of long bone fracture in young dogs below one year of age group. The dogs unless so trained, do not possess keen traffic sense, especially while crossing the roads, making them more vulnerable to road accidents, especially the young ones. Unlike pure breed dogs, the mongrel dogs are many a times let loose by the owners and thus they wander on roads and met with an automobile accidents.

In the growing age, the mineralization of bones take place and gradually bones are fully ossified and demand of calcium and other minerals increases.

However, in case of deficiency of calcium, bone may remain ricketic and more prone to get pathological fracture on external injury and trauma. Malnutrition, deficiency of vitamin A and vitamin D3 may affect calcium metabolism, proper osteosynthesis and ossification of compact and cancellous bone which may result in fracture. Further, the changes of fracture due to extrinsic causes such as automobile accidents or fall from a height are more since the bones are under the process of mineralization and complete and strength of bones is yet to achieve. The present findings can be contemplated with the observations in the present study.

**Table 2: Age wise distribution of long bone fracture in dogs**

Age	Numbers	Per cent (%)
0-6 months	5	41.66
6-12 months	3	25.00
1-2 years	2	16.66
2 yrs and above	2	16.66

#### **4.1.2 Breed wise incidence of long bone fracture**

In the present investigation the breed wise distribution of long bone fractures revealed 50% cases in non-descript, 25% in Labrador, 16.66 % in German shepherd and 8.33% in Pomeranian breeds.(Table 3) The present study indicated that percentage of incidences of long bone fractures were more in non-descript dogs. The dogs unless so trained, do not possess keen traffic sense, especially while crossing the roads, making them more vulnerable to road accidents, especially the young ones. Unlike pure breed dogs, the mongrel dogs are many a times let loose by the owners and thus they wander on roads and met with an automobile accidents.

Hansda (2008) reported highest incidence of femur fracture in non-descript dogs followed by Pomeranian, Labrador, Great Dane, German shepherd, Rottweiler and Doberman.

In the present study, the highest incidence of fracture was reported in non-descript dogs which may be due to their more exposure to automobile accidents. Because most of the time those dogs remain moving outside in search of food and water and while running at roads they undergo accidents more frequently as compare to other recognized breeds which get proper home facility by their owners.

**Table 3: Breed wise incidence of long bone fracture in dogs**

Name of Breed	Numbers	Per cent (%)
Non-descript	6	50
Labrador	3	25
German shepherd	2	16.66
Pomeranian	1	08.33

**4.1.3 Sex wise distribution of long bone fracture in dogs**

Sex wise distribution of long bone fracture in canines (Table 4) revealed higher incidence in males (66.66%) as compared to females (33.33%).

Similar findings were reported by Aithal *et al.* (1999) and Gahlod *et al.* (2002) regarding sex wise distribution. Incidence of long bone fracture in males was more as compared to females because of more activeness and alertness in males which would expose them to various accidents that is likely likely to create some injuries to the bone and may develop fracture. Male dogs are relatively more fast growers, require more calcium for proper mineralization of bones. Due to more requirement of calcium male dog are more prone to fracture in case of deficiency of dietary calcium or vitamin D as also is been reported by Aithal *et al.* (1999).

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**Table 4: Sex wise distribution of long bone fracture in dogs**

Sex	Numbers	Per cent (%)
Male	8	66.66
Female	4	33.33

**4.1.4 Distribution of types of fracture**

Different types of long bone fractures were found in the present study out of which 66.66% cases had oblique fractures and 33.33 % cases had transverse fracture.

**Table 5: Types of long bone fracture in dogs**

Types	Numbers	Per cent (%)
Oblique	8	66.66
Transverse	4	33.33

Gahlod *et al.* (2002) also reported similar findings that oblique fractures were more common (66.66%) followed by transverse fracture (25.46%) during the

study of type of fracture in canines. This could be attributed to the torsion and bending forces and the shape of long bones especially femur and humerus.

#### 4.1.5 Location wise distribution of long bone fracture in dogs

In the present investigation the radiographic examination of long bone fracture showed that maximum number of fractures were of mid-shaft (75%) followed by distal third (16.66%) and proximal third of diaphysis (8.33%).

**Table 6 : Location wise distribution of long bone fractures in dogs**

Location of fracture	Numbers	Percent (%)
Mid shaft	9	75%
Distal third	2	16.66%
Proximal third	1	8.33%

Gahlod *et al.*, (2002) and Patil *et al.*, (1991) reported similar observations that the most of the fractures in dog involves midshaft of long bones. However, Aithal (1996) reported that, out of total femur fractures in dogs, incidence of supracondylar fractures were 18.18%.

#### 4.1.6 Limbs involved in long bone fracture

During the present study, incidence of right limb fracture was highest (58.33%) than left limb (41.66%) (Table 7). Uma Rani *et al.*, (2007) reported similar observations. No significant cause of such limb fracture incidence can be specified.

**Table 7 : Limb involved in long bone fracture**

Limb involved	Numbers	Per cent (%)
Right	07	58.33%
Left	05	41.66%

#### 4.1.7 Types of bones involved in long bone fracture

In the present study, among different types of long bones involving in long bone fractures, 66.66% cases had femur fracture, 16.66% cases had humerus fracture, 8.33% cases had radius and ulna and 8.33 % case had tibia fracture. This could be attributed to the anatomical shape of the femur bone which is spiral in shape and in an attempt to escape the accidents, the dog could pass anterior part partially.

Patil *et al.* (1991), Aithal *et al.* (1999), and Roy *et al.*, (2005) also documented higher incidence of femur fracture.

**Table 8: Types of bones involved in long bone fracture**

Type of fractured bone	Number of cases	Percentage
Femur	8	66.66%
Humerus	2	16.66%
Radius and ulna	1	8.33%
Tibia	1	8.33%

#### **4.1.8 Cause of fractures**

In the present investigation, various causes of long bone fractures were automobile accidents (50%) in dogs of all age groups, followed by fall from height (33.33%), and others miscellaneous factors like various injuries (16.66%). These observations indicated that higher percentage of incidence of long bone fracture was due to automobile accidents. Aithal (1996) recorded similar observations (Table 9).

Major cause of fracture was the automobile accident. Increase in road traffic, fast moving automobiles and negligence to stray dogs while driving might have contributed to higher incidence of long bone fracture in dogs (Singh *et al.*, 1983). In the same way fall from the height was the second major cause of fracture (33.33%) and also recorded by Aithal (1996) which attributes to poor management conditions.

**Table 9 : Distribution of cause of fracture**

Cause of fracture	Numbers of cases	Percentage
Automobile accident	6	50%
Fall from height	4	33.33%
Miscellaneous	2	16..66%

## **4.2 Treatment**

### **4.2.1 Surgical Treatment**

On the basis of clinical and radiological observations, the suitable type of internal fixation techniques and devices were used for surgical intervention. The common pre-operative considerations viz. preparation of animal, premedication and anesthetic procedure were followed for dogs from group I and group II and subjected to open reduction and internal fixation of fractured bone with intramedullary Steinmann pin or V nail and bone plate.

#### **4.2.1.1 Preparation of patient**

All the dog owners of the included cases were advised to keep the patient off fed dog for twelve hours and without water for six hours prior to premedication. Fasting process was helpful to empty the stomach which enabled to reduce the chances of vomiting and aspiration during induction of anesthesia and which also decreased the BMR and body temperature (Lumbs and Johns, 1984; Hall and Clark, 1991, and Gahlod, 2007). In the present investigation, during the induction of anesthesia vomition was not observed in any of the cases.

#### **4.2.1.2 Premedication**

All the dogs which were subjected to internal fixation followed common pre-anesthetic protocol in both group I and group II. All the dogs were administered atropine sulphate @ 0.04 mg/kg body weight subcutaneously was given and after fifteen minutes inj. Xylazine hydrochloride @ 1 mg /kg body weight intramuscularly was given.

Non narcotic sedative Xylazine hydrochloride was found to be more effective as sedative, analgesic and muscle relaxant. Lumbs and Jones (1984) and Hall and Clark (1991) also recommended xylazine as a premedicant in domestic as well as wild animals. Atropine sulphate acting as the anticholinergic drug decreased the secretion of parotid glands acting as an antisialogogue and also had the anti vagolytic action which counteracted the effect of the vagal inhibition to heart caused by xylazine. Xylazine and atropine sulphate were used as premedicants which calm down the patients and it became easy to place them on operation table for administration of further anesthetic agents.

#### **4.2.1.3 Anaesthesia**

Fifteen minutes after administration of xylazine, inj. Diazepam was administered @ 0.5 mg/kg body weight intravenously followed by inj. ketamine hydrochloride @ 5 mg/kg body weight intravenously through the Ringers lactate solution.

During the present study, ketamine produced dissociative anesthesia and Diazepam, a Benzodiazepine derivative having muscle relaxant and anticonvulsant action along with Ringer's lactate solution was found safe and produced balanced anesthesia for surgical intervention. When the dog achieved the deep level of surgical anesthesia, the surgical procedure was undertaken. Gahlod (2007) and Hansda (2008) advocated similar method of administration of dissociative anesthesia.



**Plate 1 : Surgical site prepared for femur fracture repair**



**Plate 2 : Exteriorization of proximal fractured fragment**

## **Surgical Treatment for Open reduction and internal fixation of long bone fracture**

Fractures of femur and humerus are generally not amenable to conservative repair due to their anatomical shape, size and positioning and musculature around them and external fixation with proper reduction is not possible and therefore open reduction with internal fixation becomes mandatory. Open reduction and internal fixation of long bone fractures by using appropriate intramedullary implants are safe, inexpensive and successful treatment method of appropriate principles of repair are implemented. The complications can be minimized with appropriate surgical technique, preservation of regional soft tissue and vasculature and proper postoperative care.

The open reduction and internal fixation of fractured femur bone was carried out in eight dogs four each in group I and group II. The intramedullary Steinmann pin fixation was adopted in both the groups. Threaded Steinmann pin was used for intramedullary pinning in one case of femur fracture (B1), in B2 Steinmann pin coupled with hemicerclage was used for internal fixation, while in B3, B4, and B5 V nail was used. In group I, A1, A2 and A6 Steinmann pin, and in A3 Steinmann pin coupled with full cerclage, and in dog no. A4 and A5 V nail was used as an intramedullary device for internal fixation whereas in group II B6 and A7 plating was used for internal fixation of radius ulna fracture.

### **4.2.1.4.1 Open reduction and internal fixation of femur fracture**

The cranio-lateral aspect of thigh for exposing femur shaft was found more appropriate and the reduction of fracture fragment was observed to be more convenient and easier. The cranio-lateral aspect of femoral shaft was exposed by open method as described by Archibald (1974).

Drilling of hole in both the groups using trocar point of Steinmann pin introduced in retrograde fashion through the fractured end through trochanteric fossa prior to insertion of intramedullary pin made easier to pass pin or nail into medullary canal of distal fragment of femur without much resistance.

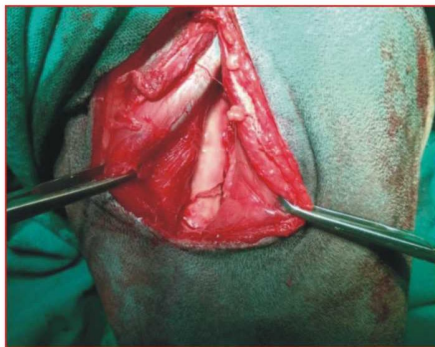
The introduction of intramedullary pin from predrilled trochanteric fossa in a normograde fashion after proper reduction was easier and no difficulty was experienced during fracture fixation technique in animals of group I and group II. The intramedullary pin embedded in the distal cortex, had a three point fixation and helped in resisting bending forces. Similar procedure was recommended by Gahlod (2007) and Hansda (2008).



**Plate 3 : Drilling through femoral proximal shaft**



**Plate 4 : Retrograde insertion of V nail in femur**



**Plate 5 : Alignment of fracture fragments  
after intramedullary pinning**

#### **4.2.1.4.2 Surgical treatment for complete fracture of humerus**

The open reduction and internal fixation of fractured humerus bone was carried out in two dogs, one each in group I and group II.

After proper sedation patient was positioned in lateral recumbency with affected forearm dorsally upwards. Under general anaesthesia a skin incision was made from the cranial border of the tubercle of the humerus to the lateral epicondyle distally to expose the humeral shaft.

During the present study the surgical methods adopted for repair of humeral fractures in both the groups included intramedullary Steinmann pinning with cerclage wire. Perfect alignment and reduction of fractured humerus was observed in each case of both the groups except in one case. However, early weight bearing and callus formation was reported in case of group II. Cerclage wiring helped in combating the torsion forces acting on the humerus shaft.

The standard craniolateral approach of humerus provided adequate exposure with minimal soft tissue and vasculature damage.

Normograde insertion starts on the proximal aspect of the crest cranial to greater tubercle, without entering the shoulder joint and the pin was directed distally and medially, the fracture was reduced, and the pin was seated in the medial aspect of the humeral condyle, avoiding the elbow joint. The pin position was evaluated on a craniocaudal radiograph immediately after surgery. Since IM pins did not provide rotational stability to the surgical repair, most IM pin fixations were complemented with cerclage wires used in addition to IM pin fixation for the repair of humeral fractures. Similar method was advocated by Denis Marcellin (1998). (Plate 6-10)

#### **4.2.1.4.3 Open reduction and internal fixation of radius ulna fracture**

The open reduction and internal immobilization of fractured radius with appropriate plate and cortical screws was found satisfactory in both the groups. Perfect alignment and reduction was observed in each case of both the groups. However, healing time of fractured radius and ulna was observed lesser in group II whereas, in other case of radius fracture in group I were noted in complications.

Similar method of reduction and immobilization of close fractures of long bones was described by Slatter (2002).

Fixation of radius by plating provided rigid fixation and stabilized the fracture and kept ulnar fractured ends in proper apposition. Thus, fixation of ulna



**Plate 6 : Surgical site prepared for Humerus fracture repair**



**Plate 7 : Exteriorization of proximal fractured fragment**



**Plate 8 : Cerclage wiring for additional support to the fractured fragments**



**Plate 9 : Cerclage wiring for additional support to the fractured fragments**



**Plate 10 : Closure of surgical would after normograde pin insertion**



**Plate 11 : Surgical site prepared for Radius fracture repair**



**Plate 12 : Fixation of Shermanns plate with screws for Radius fracture repair**

was thought unnecessary. This approach provided expected result and ulnar fracture healing was accomplished simultaneously. (Plate 11-12).

#### **4.2.1.4.4 Administration of *Symphytum Officinale* of 30 C potency**

Bone tissue being slow to heal as compared to the other soft tissues, require a longer period of 3-4 weeks for normal healing. Therefore search for the alternate therapies and medicaments that would enhance the repair process and reduce the fracture healing period. *Symphytum Officinale*, a homoeopathy drug of 30 C potency was easily available at homoeopathy chemist shop and prescribed to owners belonging to all clinical cases of group II.

Many researchers reported the effect of other herbal, non herbal biomaterial and anabolic synthetic agents on fracture healing in animals with various efficacies. Recombinant human basic fibroblast growth factor (Nakamura *et al.*, 1998), FGF-2 (Kawaguchi *et al.*, 2001), Shock wave therapy (Hsu *et al.*, 2003), BMP and TGF-beta (He *et al.*, 2003), pulsed ultrasound therapy (Kaur *et al.*, 2004), Simvastatin (Wang *et al.*, 2007) ultrasound therapy (Uma Rani *et al.*, 2010), Berberis lyceum (Ahmad *et al.*, 2011) and have been used as osteo-inducers by various researchers.

In group II, irrespective of fracture management (pinning/nailing or plating), the 30C dilution of homoeopathy drug *Symphytum Officinale* was administered @ four globules twice a day per oral for 21 days postoperatively. Chhavi *et al.*, (2011) stated that *Symphytum officinale* removes inflammation surrounding the fracture whereas, Gaur *et al.* (1992) used direct application of root paste of *symphytum officinale* to aid the union of fractured bone. Neto *et al.* (2010) concluded that *Symphytum Officinale* 6cH enhances, principally at the early stages of osseointegration, bone formation around titanium implant in rats 'tibiae'. The dogs of group II revealed reduction in partial and complete weight bearing period, which indicated the enhancement in the healing process and also showed significant anti-inflammatory activity.

### **4.3. Postoperative management**

In general, a common antibiotic regimen was followed in all dogs subjected to surgical therapy includes Ceftriaxone @ 20 mg/kg body weight I/M daily for seven days and Inj. Meloxicam @ 0.2 mg/kg body weight I/M for three days.

The bandage was changed on alternate days for 10 to 20 days. Antiseptic dressing of surgical wound was done with povidone iodine solution. Skin sutures

were removed on 10<sup>th</sup> to 12<sup>th</sup> postoperative day. The owner was advised to restrict the activities of the animal for first ten days; subsequently the dogs were allowed limited physical activities regularly.

Similar postoperative care was recommended by Tembhone (2006) and Gahlod (2007).

#### **4.4 Evaluation of fracture healing**

The primary goal of fracture treatment is to achieve a healed fracture with normal bone alignment and promote early return of normal limb usage. Therefore, optimum reduction and immobilization and retention using appropriate implants with minimal soft tissue and vasculature damage is of utmost importance for successful outcome.

The fracture healing evaluation was assessed on the basis of various clinical, radiological, hematological and biochemical parameters.

##### **4.4.1 Wound condition**

The wound healing in all dogs of group I and group II was observed for about 7 to 15 days by primary intention. Wound with serumen discharge was observed at the trochanteric fossa region on 7<sup>th</sup> day of treatment in the dog (B5) from group II of femur fracture, it was due to migration of pin at trochanteric fossa and open septic wound along with migrated pin was observed at the area of trochanteric fossa. However, Local dressing was done with povidone iodine solution and administration of Tab. Moxclave (amoxicillin and potassium clavulanate ) @ 25 mg/kg body weight orally for 5 days resulted in satisfactory recovery and wound healed subsequently.

Clinically, the dogs with open reduction of femur fracture did not show any signs of infection at the operated site from both the groups.

##### **4.4.2 Weight bearing in dogs**

###### **4.4.2.1 (a) Partial weight bearing**

The observations regarding partial weight bearing and complete weight bearing period recorded in animals of group I and II are recorded in (Table 10) .

In group I, the dogs with long bone fracture required 7 to 14 days with a mean of  $9.16 \pm 1.07$  post operative days to touch the operated limb to the ground. The foot pad of operated limb touched to the ground but due to severe pain at the operated site, animal was unable to put weight. However, in the dogs of group II, partial weight bearing was observed between 3 to 4 days with a mean of  $3.25 \pm 0.25$  days which was lesser as comparable to group I (Plate 13). In one case,



**Plate 13 : Partial Weight Bearing in Group II  
( Dog B2, day 3)**



**Plate 14 : Partial Weight Bearing and knuckling  
in Group II ( Dog B1, day 3)**



**Plate 15 : Complete weight bearing in Group II  
(Dog B1, day 28)**

although there was partial weight bearing, knuckling was also observed (Plate 14).

#### 4.4.2.2 (b) Complete weight bearing

The dogs of group I with femur fracture required 35 to 38 days with mean of  $36.16 \pm 0.47$  post operative days to bear complete weight on operated limb. The animal of group II required 26 to 32 days with a mean of  $28.5 \pm 0.88$  post operative days for complete weight bearing (Plate 15). The reduction in complete weight bearing period was found statistically significant. It was therefore concluded that the period for weight bearing was less in group II as compared to group I.

During the present study, the reduction time for partial and complete weight bearing in long bone fracture in group II was found statistically significant. This could be attributed to be administration of homoeopathy drug *Symphytum Officinale* of 30 c potency in animal of group II which possess anti-inflammatory property, enhances the bone healing process principally at the early stages of osseointegration as suggested by Neto *et al.* (2010).

The present observation of partial and complete weight bearing period in dogs of group I agreed with the findings of Uma Rani *et al.* (2007), who had reported early functional usage of limb by 1<sup>st</sup> week and full weight bearing by 6<sup>th</sup> week in femur fracture in dogs.

**Table 10: Mean  $\pm$  S.E days recorded for weight bearing in two treatment groups**

Particulars	Group I (n=6)	Group II (n=6)
Partial weight bearing	$9.16 \pm 1.07^a$	$4.66 \pm 0.49^b$
complete weight bearing	$36.16 \pm 0.47^a$	$28.5 \pm 0.88^b$

Different superscript indicated significant difference within the group

#### 4.4.2 Radiological observation

The plain radiographs were taken prior to and immediately after open reduction and internal fixation and on 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> postoperative days to ascertain type of fracture and to evaluate the status of implant, alignment of fragments, progress of fracture healing and reaction of host bone to implant and complications, if any.

##### Group I

In four dogs of group I, intramedullary pins /nails were used for open reduction and internal fixation of femur fracture. In group I, radiograph of dog (A1,

A2, A4 and A5) on day 0, i.e. before fixation revealed mid shaft femur fracture, whereas (A3 ) revealed mid shaft complete, close fracture of left humerus, and (A6) showed distal third transverse complete fracture of tibia. The radiographic anatomy of fractured femur, humerus and tibia bones was well aligned in all dogs. In A1, A2 and A6 steinmann pin, and in A3 steinmann pin coupled with full cerclage, whereas in dog A4 and A5, V nail was used as a intramedullary device for internal fixation.

Post operative radiograph on 0<sup>th</sup> day was taken to assess fracture reduction, alignment and fixation in all the animals. The intramedullary pinning/nailing/plating was found satisfactory for fixation of long bone fracture in dogs of group I. Similar observations were found by Kushwaha *et al.* (2010).

Radiograph of dog (A3) on day 0 revealed closed midshaft oblique complete fracture of right humerus (Plate 16-17).

Radiograph taken immediately after surgery revealed appropriate reduction of the fracture fragment and in aligned position with proper fixation of threaded Steinmann pin coupled with full cerclage wiring.

Radiograph obtained on 15<sup>th</sup> day revealed perfect alignment of fracture fragments of humerus. There was evidence of mild degree periosteal reaction and fracture gap was blurred (Plate 18). Rest of the dogs in this group showed similar observations. The radiograph taken on day 30<sup>th</sup> day revealed moderate periosteal proliferation. The periostitis was evident and fracture gap was clearly visible (Plate 19).

The radiograph taken on 45<sup>th</sup> day revealed healing of fractured bone, and from anterior site bridging of fracture gap and callus was visible (Plate 20).

In dog, (A1) fractured fragments of femur were reduced and immobilized with open reduction and internal fixation.

Radiograph of dog (A1) on day 0 revealed close mid shaft complete transverse fracture of femur with displaced fragments (Plate 21). The radiograph taken on 15<sup>th</sup> day showed slight to moderate periosteal reaction. Alignment of fracture fragment was noticed (Plate 22). Radiograph taken on 30<sup>th</sup> day revealed slightly deviated alignment of fractured fragments with periosteal bridging callus (Plate 23).Radiograph taken on 45<sup>th</sup> day revealed very little periosteal callus and incomplete bridging of fracture gap was visible (Plate 24).

The present findings of femur fracture in dogs of group I are in accordance with Singh *et al.* (1999) and Uma Rani *et al.* (2007), who had

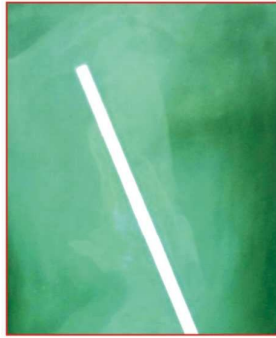


Plate 22 : Radiograph of fractured femur  
(Group I, Dog A1, 15<sup>th</sup> day postop.)



Plate 23 : Radiograph of fractured femur  
(Group I, Dog A1, 30<sup>th</sup> day postop.)



Plate 24 : Radiograph of fractured femur  
(Group I, Dog A1, 45<sup>th</sup> day postop.)



Plate 25 : Preoperative radiograph of  
femur in Group II (Dog B1, day 0)



Plate 26 : Radiograph of femur  
(Group II, dog B1, day 0 postop.)



Plate 27 : Radiograph of femur  
(Group II, dog B1, 15<sup>th</sup> day postop.)



Plate 16 : Radiograph of fractured humerus ( Group I, Dog A3, day 0)



Plate 17 : Radiograph of fractured humerus (Group I, Dog A3, day 0 postop.)



Plate 18 : Radiograph of fractured humerus (Group I, Dog A3, 15<sup>th</sup> day postop.)



Plate 19 : Radiograph of fractured humerus (Group I, Dog A3, 30<sup>th</sup> day postop.)



Plate 20 : Radiograph of fractured humerus (Group I, Dog A3, 45<sup>th</sup> day postop.)



Plate 21 : Radiograph of fractured femur (Group I, Dog A1, 0<sup>th</sup> day postop.)

reported complete fracture healing and clinical union of fracture in all animals on 8 to 12 post operative week.

## **Group II**

In group II, radiograph of dog B1 on 0<sup>th</sup> day, before fixation revealed distal third slight oblique diaphyseal complete closed fracture of right femur with displaced fragment at distal end (Plate 25), B2 revealed mid shaft oblique diaphyseal complete closed fracture of right humerus, B3 midshaft little oblique fracture of femur, B4 and B5 mid shaft transverse fracture of femur, B6 mid shaft complete fracture of right radius. The radiographic anatomy of fractured bone was found well defined in all dogs.

The radiograph taken immediately after surgical fixation of fractured bone provides the status of fracture, reduction, alignments and implant perfection. (Plate 26), In general the placement of intramedullary nails i.e. Kuntscher V nail in two dogs and Steinmann pin in one dog and threaded Steinmann pin in one dog and Steinmann pin with full cerclage was found to be satisfactory in group II. Radiograph taken soon after reduction revealed appropriate reduction and perfect alignment of fractured fragment with the help of Steinmann pin and fracture line was visible.

The 15<sup>th</sup> day radiograph of B1 revealed proper alignment of fractured fragment and periosteal reaction. Bridging of callus and obliteration of fracture gap by distinct callus was evident (Plate 27).

The radiograph taken on 30<sup>th</sup> day showed perfect union of fracture fragment with complete obliteration of fracture gap (B1) with excessive callus (Plate 28). Continuity of cortex and restoration of medullary canal was also observed in dogs.

Radiograph taken on 45<sup>th</sup> day revealed healing of fracture bone. Excessive callus was reduced suggestive of remodeling of bone that could be appreciated in radiograph (Plate 29).

The present observations are in accordance with the findings of Neto *et al.* (2010) who reported the acceleration of tibial fracture healing immobilized by titanium implants in rats, along with *Symphytum Officinale* 6cH treatment.

In one dog of group II, the fracture of humerus was reduced by open method and internal fixation in addition to cerclage wiring.

Radiograph of dog (B2) before open reduction and internal fixation with Steinmann pin and hemi cerclage wiring, revealed mid shaft oblique complete closed fracture of humerus (Plate 30-31).

The radiograph of dog taken on 15<sup>th</sup> day revealed proper alignment with beginning of bridging callus.

The radiograph of dog taken on 30<sup>th</sup> day revealed proper alignment of fracture fragment along with evidence of moderate periosteal proliferation and fracture gap was invariably blurred and appeared irregular (Plate 33).

Radiograph taken on 45<sup>th</sup> day showed periosteal bridging callus at the anterior site. Both the proximal and distal bone fragments were united perfectly (Plate 34).

The observations are in accordance with the findings recorded by Gaur *et al.* (1992) who used direct application of root paste of *Symphytum officinale* to enhance the union of fractured bone.

Singh *et al.* (1998) who reported the faster initiation of healing process of radius ulna in dogs treated with *Coriandrum sativum* on the basis of clinical, radiological and histological observations.

Asgar *et al.* (1999) had reported that feeding of *Cissus quadrangularis* and *Uraria pictalepidium sativum* for 20 days enhanced the rate of fracture healing, calcification and functional restoration of limb in close fracture of radius and ulna immobilized with plaster of paris cast in dogs.

Mishra *et al.* (2010) also reported that the fractured radius ulna was completely healed in 21 days treated with *Cissus quadrangularis* extract and plaster of paris cast.

Early resolution of functional activity and weight bearing were observed in animal of treated group II than control group I.

Radiographs taken at scheduled intervals showed accelerated fracture healing with complete bridging of fragment, mineralization along with periosteal and medullary continuity across the fracture site in animals of treated group II whereas radiographic union and periosteal bridging at fracture site although satisfactory were relatively slow and incomplete in animals of group I till the end of observation period.

The enhancement of fracture healing and calcification process in animals of group II could be attributed to the therapeutic efficacy of *Symphytum officinale* homoeopathy drug which acted as an anti-inflammatory, analgesic drug with



Plate 28 : Radiograph of femur  
(Group II, dog B1, 30<sup>th</sup> day postop.)



Plate 29 : Radiograph of femur fracture  
(Group II, dog B1, 45<sup>th</sup> day postop.)



Plate 30 : Radiograph of a humerus  
(Group II, Dog B2, day 0)



Plate 31 : Radiograph of a humerus  
(Group II, Dog B2, day 0)



Plate 32 : Radiograph of fractured  
humerus (Group II, Dog B2, day 15 postop.)

specific osteoinductive properties along with the proper fracture fixation technique as demonstrated by several workers such as Youngken (1950), Goldman *et al.*(1985), Saito and Oliveira (1986), Asgar *et al.* (1999), Martindale (2002), Cunha *et al.* (2003), Kucera *et al.*, (2004), Predel *et al.* (2005), , Grube *et al.* (2007), Staiger (2007).

#### **4.4.3 Haematological studies**

Any surgical intervention produces stress in the animal. Depending upon the surgical intervention being undertaken, duration of surgery, haemorrhage and soft tissue damage, type of anaesthetic used and duration surgery, the stress may vary. Thus the stress induces various metabolic and other intrinsic changes in the body, which are reflected in various clinical, haematological biochemical parameters. Further, a homoeopathic drug *Symphytum officinale* was administered to the dogs of group II for 21 days period. Therefore in order to assess the effect of surgery and the administration of the drug, various haematological and biochemical parameters were studied. The results are presented hereunder-

The haematological parameters were assessed to evaluate the effect of treatment on the clinical health status of animals in both the groups.

##### **4.4.3.1 Total erythrocyte count (million/cu.mm)**

In group I, the mean total erythrocyte count on 0<sup>th</sup> day was  $5.2 \pm 0.24$  million/cu.mm and it was  $5.20 \pm 0.17$  million/cu.mm,  $4.88 \pm 0.38$ ,  $5.01 \pm 0.187$  and  $4.93 \pm 0.197$  million/cu.mm on 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> day, respectively. However the variation in values at different interval was statistically non-significant.

The animals of group II had mean value of TEC  $5.59 \pm 0.04$  million/cu.mm on 0 day and increased on day 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> i.e.  $5.83 \pm 0.058$ ,  $6.27 \pm 0.13$ ,  $6.50 \pm 0.073$ , and  $6.72 \pm 0.06$  respectively (Table 11).

There was constant and continuous inclining trend of TEC value in group II, but the inclining trend was statistically significant. On day 5, the rise in TEC count in group II could be due to anabolic effect of *symphytum officinale* drug which has stimulated and coapted the reticuloendothelial system. Similar findings have been reported by Tembhurne (2006) during her studies on anabolic agent in femoral fracture healing in dogs.



Plate 33 : Radiograph of fractured humerus  
(Group II, Dog B2, day 30 postop.)



Plate 34 : Radiograph of fractured humerus  
(Group II, Dog B2, day 45 postop.)

**Table 11: Mean ± S.E of total erythrocytes count (million/cumm) in two treatment groups**

Group	N	0 <sup>th</sup> Day	3 <sup>rd</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
I	6	5.2±0.24	5.20±0.17	4.88±0.38	5.01±0.18	4.93±0.19
II	6	5.59±0.04 <sup>a</sup>	5.83±0.058 <sup>a</sup>	6.27±0.13 <sup>b</sup>	6.50±0.073 <sup>a</sup>	6.72±0.06 <sup>a</sup>

Different superscript indicated significant difference within the group

#### 4.6.2 Total Leucocytes Count

The animals of group I revealed the mean total leukocyte count  $14.96 \pm 0.412$  thousand/cumm on 0<sup>th</sup> day. The mean value thereafter showed undulating trend upto 14<sup>th</sup> day. The value on 14<sup>th</sup> day was  $14.81 \pm 0.35$  thousand/cumm. The differences between the intervals were non-significant.

The animals of group II showed the mean total leukocyte count value on 0 day as  $14.49 \pm 0.31$  thousand/cumm which inclined on 7<sup>th</sup> day to  $14.71 \pm 0.26$  thousand/cumm. The value thereafter showed undulating trend up to 14<sup>th</sup> day. The mean value on 14<sup>th</sup> day was  $14.71 \pm 0.26$  thousand/cumm (Table 12). The differences between the intervals were non-significant.

The mean values of TLC revealed undulating pattern and the values were statistically non significant in both the groups. The variation of TLC could be due to trauma and inflammatory condition of surgical sites and surgical stress during the treatment period. Similar observations were also recorded by singh *et al.* (1994).

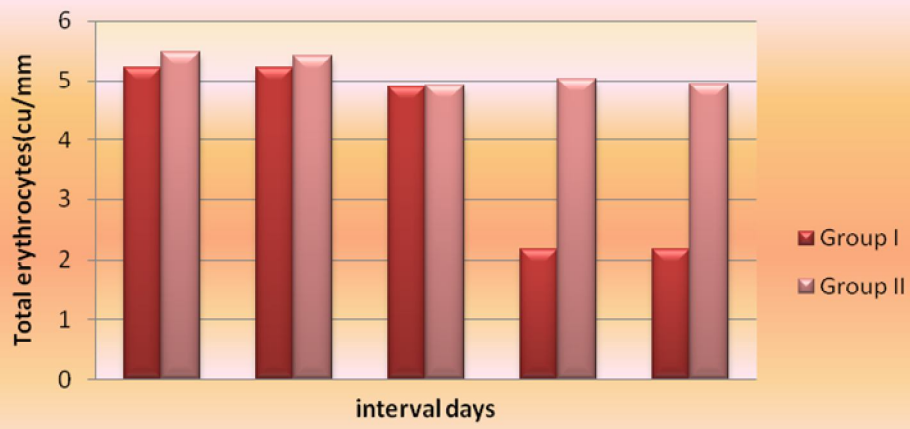
**Table 12: Mean ± S.E of total leucocytes count (thousand/cumm) in two treatment groups**

Group	N	0 <sup>th</sup> Day	3 <sup>rd</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
I	6	14.96±0.41	15.30±0.38	14.96±0.324	15.00±0.441	14.81±0.35
II	6	14.49±0.31	14.71±0.26	14.92±0.31	14.89 ±0 .41	14.43±0.34

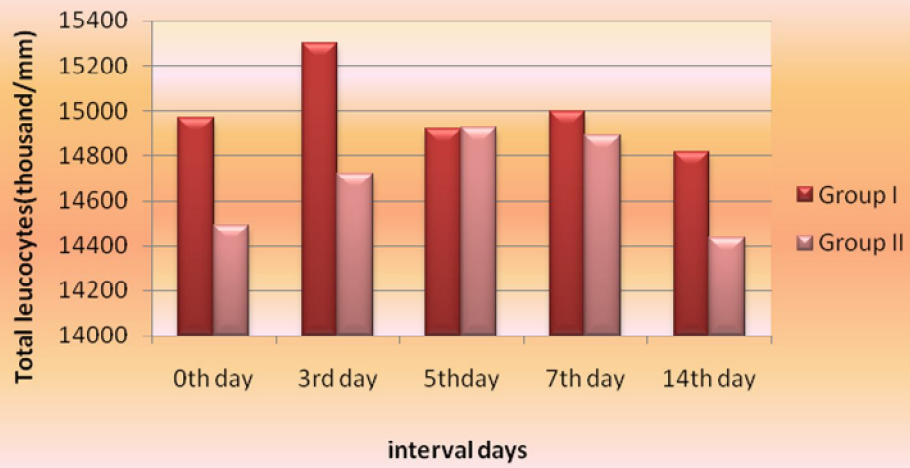
#### 4.6.3. Hemoglobin (gm %)

The animals of group I had the mean hemoglobin value  $10.68 \pm 0.46$  gm percent on 0<sup>th</sup> day. The values were found declined on 3<sup>rd</sup> day i.e.  $10.56 \pm 0.34$  gm percent. Thereafter, the mean value of haemoglobin showed increasing trend up to 14<sup>th</sup> day. The value on 14<sup>th</sup> day was  $10.78 \pm 0.31$  gm percent. The mean hemoglobin value on 0 day in group II was  $11.23 \pm 0.33$  gm%. The value showed inclining trend up to 14<sup>th</sup> day. The value on 14<sup>th</sup> day was  $12.82 \pm 0.26$  gm percent.

**Fig1 . Mean  $\pm$  S.E of total erythrocyte count in two treatment groups**



**Fig. 2 Mean  $\pm$  S.E of total leucocyte count in two treatment groups**



(Table 13). The variations although non-significant in hemoglobin values in group I and group II could be attributed to the surgical stress, and the little anabolic effect of *symphytum officinale* homoeopathy drug which was given per oral for 21 days in group II. However, the changes in present investigation were non-significant. Therefore, it can be concluded that haemoglobin levels did not alter due to surgical or symphytum therapy.

**Table 13: Mean  $\pm$  S.E of hemoglobin (gm %) in two treatment groups**

Group	N	0 <sup>th</sup> Day	3 <sup>rd</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
I	6	10.68 $\pm$ 0.46	10.56 $\pm$ 0.34	10.56 $\pm$ 0.34	10.58 $\pm$ 0.28	10.78 $\pm$ 0.31
II	6	11.23 $\pm$ 0.33	11.36 $\pm$ 0.36	12.24 $\pm$ 0.33	12.46 $\pm$ 0.28	12.82 $\pm$ 0.26

#### 4.6.4 Differential leucocyte count

##### 4.6.4.1 Neutrophil (%)

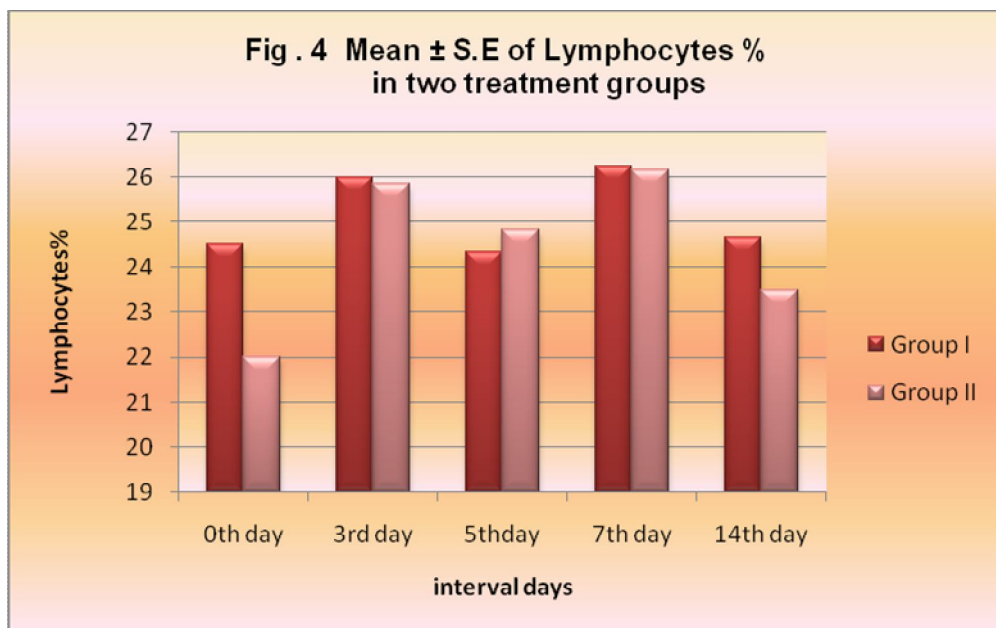
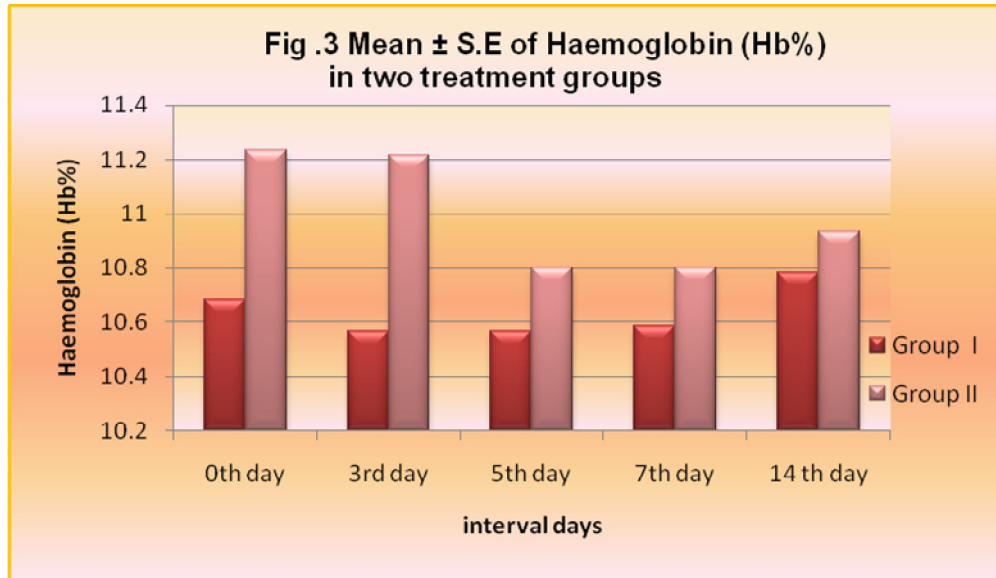
The mean total neutrophil value on 0 day in group I was 53.60  $\pm$  10.61 per cent. mean value thereafter showed undulating trend up to 14<sup>th</sup> day and it was 52.77  $\pm$  10.44 per cent.

In group II, the mean neutrophil value on 0 day was 68.16 $\pm$ 1.07 per cent. The value thereafter showed declining trend up to 14<sup>th</sup> day. The mean value on 14<sup>th</sup> day was 64.83 $\pm$ 65 per cent (Table 15).

In the present study, the mean values of neutrophil showed a continuous varying pattern and the values were statistically non significant. The transient increase of neutrophil could be due to trauma and inflammatory condition of the surgical site and surgical stress during the treatment period. The similar observation was reported by Gahlod (2007).

**Table 14: Mean  $\pm$  S.E of neutrophil (%) in two treatment groups**

Group	N	0 <sup>th</sup> Day	3 <sup>rd</sup> <sup>th</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
I	6	53.60 $\pm$ 10.61	52.77 $\pm$ 10.43	53.60 $\pm$ 10.61	52.77 $\pm$ 10.44	52.77 $\pm$ 10.44
II	6	68.16 $\pm$ 1.07	64.66 $\pm$ 1.54	64.83 $\pm$ 1.10	63.33 $\pm$ .954	64.83 $\pm$ 65



#### 4.6.4.2 Lymphocyte (%)

The mean total lymphocyte count on day 0 in group I was  $24.5 \pm 1.14$  per cent. The value on 3rd day was increased and showed undulating trend up to 14<sup>th</sup> day. The value on 14<sup>th</sup> day was  $24.5 \pm 1.14$  percent.

The mean lymphocyte value on 0 day in group II was  $22.00 \pm 0.63$  per cent. The value showed undulating trend up to 14<sup>th</sup> day. The value on 14<sup>th</sup> day was  $23.5 \pm 0.56$  per cent. (Table 14). The variation in lymphocyte values was found statistically significant at 1% level.

**Table 15: Mean  $\pm$  S.E of lymphocyte (%) in two treatment groups**

Group	N	0 <sup>th</sup> Day	3 <sup>rd</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
I	6	$24.5 \pm 1.14$	$26.00 \pm 0.59$	$24.33 \pm 0.91$	$26.25 \pm 0.70$	$24.66 \pm 0.80$
II	6	$22.00 \pm 0.63^c$	$25.85 \pm 1.10^a$	$24.83 \pm 0.54^{ab}$	$26.16 \pm 0.30^a$	$23.5 \pm 0.56^{bc}$

Different superscript indicated significant difference within the group

#### 4.6.4.3 Monocyte (%)

In group I, the mean monocyte value on 0 day was  $9.00 \pm 0.51$  per cent. The mean values thereafter showed undulating trend up to 14<sup>th</sup> day. The mean value on 14<sup>th</sup> day was  $9.33 \pm 0.33$  per cent.

In group II, the mean monocyte value on 0 day was  $7.66 \pm 0.80$  per cent. The mean value showed inclining trend up to 14<sup>th</sup> day. The value on 14<sup>th</sup> day  $9.66 \pm 0.21$  per cent (Table 16).

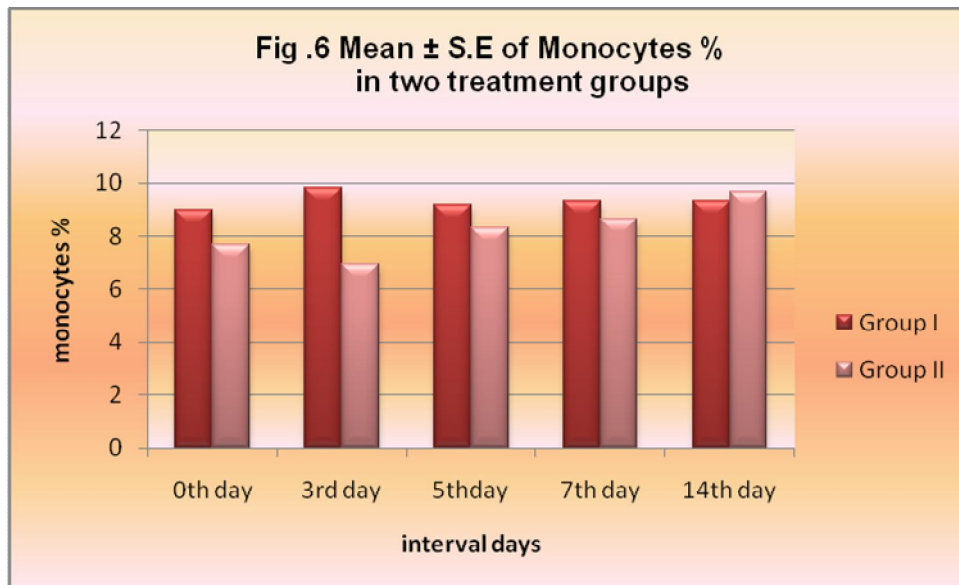
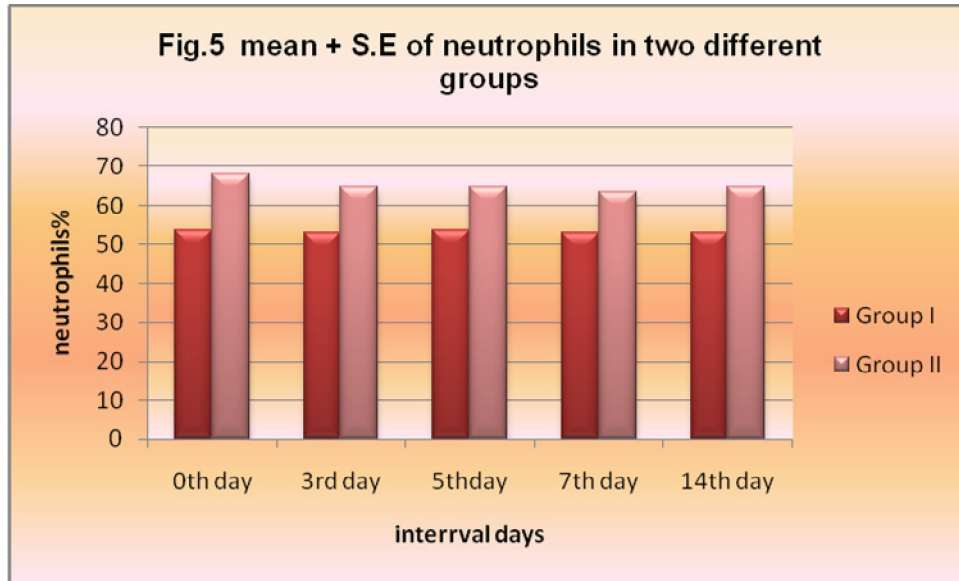
The mean values of monocyte showed an undulating trend and the changes were statistically non-significant. The transient non-significant increase in mean values of monocyte in group I and group II dogs could due be to the trauma and stress during postoperative period. The variations of monocytes count were observed by Tembhone (2006) and Gahlod (2007).

**Table 16: Mean  $\pm$  S.E of monocyte (%) in two treatment groups**

Group	N	0 <sup>th</sup> Day	3 <sup>rd</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day
I	6	$9.00 \pm 0.51$	$9.80 \pm 0.29$	$9.16 \pm 0.60$	$9.31 \pm 0.42$	$9.33 \pm 0.33$
II	6	$7.66 \pm 0.80$	$6.98 \pm 0.76$	$8.33 \pm 0.76$	$8.66 \pm 0.60$	$9.66 \pm 0.21$

#### 4.6.4.4 Eosinophil (%)

In group I, the mean value of eosinophil on day 0 was  $3.0 \pm 0.36$  per cent which was increase on 7<sup>th</sup> day i.e.  $3.3 \pm 0.42$  per cent. The mean value decreased on 14<sup>th</sup> day and remains constant on 14<sup>th</sup> day  $2.6 \pm 0.33$  per cent.



In group II, the mean eosinophil value on 0 day was  $3.1 \pm 0.30$  per cent. The mean value increases on 7<sup>th</sup> day  $4.3 \pm 0.88$  per cent. Thereafter, the value showed undulating trend up to 14<sup>th</sup> day. The value on 14<sup>th</sup> day was  $3.3 \pm 0.49$  per cent (Table 17).

In the present study, the mean values of eosinophil increases up to 7<sup>th</sup> day and the values were statistically non significant. The transient non-significant increase of eosinophil could be attributed to the trauma and inflammatory condition and surgical stress during the treatment periods. The observation was accordance with Tembhurne (2006) and Gahlod (2007).

**Table 17: Mean  $\pm$  S.E of eosinophil (%) in two treatment groups**

Group	n	0 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	28 <sup>th</sup> Day
I	6	$3.0 \pm 0.36$	$3.3 \pm 0.42$	$2.6 \pm 0.33$	$2.6 \pm 0.33$
II	6	$3.1 \pm 0.30$	$4.3 \pm 0.88$	$3.1 \pm 0.54$	$3.3 \pm 0.49$

#### 4.6.4.5 Basophil (%)

In group I, the mean basophils count on day 0 was  $0.5 \pm 0.33$  per cent, while it was  $0.5 \pm 0.28$  percent,  $0.5 \pm 0.22$  percent and 0.00 on 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup> day, respectively.

In group II, the mean basophil count on day 0 was  $0.33 \pm 0.30$  per cent, while it was  $0.5 \pm 0.22$  percent,  $0.5 \pm 0.22$  percent and  $0.33 \pm 0.21$  percent at 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup> day, respectively (Table 18). The variations in basophil per cent in group I and group II was basophil count non significant.

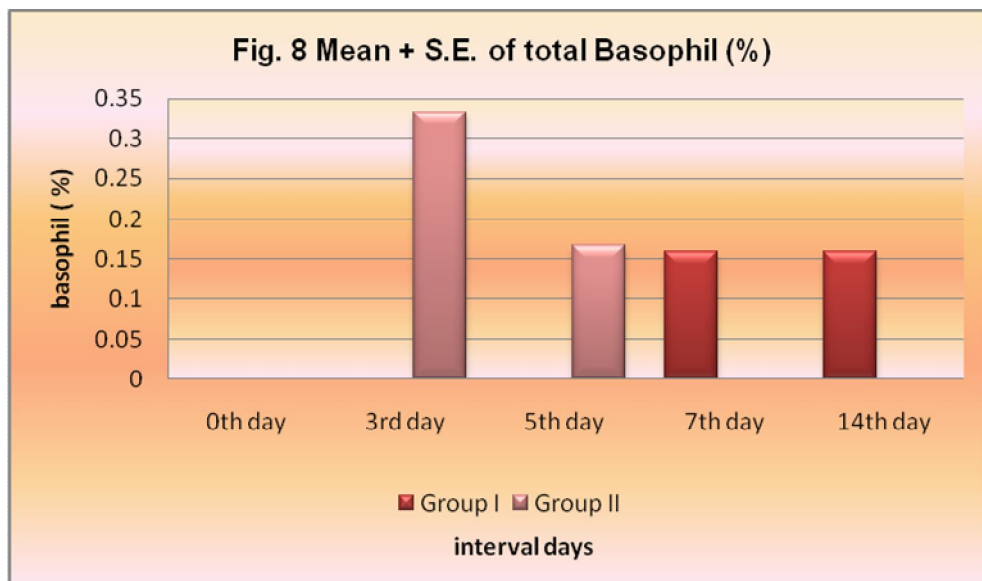
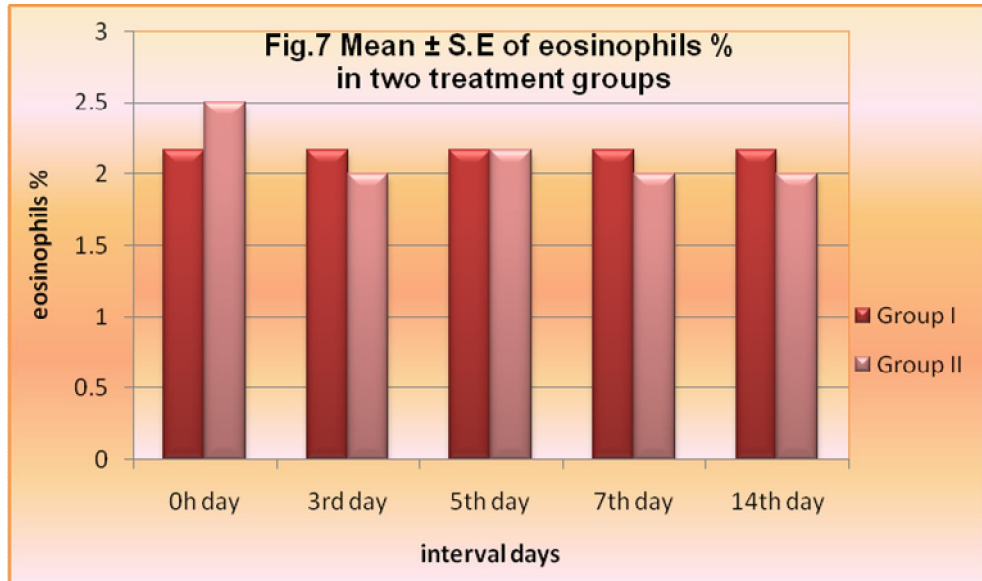
**Table 18: Mean  $\pm$  S.E of basophil (%) in two treatment groups**

Group	N	0 <sup>th</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	28 <sup>th</sup> Day
I	6	$0.5 \pm 0.33$	$0.5 \pm 0.28$	$0.5 \pm 0.22$	0.00
II	6	$0.33 \pm 0.30$	$0.5 \pm 0.22$	$0.5 \pm 0.22$	$0.33 \pm 0.21$

## 4.7 Biochemical Studies

### 4.7.1 Serum alkaline phosphatase activity (U/L)

In group I, the mean values of serum alkaline phosphatase value on 0<sup>th</sup> day was  $356.5 \pm 02.80$  U/L, and it was found elevated on 7<sup>th</sup> day i.e.  $446.66 \pm 07.96$  U/L. Later on, adopted regular declining trend on 15<sup>th</sup> day, 30<sup>th</sup> day and 45<sup>th</sup> day i.e.  $321.33 \pm 02.12$  U/L, and  $134.16 \pm 5.86$ , and  $122.33 \pm 4.75$  U/L



respectively. The values of Serum Alkaline Phosphatase were statistically highly significant from day 7 onwards.

In group II, the mean values of Serum Alkaline Phosphatase value on 0<sup>th</sup> day was 357.85±05.24 U/L and adopted regular declining trend on 7<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day postoperative i.e. 345.0±21.90 U/L, 227.58±16.81 U/L, 157.03±4.00 U/L and 140.03±02.65, respectively (Table 19). The variation in Serum Alkaline Phosphatase levels was highly statistically significant from day 15 onwards.

Therefore the Serum Alkaline Phosphatase levels in both the groups showed significant decline as compared to the day 0 values.

Alkaline phosphatase are group of membrane associated zinc metallo enzymes that are present in most tissue. However, increased serum alkaline phosphatase activity is usually associated with either skeletal or hepatobiliary diseases. The metabolic changes during bone healing or the diseases of the musculoskeletal system increases osteoblastic activity that accounts for the increased serum alkaline phosphatase activity, since the Rate of release of enzyme into the serum will exceed its rate of inactivation (Hill and Sammons, 1967). In the present investigation, the Serum Alkaline Phosphatase levels were significantly high in both the groups postoperatively. The values decreased significantly in both the groups during the period of observations. However, when two groups were compared, the reduction in levels were more in group II, which could be attributed to the anti-inflammatory activity of the drug *Symphytum officinale*.

Singh *et al.* (1976) and Nagaraja *et al.* (2003) advocated similar findings, who reported non-significant variations in post operative serum alkaline phosphatase values from that of preoperative values; however, the values declined from 30<sup>th</sup> postoperative day onwards.

The present observations supports findings of Singh *et al.* (1976) and Nagaraja *et al.* (2003) who reported non-significant variations in post operative values from that of preoperative values; however, the values declined from 30<sup>th</sup> postoperative day onwards.

The present study reflected that the initial significant increase in the concentration of total serum alkaline phosphatase postoperative and subsequent decrease might reveal normal healing process due to the increase in osteoblastic activity of the fracture fragment of the bone, as reported by Nakagawa *et al.* (2006). However, when two groups were compared, it was observed that the

levels were significant from the beginning in group I whereas in group II levels were significant after 15 days.

Alkaline phosphatase is involved in bone formation and healing of fractures. The enzyme, secreted by the osteoblast accelerates the process of mineralization either by increasing the local concentration of inorganic phosphate.

Values of serum alkaline phosphatase during fracture healing could be an additional tool in evaluating the process of fracture healing in dogs and also to assess in potential as a poor surgical prognostic indicator (Komnenou *et al.*, 2005).

During the present study, Alkaline phosphatase was found to be elevated in early postoperative period may be due to adrenal hyper function due to stress, skin and muscle trauma and increase osteogenic activity and deposition of calcium salts at the site of fracture as opined by Wilkinson (1980) and Pardeshi and Ranganath (2009). While Mahendra *et al.* (2007) reported significant increase in serum alkaline phosphatase activity throughout the observation period in treated as well as control group.

**Table 19: Mean±S.E of alkaline phosphatase (U/L) in different two groups**

Group	N	0 <sup>th</sup> Day	7 <sup>th</sup> Day	15 <sup>th</sup> Day	30 <sup>th</sup> Day	45 <sup>th</sup> Day
I	6	356.5±02.80 <sup>b</sup>	446.66±7.96 <sup>a</sup>	321.33±02.12 <sup>c</sup>	134.16±05.86 <sup>d</sup>	122.33±4.75 <sup>d</sup>
II	6	357.85±05.24 <sup>a</sup>	345±21.90 <sup>a</sup>	227.58±16.81 <sup>b</sup>	157.03±4.00 <sup>c</sup>	140.03±2.65 <sup>c</sup>

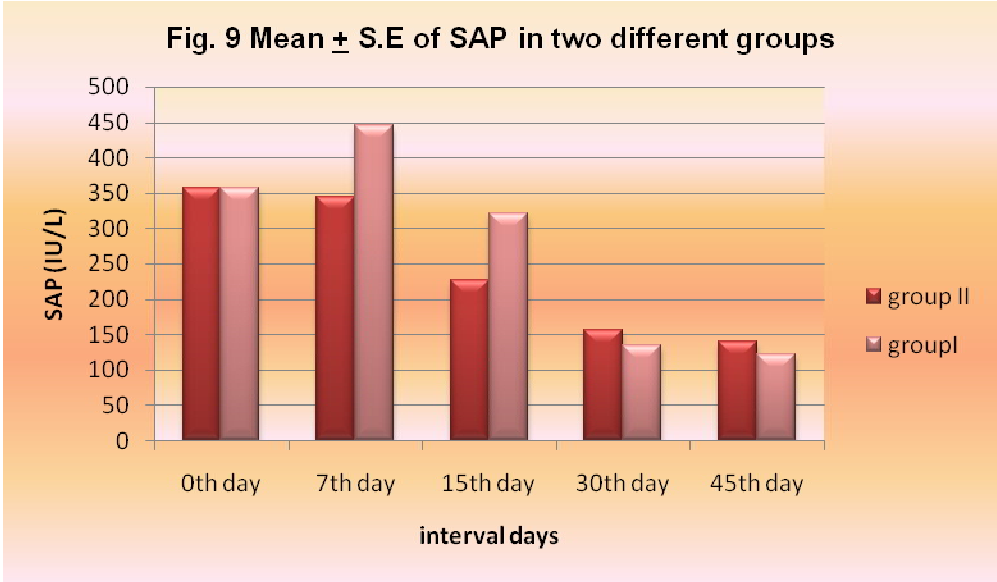
#### 4.8 Complications

The observations on the complications related to the wound and fracture like wound infection, swelling of paw, loosening of external fixation (plaster cast), migration of intramedullary implant, host implant reaction, rotational movement of the fractured bone were also recorded during the present study.

However, during the study period no major complications were recorded except Wound at the trochanteric fossa region on 7<sup>th</sup> day of treatment was observed in the dog (B5) from group II of femur fracture, it was due to migration of pin at trochanteric fossa and open septic wound along with migrated pin was observed at the area of trochanteric fossa, which was controlled by daily dressing with extended antibiotic course for 5 days.

#### 4.9 Role of *Symphytum officinale* in fracture healing process

Homoeopathy is a popular form of complementary medicine which was founded on the medical traditions and beliefs of the Greeks and Romans many



thousands of years ago. Symphytum is a homoeopathic drug derived from the herb *Symphytum officinale* L. The available literature revealed that this drug has a potential anti-inflammatory and analgesic properties without any side effects.

Bone fracture healing is a very remarkable process since, unlike soft tissue healing, which leads to scar formation, the end result of normal healing is the regeneration of the anatomy of the bone and complete return to function. The fracture generally heals in 6-8 weeks after the initial injury. The major phases of fracture healing are, inflammatory phase, reparative phase (fibrocartilage callus formation and bony callus formation) and remodelling phase. The aim of the clinician is to complete these phases as early as possible without any adverse results.

Further use of anti-inflammatory and analgesic drugs is very common in veterinary practice, especially in orthopaedic cases; these drugs are widely used even for longer duration with the belief that the pain due to bone injury is very acute and long lasting. Therefore the analgesic drugs are mandatory in such cases. Uses of COX-2 inhibitors have gained popularity in human and veterinary medicines since they have least side effects such as acidity, gastric ulcerations and renal damage. Such drugs are used for a longer period in orthopaedic cases for alleviating the pain. However, Herbenick *et al.* (2008) studied the effects of Cyclooxygenase 2 Inhibitor on Fracture Healing in a Rat Model. They observed a statistically significant difference in the biomechanical strength of fracture in the pooled data from their treatment rats and control rats, but they did not find any statistically significant difference in biomechanical strength at 4 sacrifice intervals. At the 8-week interval, more callus was noted in treatment group relative to the control group. However, no statistically significant difference was found in mean elliptical area of callus formation at any interval. From their studies they suggested that COX-2 enzyme function is important for fracture healing and that caution should be used when considering use of COX-2 inhibitors in patients with fractures. If the findings of the researchers are to be observed and followed, then the option left with us is to explore the possibility of alternate analgesic and anti-inflammatory drugs in orthopaedic cases. The use of *Symphytum officinale* and such other drugs can overcome the problem. Hence an attempt has been made to reduce the duration of fracture healing by administering the homoeopathic drug.

- i) The results of the present investigation revealed that there was an early partial weight bearing and complete weight bearing observed in group II as compared to group I. The radiological observations also revealed that the bony callus formation and bone union was better and early in group II. The hematobiochemical parameters did not show any adverse changes in group II, on the contrary, the levels of serum Alkaline Phosphatase returned to normal early in group II, therefore it evident that the group II revealed better fracture healing process without any adverse side effects. Therefore it is concluded that the administration of Symphytum leads to reduction in partial and complete weight bearing time, suggestive of its anti-inflammatory, analgesic and specific osteoinducing properties along with the proper fracture fixation technique.

# **SUMMARY AND CONCLUSIONS**

## SUMMARY AND CONCLUSIONS

The study on healing of long bone fractures in dogs with the use of bone healing agent osteoinducers was undertaken with the objective to study the efficacy of *Symphytum officinale*, a homoeopathy drug of 30 c potency as an osteoinducer drug in the process of bone healing. The data of clinical cases in dogs was collected from Teaching Veterinary Clinical Complex, North Ambazari Road, Nagpur from February 2014 to July 2014 to study the prevalence and incidence of long bone fracture. Among these, twelve cases of long bone fractures were used as material for research work.

The study was carried out on twelve clinical cases of long bone fractures in dogs which were divided in two equal groups I and II, irrespective of breed, age and sex. In group I, immobilization of fractured bone by applying various techniques was done. In group II, immobilization of fractured bone was done along with the administration of *Symphytum officinale* of 30 c potency @ 4 globules twice a day per oral for 21 days postoperative days. The study included the role of *Symphytum officinale* as an osteoinducer in long bone fracture repair in canine and the results were compared on the basis of clinical, radiological, hematological and biochemical observations in groups I and II.

The study on twelve clinical cases of long bone fractures revealed that higher breed wise prevalence of long bone fracture were in non - descript breed of dogs (50%), followed by Labrador (25%), in German shepherd (16.66 %) and Pomeranian breeds (8.33%). The present study recorded that percentage of breed wise incidence of long bone fractures were more in non-descript breed of dogs.

The incidence of long bone fracture was highest among younger dogs of 0 to 6 months age group (41.66%), followed by 6 to 12 months age (25%) and 1 to 2 years (16.66%) and 2 yrs and above (8.33%). Incidence of long bone fracture was higher (33.32%) in the dogs below 6 months of age. Sex wise distribution of long bone fracture in canines revealed higher incidence in males (66.66%) as compared to females (33.33%).

Among the long bone fractures found in the present study, 66.66% cases had oblique fractures and 33.33 % cases had transverse fracture.

In the present study the radiographic examination revealed that maximum number of fractures were of mid-shaft (75%) followed by distal third (16.66%) and proximal third of diaphysis (8.33%).

The percentage involvement of various long bone fractures were 66.66% cases in femur, 16.66% cases in humerus, 8.33% in radius and ulna and 8.33 % in tibia.

Among the types of bone involved and the types of internal fixation done, the intramedullary pinning/nailing was done in 8 (66.66%) femur fractures and 1(8.33%) tibial fracture, intramedullary pinning with cerclage wiring 2 (16.66%) humerus fractures and bone plating was done in 1(8.33%) case of long bone fracture.

The immobilization of fractured bones in dogs of both the groups was done depending on type of fracture and bone involved. Among different types of fractured bone, internal fixation by intramedullary pinning was done 8 (61.66%) in femur fracture cases and intramedullary pinning of humerus was done by intramedullary pinning complemented by cerclage wiring in 2 (16.66 %) radius ulna fracture of both the groups, bone plating was done in one case of radius fracture(8.33%) and intramedullary pinning of tibial fracture in one case (8.33%)

In group II the effect of *Symphytum officinale* proved its efficacy as anti-inflammatory, analgesic, granulation promoting and induced osteogenic activity.

The wound healing action of comfrey has been attributed to the presence of allantoin. Anti-irritating, hydrating and the anti-inflammatory properties are attributed to mucilage and tannins, which are astringent and haemostatic.

The dogs were subjected to the treatment according to diagnosis based on clinical and radiological observations. The common preoperative consideration viz. preparation of animal, premedication and anaesthetic procedure was followed for four dogs each from groups I and II and subjected to open reduction and internal fixation of fractured bone with intramedullary pin/nail. Two dogs each with fracture of humerus in group I and II were subjected to intramedullary pinning along with cerclage wiring and plating was done in one case of radius fracture in group II.

The dogs were kept off fed for 12 hrs while water was withheld 6 hours prior to premedication. The dogs were administered inj. Xylazine hydrochloride @ 1 mg/kg body weight intramuscular and Atropine sulphate @ 0.04 mg/kg body weight subcutaneous. Xylazine and Atropine sulphate were used as a premedicant which calm down the patients and also adopted a restrained posture on operation table that was suitable for administration of anaesthetic agents. Diazepam was administered @ 0.5 mg/kg body weight intravenously followed by

Ketamine hydrochloride @ 5 mg/ kg body weight intravenously along with Ringers lactate solution. As soon as animal entered into profound anaesthesia and attain a deep level of surgical anesthesia the surgical procedure initiated.

The surgical procedure of intramedullary pinning and nailing was carried out in four dogs each in group I and group II. The cranio lateral aspect of thigh for exposing femur shaft was found more appropriate and the reduction of fracture fragment was observed to be more convenient and easier. The introduction of intramedullary pin from proximal fragment of fractured femur to trochanteric fossa in a retrograde fashion after proper reduction was easier and no difficulty was experienced.

The open reduction and internal fixation of fractured humerus with intramedullary pinning along with cerclage wiring in both the groups was found satisfactory. The perfect alignment and reduction was observed radiographically in both the groups. However, the reduction in healing time of humerus was observed in group II as compared to group I.

The postoperative regimen was followed in all dogs subjected to surgical therapy includes Ceftriaxone @ 20 mg /kg body weight I/M daily for seven days and Inj. Meloxicam @ 0.5 mg/kg body weight I/M for three days.

The bandage was changed on alternate days for 10 days. Antiseptic dressing of surgical wound was done with povidone iodine solution. Skin sutures were removed on 10<sup>th</sup> to 12<sup>th</sup> postoperative day. The owner was advised to restrict the activities of the animal for first ten days; subsequently the dogs were allowed limited physical activities regularly.

The process of fracture healing was assessed on the basis of clinical, radiological, hematological and bio-chemical observations recorded at scheduled intervals.

In present study, the wound healing in all dogs of group I and group II was observed from 7 to 12 days by primary intention. Wound at the trochanteric fossa was observed on 5<sup>th</sup> day of treatment was noticed in the dog from group II B5 femur fracture, it was due to protrusion of v nail in trochanteric fossa because of excessive motility of dog. Local dressing was done with povidone iodine solution and administration of Tab. Moxclav (@ 25 mg/kg body weight) orally for 5 days resulted in satisfactory recovery.

The variable time required for complete and partial weight bearing by the affected limb in group I and II were recorded. The dogs of group I having femur

fracture required 6 to 7 days with a mean of  $6.75 \pm 0.25$  post operative day to bear partial weight and required 35 to 36 days with mean of  $35.00 \pm 0.70$  postoperative days for complete weight bearing on operated limb. The dogs of group I with humerus fracture required 9 to 10 days with a mean of  $9.0 \pm 1.00$  days for partial weight bearing and required 35 to 36 days with mean of  $35 \pm 1.00$  days for complete weight bearing by affected limb. In the present study, animals of group II, required less time for partial weight bearing i.e.  $3.5 \pm 0.50$  and  $25.00 \pm 1.00$  postoperative days for complete weight bearing in humerus fracture and weight bearing period was statistically significant.

In group I and group II radiograph taken immediately after fixation provides the status of fracture reduction, alignment and fixation. The intramedullary pinning was found satisfactory for fixation of femur fracture in most of the dogs and the radiographs taken subsequently on 15, 30 and 45 day postoperatively for evaluating the status of implant, progress of fracture healing, alignment of fragments and reaction of host bone to implant and complications if any.

In group I, radiograph taken on day 15 revealed perfect alignments of fracture fragments of femur. There was evidence of mild degree periosteal reaction and fracture gap was blurred while in group II on day 15 excessive periosteal reaction, bridging of callus and obliteration of fracture gap by distinct callus formation.

In group II the radiograph taken on day 30<sup>th</sup> revealed moderate periosteal proliferation, periostitis and bridging of fracture gap was not completed. The radiograph taken on day 45 revealed, healing of fractured bone from anterior site and incomplete bridging of fracture gap and little callus was visible.

In two dogs each from group I and II humerus fracture was corrected by intramedullary Steinmann pinning along with cerclage wiring. In group I, radiograph taken on 15<sup>th</sup> day showed slight to moderate periosteal reaction. Proper alignment of fracture fragment was noticed and radiograph on day 30<sup>th</sup> revealed slightly deviated alignment of fractured fragments with periosteal bridging callus and 45<sup>th</sup> day radiograph revealed very little periosteal callus and incomplete bridging of fracture gap was visible.

In group II, radiograph taken on 15<sup>th</sup> day revealed slight elevated fracture fragment. There was evidence of moderate periosteal proliferation and fracture gap was invariably blurred and appeared irregular. Radiograph taken on 30<sup>th</sup> day

showed periosteal bridging callus at the anterior site. Bone fragments appeared united perfectly. The 45<sup>th</sup> day radiograph revealed complete healing of fractured bone. The continuity of medullary canal was also evident.

Radiographs taken at scheduled intervals showed accelerated fracture healing with complete bridging of fragment, bony deposition along with periosteal and medullary continuity across the fracture site in animals of treated group II whereas radiographic union and periosteal bridging at fracture site was relatively slow and incomplete in animals of group I. The enhancement of the rate of fracture healing and calcification process in animals of group II could be attributed to the therapeutic efficacy of *Symphytum Officinale* acted as anti-inflammatory, analgesic with specific osteoinductive properties along with the proper fracture fixation technique.

The animals of group I and group II revealed non significant changes of hematological values such as hemoglobin, total erythrocyte count, total leucocyte count, differential leucocyte count which does not affect the bone healing. The variations in serum alkaline phosphatase level in both the groups revealed decreasing trend which was statistically non-significant. The study revealed that initial increase in the concentration of total serum alkaline phosphatase after surgery and subsequent decrease might reflect normal healing process due to the increase in osteoblastic activity on the fracture fragment of the bone. The administration of *symphytum officinale* homoeopathy drug of 30 c potency @ four globules twice a day for 21 days postoperatively irrespective of fracture management (pinning /nailing/plating) in group II revealed reduction in partial and complete weight bearing period, which indicated the enhancement of the healing process and showed significant osteogenic activity. The radiographical and haemato-biochemical parameters also reported the clinical observations.

In the present study, during the study period no major complications were recorded except wound at the trochanteric fossa region on 7<sup>th</sup> day of treatment was observed in the dog (B5) from group II of femur fracture, it was due to migration of pin at trochanteric fossa and open septic wound along with migrated pin was observed at the area of trochanteric fossa, which was controlled by daily dressing with extended antibiotic course for 5 days. In other case of tibial fracture patient escaped from shelter due to care taker's mistake and one case of radius ulna fracture in group I A7 expired on day 10<sup>th</sup> post operative. In other case of humerus fracture group II B7 undergone in malunion.

**CONCLUSIONS:**

1. The incidence of long bone fracture was more in dogs below 6 months of age, higher in males as compare to females and in non descript breeds of dogs.
2. Administration of *Symphytum officinale* homoeopathy drug of 30c potency leads to reduction in partial and complete weight bearing time, suggestive of its anti-inflammatory, analgesic and specific osteoinducer properties along with the proper fracture fixation technique.
3. Supplementation of *Symphytum officinale* acted as anti-inflammatory drug with osteoinducer properties as indicated by clinical, radiographical and haemato-biochemical parameters.

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# **APPENDIX**

## APPENDICES

### APPENDIX - I

Analysis of variance for Hb in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	0.433	0.103	0.121	0.970
Error	25	22.546	0.908		
Total	29				

Coefficient of Variation = 8.873

Treatments found to be Non Significant

Analysis of variance for Hb in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	1.606	1.606	0.401	0.579
Error	25	.750	17.610	0.704	-
Total	29	-	-	-	-

Coefficient of Variation = 7.540

Treatments found to be Non Significant

Analysis of variance for TEC in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	0.539	0.132	0.350	0.844
Error	25	9.517	0.386	-	-
Total	29	-	-	-	-

Coefficient of Variation = 12.221

Treatments found to be Non Significant

Analysis of variance for TEC in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	1.676	0.416	3.383	0.022
Error	25	3.089	0.125	-	-
Total	29	-	-	-	-

Coefficient of Variation = 6.540

Treatments found Significant at 5% level of Significance CD(0.05)= 0.410

Analysis of variance for TLC in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	790000.000	197500.000	0.221	0.926
Error	25	22430000.000	897200.000	-	-
Total	29	-	-	-	-

Coefficient of Variation = 6.317

Treatments found to be Non Significant

Analysis of variance for TLC in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	1210833.333	302708.333	0.444	0.772
Error	25	16914583.333	676583.333	-	-
Total	29	-	-	-	-

Coefficient of Variation = 5.597

Treatments found to be Non Significant

Analysis of variance for Lymphocyte in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	19.533	4.883	1.114	0.378
Error	25	109.546	4.386	-	-
Total	29	-	-	-	-

Coefficient of Variation = 8.320

Treatments found to be Non Significant

Analysis of variance for Lymphocyte in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	71.743	17.933	6.406	0.000
Error	25	70.046	2.806	-	-
Total	29	-	-	-	-

Coefficient of Variation = 6.842

Treatments found Significant at 1% and 5% level of significance

CD(0.01) = 2.692 CD(0.05) = 1.997

Analysis of variance for Neutrophil in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	15.533	3.883	1.855	0.140
Error	25	52.203	2.083	-	-
Total	29	-	-	-	-

Coefficient of Variation = 2.271

Treatments found to be Non Significant

Analysis of variance for Neutrophil in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	77.000	19.250	2.622	0.059
Error	25	183.666	7.346	-	-
Total	29	-	-	-	-

Coefficient of Variation = 4.152

Treatments found to be Non Significant

Analysis of variance for Monocyte in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	2.136	0.536	0.442	0.771
Error	25	30.155	1.202	-	-
Total	29	-	-	-	-

Coefficient of Variation = 11.778

Treatments found to be Non Significant

Analysis of variance for Monocyte in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	23.776	5.946	2.344	0.083
Error	25	63.403	2.533	-	-
Total	29	-	-	-	-

Coefficient of Variation = 18.674

Treatments found to be Non Significant

Analysis of variance for Eosinophil in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	0.000	0.000	0.000	1.000
Error	25	4.166	0.166	-	-
Total	29	-	-	-	-

Coefficient of Variation = 18.842

Treatments found to be Non Significant

Analysis of variance for Eosinophil in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	1.133	0.283	3.037	0.030
Error	25	2.333	0.093	-	-
Total	29	-	-	-	-

Coefficient of Variation = 14.325

Treatments found Significant at 5% level of Significance  $CD(0.05) = 0.363$

Analysis of variance for Basophil in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	0.200	0.050	0.750	0.563
Error	25	1.666	0.066	-	-
Total	29	-	-	-	-

Coefficient of Variation = 387.293

Treatments found to be Non Significant

Analysis of variance for Basophil in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	0.533	0.133	1.534	0.226
Error	25	2.166	0.086	-	-
Total	29	-	-	-	-

Coefficient of Variation = 294.390

Treatments found to be Non Significant

Analysis of variance for ALP in group I

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	488354.466	122088.616	765.668	9.570
Error	25	3986.333	159.453	-	-
Total	29	-	-	-	-

Coefficient of Variation = 4.578

Treatments found Significant at 1% and 5% level of significance

CD(0.01) = 20.315 CD(0.05) = 15.013

Analysis of variance for ALP in group II

ANOVA TABLE					
S.V.	D.F.	S.S.	M.S.S.	F cal	F prob
Treatments	4	250760.090	62690.025	64.249	8.696
Error	25	24393.398	975.739	-	-
Total	29	-	-	-	-

Coefficient of Variation = 12.727

Treatments found Significant at 1% and 5% level of significance

CD(0.01) = 50.263 CD(0.05) = 37.151

**VITA**

## VITA

The author of this dissertation Swaroop Chandel born on 14<sup>th</sup> July 1987 at Mirzapur district of state Uttar Pradesh state.

He has passed his S.S.C. examination from Little Flower High School, Kakarmatta, Varanasi in the year 2002 and H.S.S.C. examination from Central Hindu Boys School, Kamachha , Varanasi in the year 2004.

Author joined Nagpur Veterinary College, Nagpur (Maharashtra Animal and Fishery Sciences University) in the year 2007 to undertake undergraduate studies and obtained the degree Bachelor of Veterinary Science and Animal Husbandry (B.V.Sc. & A.H.) in July 2012 with 83.20% marks. Author was recipient of National Talent Scholarship provided by I.C.A.R throughout his graduation studies.

Author joined the department of Veterinary Surgery and Radiology, Nagpur Veterinary College, Nagpur in September 2012. He actively participated in various animal welfare programmes and was consultant to an active N.G.O related to animal welfare in the city Nagpur.

He has actively participated in National Service Scheme camping programs during his college life. He has participated in many Clinical Animal Health camps held during the course of undergraduate and postgraduate studies organized by the college and various organizations and associations.

He was recipient of senior veterinary foundation award for getting highest marks in animal genetics and breeding during undergraduate studies. He was awarded NCC 'C' certificate during his graduation studies.

# **THESIS ABSTRACT**

### THESIS ABSTRACT

- a) Title of thesis : ROLE OF *SYMPHYTUM OFFICINALE* AS AN OSTEOINDUCER IN LONG BONE FRACTURE REPAIR IN CANINE
- b) Full Name of Student : SWAROOP CHANDEL
- c) Name & Address of Major Advisor : Dr. P. T Jadhao  
Professor & Head,  
Department of Veterinary Surgery and Radiology,  
Nagpur Veterinary College, Nagpur
- d) Degree to be awarded : MASTER OF VETERINARY SCIENCE
- e) Year of award of degree : 2014
- f) Major subject : VETERINARY SURGERY AND RADIOLOGY
- g) Total number of pages in the thesis : 67
- h) Number of words in the thesis abstract : 670
- i) Signature of student :
- j) Signature, Name & Address of forwarding authority :

**Associate Dean**  
Nagpur Veterinary College,  
Nagpur

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### ABSTRACT

The study was undertaken to evaluate the efficacy of *Symphytum Officinale* as a stimulating agent in the process of long bone fracture healing. Twelve clinical cases of long bone fracture in dogs presented at Teaching Veterinary Clinical Services Complex, Nagpur reported during February 2014 to July 2014, were divided into two equal groups and subjected to the treatment as per schedule.

In group I, immobilization of fractured bone were carried out by open reduction and internal fixation. In group II, in addition to immobilization of fractured bone, the *Symphytum Officinale* homoeopathy drug was administered @ four globules twice a day for days for 21 days postoperatively.

In the present investigation breed wise distribution of long bone fracture revealed highest incidence 50 percent in non-descript, 25 percent in Labrador, 16.66 percent in German shepherd and 8.33 percent in Pomeranian breeds. The present study indicated that percentage of incidences of long bone fractures were more in non-descript dogs. Incidence of long bone fracture were highest among younger dogs of 0 to 6 months age group .

The immobilization of long bone fracture of both the groups was done depending on the type of fracture and fractured bone. Among different types of fractured bone, internal fixation by intramedullary pinning/nailing was done in nine cases of long bone fracture (75%) and intramedullary pinning along with cerclage wiring in two cases of humerus fracture (16.66%), bone plating was done in one case of fracture of radius ulna (8.33%).

The method adopted for the administration of the *symphytum officinale* homoeopathy drug of 30 c potency for oral administration in the dogs with fracture of long bones during the present study was found suitable and easy and the quantity of globules administered was found satisfactory as an osteoinducer.

The common preoperative consideration viz., preparation of animal, premedication and anaesthetic procedure was followed for four dogs each from groups I and II and subjected to open reduction and internal fixation of femur bone with intramedullary pin/nail. The two dogs one each with fracture of humerus in group I and II were subjected to be open reduction and internal fixation with intramedullary pinning coupled with cerclage wiring .

The open reduction and internal fixation of fractured humerus with intramedullary pinning in addition with cerclage wiring in both the groups were found satisfactory; however, the perfect alignment and reduction was not observed radiographically in both the groups. The reduction in healing time of humerus fracture was observed in group II as compared to group I.

The process of fracture healing was assessed on the basis of clinical, radiological, hematological and biochemical observations recorded at scheduled interval.

The wound healing in dogs of group I and group II was observed for about 7 to 12 days by primary intention.

The animals of group II with long bone fracture required less time for partial weight bearing  $3.25 \pm 0.25$  postoperative days and complete weight bearing  $25.75 \pm 2.17$  postoperative days as compared to group I and reduction in weight bearing was statistically significant.

Radiographs taken at schedule intervals showed accelerated fracture healing with complete bridging of fragment, bony deposition along with periosteal and medullary continuity across the fracture site on animals of treated group II whereas radiographic union and periosteal bridging at fracture site were relatively slow and incomplete in dogs of group I at schedule interval.

The animals of group I and group II revealed non-significant changes of hematological values such as hemoglobin, total erythrocyte count, total leucocyte count, differential leucocyte count which does not affect the bone healing process. The serum alkaline phosphatase level revealed decreasing trend in both the group I and II which was statistically significant.

The enhancement of rate of fracture healing and reduction in partial and complete weight bearing in animals of group II could be understood to the therapeutic efficacy of symphytum officinale homoeopathy drug of 30 c potency @ four globules twice a day as an anti-inflammatory, analgesic and specific osteoinductive property along with the proper open reduction and internal fixation of fractured bone.

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