1. INTRODUCTION

Bone is a multifunctional tissue that provides structure to the body. It acts as a protective cage for visceral organs, acts as a fulcrum for the muscles, a bank of calcium and phosphorous and is the site for formation of blood and immune cells (Caplan, 1991 and Hing, 2004). Fracture is the breach in the continuity of hard tissue. Bone is unique among tissue, in that, it heals by regeneration and not by scar formation as is evident in muscle, skin, heart etc.

Bone healing is a complex physiological process and involves numerous mechanisms at tissue and cellular level. This phenomenon differs from that of soft tissue because of its morphology and composition. Healing of bone is a slow process as a result of which there is a chance of failure of the implant. Whenever an implant is used the surgeon is often worried about the implant migration, since the animals cannot be kept confined at a place for longer duration of time and it becomes difficult to avoid running and jumping.

Management of long bone fracture with implants have certain complications including excessive trauma to soft tissue, migration ,bending, loss of bone implant contact and delayed healing due to reduced blood supply. Orthopaedician often implies various methods so as to limit the incidence of implant failure, in this regard they often think of any mechanism that can increase the bone- implant contact often referred as BIC. A thorough review of the literature reveals that the low level laser therapy increases the bone –implant-contact (Pereira et al., 2009).

Internal immobilization of long bones along with the use of low level laser therapy may lead to healing of bone fracture without any complication and may prove a milestone for establishment of an effective technique for fracture management. The principle of using low level laser therapy (LLLT) is to supply direct biostimulative light energy to body cells. Absorbed laser energy causes stimulation of molecules and atoms of cells. It increases the metabolic rate of cell and helps in fast fibroblast and osteoblast formation (Khadra et al., 2004). On the other hand, it has biochemical stimulation effects on cells, which creates multiple biological changes. These types of radiation affect the photoreceptor of cells and by stimulating the electron transport chain, modulate the cellular action (Mester et al., 1985).
With respect to the bone, LLLT has shown to modulate inflammation, accelerate cell proliferation and enhance healing (Khadra et al., 2005). Several studies have demonstrated that LLLT has stimulating effects on stem cells of the bone and accelerates the repair process of the bone (Garavello-Freitas et al., 2003). It also causes release of growth factors (Peplow et al., 2011) and also enhances the production of endorphins which are natural pain killers (Hussain et al., 2011).

Effect of laser on bone healing along with implant is scarcely reported owing to the assumption that the laser can cause a heating effect on implant and thus lead to tissue charring. However, going through the mechanism of action of laser therapy it was found that low level laser therapy have photochemical rather than thermal effect because low irradiation level are used which does not cause appreciable rise in temperature (Kitchen and Partridge, 1991).

The effect of low level laser therapy on bone healing is still an untouched field. Moreover, it is often reported that low level laser therapy acts as a catalyst for transformation of none differentiated mesenchymal cells into osteoblast which further changes rapidly to osteocytes (Pinheiro et al., 1997).

Takeda (1988) on histopathological examination observed that proliferation of fibroblasts and formation of trabecular osteoid tissue was more prominent in the laser irradiated group of rats.

Since, fracture of long bones is most commonly observed in dogs and even after doing internal immobilization, it has lots of post-operative complications. Therefore, looking in the importance of laser therapy in the healing of fracture in laboratory animals and its clinical application. The present study was undertaken with the following objective:

**Objectives**

1. To study the efficacy of low level laser therapy on fracture healing of long bones in dogs.

2. Comparative evaluation of laser therapy on the basis of clinical, radiographic and haemato-biochemical attributes.
2. REVIEW OF LITERATURE

2.1 Intramedullary pinning

Dixon et al., (1994) observed the effects of three different techniques of intramedullary (IM) pin placement on pin location and incidence of stifle joint injury were evaluated using 70 cadaver canine tibiae after mid diaphyseal osteotomy and concluded that non-directed retrograde pinning cannot be recommended for fracture treatment but retrograde pins directed craniomedially may be accepted for the repair of proximal to mid-diaphyseal tibial fractures, if care is taken to properly seat the pins.

Hulse et al., (1997) carried out in-vitro investigation on reduction in plate stress by addition of an intramedullary pin. The combination of a bone plate and intramedullary pin was superior in reducing plate stress when compared with the plate alone and functioned as two beams acting in concert.

Nath et al., (2001) treated a three month old tiger cub having mid-shaft femoral fracture by intramedullary pinning. They reported partial weight bearing of the limb from 21 days post operatively.

Saravanan et al., (2002) used intramedullary pin, circlage wire and neutralization bone plate for the management of comminuted femoral fracture in dogs and observed various clinical and post-operative complications.

Agnihotri (2007) evaluated fracture healing by using intramedullary pin along with indigenous plant for long bone fracture in dogs and found various clinical and haemato biochemical fluctuation.


2.2 Low Level Laser Therapy (LLLT)

Takeda et al., (1988) evaluated the effect of low energy laser on extraction socket healing and observed fibroblast formation of trabecular osteoids more prominent after laser treatment in rats.
Honmura et al., (1992) studied the therapeutic effect of laser irradiation on experimentally induced inflammation in rats and concluded that it reduces inflammation and pain.

Pinheiro and Frame (1992) studied the effect of LLLT and concluded that non-differentiated mesenchymal cells could be biomodulated positively to osteoblasts that would more rapidly change to osteocytes.

Luger et al., (1998) evaluated the effect of LLLT in tibial fracture healing in rats. They removed the tibia after irradiation of the fracture for four weeks and tested for its tension failure and found that laser irradiated tibial stiffness was significantly increased.

Ozawa et al., (1998) studied the stimulatory effect of low power laser irradiation on bone formation in vitro and reported that LLLT increases cellular proliferation, bone nodule formation and alkaline phosphatase activity.

Guzzardella et al., (2002) evaluated the effect of laser on bone defect healing in femur of rat and found that alkaline phosphatase, total protein and nitric oxide was significantly higher in the laser group. He further concluded that low level laser therapy stimulation could accelerate bone healing.

Khadra et al., (2004) studied the effect of low level laser therapy on bone implant interaction and concluded that LLLT enhances the functional attachment of implant to bone and promotes bone healing and mineralization.

Khadra et al., (2005) observed growth and differentiation of osteoblasts, the bone forming cells around the implant. They concluded that biostimulation by LLLT may enhance the osteogenic potential of these cells.

Weber et al., (2006) used LLLT in conjunction with autogenous bone graft and barrier membrane for the treatment of intra bony defects. It was found that quantity and quality of bone remodeling was more evident in irradiated animals than non-irradiated animals.

Ribeiro and Matsumoto (2008) evaluated the effect of LLLT on bone repair on rats treated with anti inflammatory drugs and concluded that laser therapy improves bone repair because of an up regulation of sacculo-oxygenase to expression in bone cells.
Stein et al. (2008) evaluated the effect of low level laser therapy on growth and differentiation on human osteoblast like cells and concluded that laser has a biostimulatory effect on osteoblast cells during the first 72 hr of irradiation.

Bouvet-Gerbettaz et al. (2009) evaluated the effect of LLLT on proliferation and differentiation of murine bone marrow cell. They administered laser thrice a week on murine bone marrow cells containing osteoblast progenitors and concluded that laser does not alter murine bone cell proliferation and differentiation.

Petri et al., (2010) investigated the effect of LLLT on human osteoblastic cell grown on Titanium and revealed that LLLT affected cell responses in a complex way. The author further suggests that LLLT might have possible benefit on implant osteointegration despite a transient effect immediately after laser irradiation.

Nazrul et al. (2011) studied the effect of laser healing in human and reported that laser therapy showed better stable fracture and an earlier alleviation of pain and inflammation.

2.3 History

Aithal et al., (1999) surveyed on 403 dogs and reported that the incidence of fracture was highest in dogs of younger age (54%) less than one year and the occurrence was highest in non-descript indigenous breeds, they further observed that the most commonly fractured bone is femur (38.56%), followed by tibia -fibula (17.16%), humerus (7.71%) and males (63%) are more prone for fracture then females (37%).

Singh et al., (1999) studied the incidence of fracture in canines and found that the fracture were more in hind limb(60%) then forelimb (40%), with higher incidence in femur (36.6%) followed by tibia (23.3%).

Gahlod, B.M. (2007) evaluated femoral fracture repair in canine with special reference to use of different implants and anabolic hormones and observed that the incidence of fracture was higher in pomererian (33.32%), followed by german shepherd (25%) and non-descript breed of dogs (25%). He further suggested that the incidence of fracture was higher in pups below 6 months (33.32%) of age followed by 6-12 months(25%) of age with higher incidence in males (58.33%) as compared to females (41.66%).
Simon et al., (2011) conducted a survey on 331 cases of pectoral limb fractures in dogs and found that the incidence was highest in young animals over one and half month to 6 months (36.25%) with majority of fractures were in non-descript breeds of dogs (37.76%).

Hansda et al., (2012) compared steinmann pin, kuntscher nail and interlocking nail for femoral fracture repair in dogs and reported that the prevalence of femur fracture was highest in non-descript dogs (50%), and it was higher in younger dogs of 2-4 months of age (55.55%).

Ben Ali (2013) studied the occurrence and pattern of fracture in small animals (dogs and cats) from year 2005 to 2010 and reported that the automobile accident was the major cause of fracture (36.36%) followed by indoor trauma (25%).

Rajhans (2013) studied stabilization of splinters of long bone fracture in dogs and reported that Male dogs (83.3%) are more prone for fracture and automobile accident (58.3%) was most common etiology followed by fall from height (41.6%).

Jani et al., (2014) in a retrospective study of five years, on occurrence of fracture in dogs, reported that the non-descript breed (32.97%) and young dogs of 3-6 months (27.47%0) were most commonly affected and the occurrence of fracture was higher in male dogs (75.83%) then female (24.17%), with higher incidence of road traffic accident (35.16%) followed by fall from height (3.77%).

Sran et al., (2016) conducted a retrospective study on frequency of long bone fracture in dogs and concluded that the dogs of younger age, 6-12 month showed the highest occurrence (21.89%) and femoral fracture was (44.48%) most common followed by tibia/fibula (21.19%) and humerus (11.21%). He further observed that automobile accident was recorded as the major cause of fracture (56.39%) followed by fall from height (26.97%).

2.4 Radiographic evaluation

Herrmann et al., (2002) suggested that early knowledge about the individual prognosis of a fracture could help to prevent severe complications (e.g., pseudoarthrosis) and enable the physician to modify therapy. Plain radiography remains the standard method to monitor fracture healing but it documents delay healing only late in the course.
Agarwal (2007) performed ILN in fracture of long bones in dogs and reported that the radiograph of all dogs taken on 45th post operative day showed bridging of callus between intercortical and periosteal adjoining area and adequate embedding of periosteal callus over the cerclage wire. Whereas, radiograph of 60th day in all the cases revealed bridging of callus at fractured gap, callus deposition between cortical and intercortical at places and sign of cortical continuity. Implant failure and implant related complications was observed as nil except in one case.

Gahlod (2007) studied fracture healing of femur in canine with special reference to use of different implants and anabolic hormone and observed reabsorption of excessive callus, reduced periosteal reaction and remodeling of bone in a radiograph at 40th day.

George et al., (2007) carried out study on 12 dogs divided equally in to two groups, the study was conducted to evaluate the clinical healing of fractures of femur immobilized with intramedullary pinning along with stainless steel in group I and intramedullary pinning with acrylic external skeletal fixator in group II. He reported that the dogs of both the groups showed progressive improvement in functional limb usage but the healing was excellent with minimal complications in group II.

Singh et al., (2007) conducted a study on bilateral femur fracture with static intramedullary interlocking nailing in three dogs and reported that the left femur of dog 1 showed sign of delayed healing, whereas the right femur showed sign of callus formation and intact implant in the follow up radiographs. In dog 2, signs of periosteal reaction were evident by 20th post operative radiograph and the sign of bridging callus were evident in both limbs in three month post operative radiograph. full weight bearing was evident by the end of 4th month due to establishment of cortical and medullary cavity. In dog 3, little weight bearing was observed and periosteal reaction was evident at the fractured site on 20th post operative radiograph.

Hayashi et al., (2008) used a radiographic scoring system for assessment of fracture healing, saopaulo- 2007 in which a score from (0 to 6) was allotted to specific radiographic sign.
De’ Souza (2012) observed periosteal callus formation, a decrease in fracture gap and variable loss of opacity of the fracture margin in most of the dogs which were treated for a fracture using a cross pinning or a LCP. Bridging callus formation was observed on 45\textsuperscript{th} post operative day in both the groups.

Hansda et al., (2012) conducted a study on comparative evaluation of steinmann pin, kuntscher nail and interlocking nail for femur fracture repair in 18 dogs and the radiographic interpretation on 20\textsuperscript{th} day revealed, moderate quantity of callus in dogs subjected with steinmann intramedullary pin.

Rathod et al., (2014) conducted a study on 6 dogs presented for femoral fracture and found that the radiographic interpretation on 60\textsuperscript{th} days showed excellent healing with minimum endosteal and periosteal callus at the fractured site.

### 2.5 Clinical examination

Agnihotri (2007) recorded the temperature, pulse and respiration rate after the use of indigenous plants along with intramedullary pinning for long bone fracture in dogs. She further reported that there was an increase in temperature on the 1\textsuperscript{st} post operative day, followed by its decrease. However, no significant variation was observed in respiration and pulse rate.

De’ Souza (2012) performed internal fixation of distal third fracture of long bone in dogs and observed an initial increase followed by decrease in temperature, pulse, and respiration in the post operative period.

Rajhans (2013) studied stabilization of splinters of long bone fracture in dogs and found that rectal temperature, pulse rate, and respiratory rate fluctuated within normal range and did not showed any significant changes at different time intervals.

Gupta (2015) carried out fracture healing in dogs using dynamic compression plating and reported that the values of temperature, pulse and respiration was fluctuating within the normal range.

Singh (2015) performed techniques of fracture healing in dogs using composite mesh guided tissue regeneration in dogs and reported that
there was an increase in temperature preoperatively due to inflammatory mediators i.e. prostaglandins, interleukins and bradykinins. He also observed decrease in the temperature as well as inflammation post operatively.

2.6 Exudation and inflammation

De’ Souza (2012) observed complete absence of exudation at fracture site on 30\(^{th}\) post operative day in the distal fracture of long bones in dogs immobilized by internal fixation.

Gupta (2015) reported inflammation at fractured site till 3\(^{rd}\) post operative day in the goats, immobilized by dynamic compression plates with hydroxyl appetite bone substitute, implanted at fractured site.

Singh (2015) reported reduction of inflammation on 7\(^{th}\) post operative day after immobilization with DCP along with β-tricalcium phosphate in long bone fracture of dogs.

2.7 Weight bearing and limb function

Raghunath and Singh (2003) treated 4 clinical cases of comminuted segmental fractures of dog femur with static ILN and found that all dogs were able to touch the paw on ground within 5 days and obtained complete good limb function in less than 3 weeks.

Aithal et al., (2004) studied modified technique of single pin fixation and cross intramedullary pin fixation technique for supracondylar femoral fracture in dogs and stated that weight bearing in immediate postoperative period was better in animals treated with cross pinning than single pin fixator.

Ganesh et al., (2004) described the repair of transverse mid shaft fracture of right femur in dogs using dynamic compression plates and stated that dog started bearing partial weight on the operated limb by 3\(^{rd}\) postoperative day and full weight bearing by the end of 2\(^{nd}\) week.

Gupta et al., (2008) treated clinical cases of humerus and tibial shaft fracture in 8 dogs by ILN with the help of C-arm and found good fracture healing and weight bearing in 7 dogs.

Kushwaha et al., (2011) reported full weight bearing at 18\(^{th}\) day on affected limb after internal fixation of radius – ulna in a non descript dog.
Rathod et al., (2014) conducted a study on 6 dogs presented for femoral fracture, the fracture was repaired by intramedullary pinning and in addition concentrated bone marrow stem cells were deposited at the fractured site. Among six animals, five animals showed normal weight bearing on third post operative day and made a complete functional recovery by 15th post operative days.

Singh (2015) reported that weight bearing score was higher at 30th, 60th and 90th post operative day in dogs of T3 group (internal fixation with β-TCP and platelet rich plasma) as compared to the T2 group (internal fixation with β-TCP alone) and T1 group (internal fixation alone).

2.8 Haemato-biochemical changes

Aithal et al., (1998) studied modified technique of single pin fixation and cross intramedullary pin fixation technique for supracondylar femoral fracture in dogs and reported that total leukocyte count markedly increased and remained significantly high up to 15th post operative day in dogs.

Zama et al., (1999) used post-operative acupuncture therapy in femoral fracture repair in dogs and reported that neutrophil count increased significantly along with the corresponding decrease in the lymphocyte count and a non significant variation in monocyte and eosinophil count.

Rao et al., (2001) observed non significant changes in serum calcium, phosphorous and alkaline phosphatase following ulnar segmental defect repair in canines.

Komnenou et al., (2005) concluded that serial determination of serum ALP activity during fracture healing of long bones in dogs, could be an additional tool in predicting fractures at risk of developing a non-union, helping the clinician to choose the appropriate intervention.

Agnihotri (2007) used certain indigenous plants for fracture healing in dogs and reported that total leucocyte count increased significantly with a maximum value on 10th post operative day and attributed it to the inflammatory response to surgical trauma and stress of fracture and surgery.
Gahlod (2007) studied healing of femur fractures in canine and reported non-significant changes in Hb, TEC, TLC and DLC. The serum alkaline phosphatase activity was increased in all groups from 0 to 20th days indicating osteoblastic activity at the fracture site.

Hegade et al., (2007) compared the fracture healing in dogs and recorded significantly higher level of alkaline phosphatase on operative day than on post operative days in all the groups of animals.

Tembhurne et al., (2010) reported lymphocytopenia on 10th post operative days after horn peg fixation of a femoral fracture in canines.

During assessment of bone fracture healing in dogs, Sousa et al., (2011) observed significant correlation between total alkaline phosphatase (t-ALP) and bone specific isoforms of alkaline phosphatase (BALP) serum activities and Ca and Mg in the fracture healing group and between tartrate-resistant acid phosphatase (TRAP) and Ca, TRAP and Mg and Ca and Mg in the non union group.

Rajhans (2013) studied the stabilization of splinter in long bone fracture in dogs and reported non-significant changes in various haematological parameters i.e. TEC, TLC, PCV, DLC, HB% and PCV and these parameters remains within normal range, whereas the neutrophils were recorded within upper range of normal values. The value of serum calcium and serum phosphorous remains significantly high on 0 day interval as compared to 45th day interval, however serum alkaline phosphatase showed significant decrease on 7th, 28th and 45th post operative day respectively.

Singh (2015) evaluated haemato-biochemical parameters during fracture repair in dogs and reported that the value of serum alkaline phosphatase shows a decrease at 30th day and thereafter, an increase was noticed at 60th day onwards the elevation in values of serum alkaline phosphatase was marked by 90th day in all the groups. However, no significant difference was observed in values within or among the groups at different time intervals. Whereas, the value of serum calcium increased marginally at different time intervals in all the three groups. However, this increase was none significant.
2.9 Complications

The immediate complication of fracture healing are due to soft tissue damage while later complication are delayed union, non-union and mal union (Hickman, 1964).

Hunt et al., (1980) reported 24 percent complication in the fracture of femur. They reported that osteomyelitis is one of the most serious complication. Other complication of internal fixation are caused by errors of technique, such as choice of inappropriate method of fixation, the use of an implant of inadequate size and strength to stabilize the fracture, errors in application of the implant and excessive damage to the tissues during surgery.

Saravanan et al., (2002) recorded different complications associated with comminuted diaphysial femoral fracture in dogs with three internal fixation technique and found that two dogs with intramedullary pinning and cerclage wiring showed signs of fixation failure due to proximal pin migration and osteomyelitis whereas bending of the bone plate and screw loosening was evident in one dog.

Gupta et al., (2008) used the interlocking nailing for repair of humerus shaft fracture using image intensifier in four dogs and observed radial nerve neuropraxia in the form of dropped elbow in one out of four animals.

Hansda et al., (2012) conducted a study on comparative evaluation of steinmann pin, kuntscher nail and interlocking nail for femur fracture repair in 18 dogs and observed complications of seroma formation, and pin migration in group I whereas rotational malposition was observed in group II.
3. MATERIAL AND METHODS

3.1 Location and place of work

The study was conducted in the Department of Veterinary Surgery and Radiology, Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur.

3.2 Meteorological data and features of the place

Jabalpur is situated at 23.17° latitude and 79.57° longitude at 410.87 MSL in southern part of the second agro climatic zone, including Satpura Plateau and Kymore hills. It has a tropical climate having average rainfall of 1241 mm.

3.3 Duration of work

The study was carried out for a period of nine months from June, 2016 to March, 2017.

3.4 Animal

The clinical efficacy of low level laser therapy was evaluated in fracture healing on 18 dogs having long bone fractures, brought to TVCC, College of Veterinary Science and Animal Husbandry, Jabalpur, during the study period. Apparently healthy dogs aged between 1 to 8 years, having long bone fracture were selected in the present study irrespective of their sex, breed and body weight. These dogs were divided randomly into 3 groups, each consisting of 6 animals having long bone fracture i.e. humerus, femur and tibia-fibula.

3.5 Experimental design

The animals included in the present study were randomly divided into three groups consisting of 6 animals each.

Group I: (Control group; n=6)

This group was treated as control group. After surgical repair of fracture by intra- medullary pinning, post operative dressing of the wound was done by using povidine iodine, followed by local application of neomycin
sulphate, zinc bacitracin containing ointment and pulv containing polymixin-b sulphate, neomycin sulphate, and zinc bacitracin till complete healing of wound. Antibiotic amoxycillin sulbactum 10mg/kg b.wt I/M was started 24 hour prior to surgery and was continued upto 7th post operative day twice daily and injection meloxicam @ 0.2 mg/kg b.wt I/M once daily for 3 days.

**Group II: (single side laser irradiated group; n=6)**

Intramedullary pinning, dressing of wound, antibiotics and analgesic was done as in group I, in addition to this all the animals was treated by low level laser therapy (LLLT) for 6 minutes @ 10 Hz and 2.4 J energy, for continuous 7 days, post operatively on one side of the fractured bone (Plate 01).

**Group III: (both side laser irradiated group; n=6)**

Intramedullary pinning, dressing of wound, antibiotics and analgesic was done as in group I, in addition to this low level laser therapy (LLLT) for 3 minutes each on both the side of the fractured bone with 10 Hz , 2.4 J energy, for continuous 7 days post operatively (Plate 02).

**Treatment design**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>No. of Animals (n)</th>
<th>Treatment protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>n=6</td>
<td>Internal fixation by I/M pinning alone.</td>
</tr>
<tr>
<td>Group 2</td>
<td>n=6</td>
<td>Internal fixation by I/M pinning and low level laser therapy for 6 minutes @ 10 Hz and 2.4 joules on one side of fractured bone for 7 days post operatively.</td>
</tr>
<tr>
<td>Group 3</td>
<td>n=6</td>
<td>Internal fixation by I/M pinning and low level laser therapy for 3 minutes each on both the side of the fractured bone @ 10 Hz and 2.4 joules for 7 days post operatively.</td>
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3.6 Instrumentation

EC LASER 306\(^1\) machine having laser tube of He-Ne + infrared with a wavelength of 632.8nm, power output of 10mW and laser diode of 830nm accompanied with LCD computerized screen control for various frequency and time adjustments with automatic electronic treatment timer, was used for undertaking the laser treatment (Plate 03).

Frequency and amplitude on x-axis and y-axis were selected as per the type of bone involved. Duration of treatment was calculated as per Mester’s protocol (Rogers and James, 1987). The energy (Joules) was calculated using following formula.

\[
\text{Energy (Joules)} = \text{Power (Watt)} \times \text{Time (Seconds)}
\]

Pulsed continuous scanning laser application mode was used and the treatment was carried out in a semi dark room. The laser beam was kept perpendicular to the fracture fragments and distance from table to laser tube was kept at 80 cm. Protective goggles\(^2\) were used during the entire treatment course by both practitioner and the restrainer.

Orthopaedic instruments required during the procedure of intramedullary pinning were orthopaedic instrument box, steinmannn pins (both threaded and none threaded), orthopaedic scale, bone holding forcep, pinchuck with key, periosteal elevator, bone cutter, bone nibbler, muscle retractor, circlage wires, wire passer, pin cutter and pin rasper (Plate 04). The entire laser treatment was carried at the physiotherapy section and operation was carried out in the operation theater of TVCC, Jabalpur.

3.7 Surgical procedure and intramedullary pinning technique

3.7.1 Preparation of animals:

The animals were kept off feed for 12 hours and off water for 6 hour prior to surgery. On the day of surgery the affected limb was shaved, depending upon the site of fracture and bone involved, povidine iodine was applied 3-4 times at the prepared site.

\(^1\) EC LASER- 306 Technomed Electronics Ltd., Perungudi, Chennai.
3. Toshiba c-arm –Toshiba electron tube and devices co.Ltd., Goa, India.
3.7.2 Anaesthetic protocol:

The animal was premedicated with atropine sulphate @ 0.04mg/kg bwt. I/M and Xylazine Hydrochloride @ 1.5 mg/kg bwt. I/M followed 10 minutes later by Ketamine Hydrochloride @ 6.0 mg/kg bwt I/M, as general anaesthetic. Maintenance of the anaesthesia was done by Ketamine Hydrochloride I/V as per the requirement.

3.7.3 Anatomical approach for various long bones.

a. Humerus

Animal was kept in lateral recumbency and an incision was made on anterio-lateral aspect of humerus over an imaginary line drawn from the greater tubercle of humerus proximally to the point of elbow distally. Just beneath the skin, fascia was incised to reach the muscle bellies. The bellies of biceps brachia cranially and lateral head of triceps brachii caudally were separated bluntly to expose the fracture site of humerus. Soft tissue from the lateral aspect of the fractured bone were removed by periosteal elevator taking care to avoid any injury to the radial nerve. Suitable size of Steinman pin were selected by measuring the pin on the radiograph of fractured bone and the pin was inserted first into the proximal segment and then the fractured segments were lifted and brought in apposition by using traction and counter traction, once the fractured segment were reduced the steinmen pin was inserted into the distal segment of the bone, up to the lower epiphysis of the fractured bone to immobilize and to bring in alignment the fractured bone segments.

b. Femur

The animal was kept in lateral recumbency. An incision was made on the anterio-lateral aspect of the thigh on an imaginary line extending from the greater trochanter to the stifle joint. Skin and fascia were incised to expose the bellies of vastas lateralis, biceps femoris and semi membranous muscles, bellies of vastus lateralis were reflected cranially and that of biceps femoris and semimembranous muscle caudally to expose the fractured bone. Soft tissue from the lateral aspect of the fractured bone were removed by
periosteal elevator. Suitable size of Steinmann pin were selected by measuring the pin on the radiograph of fractured bone and the pin was inserted first into the proximal segment in retrograde direction and then the fractured segments were lifted and brought in apposition by using traction and counter traction, once the fractured segment were reduced the steinmann pin was inserted into the distal segment of the bone in normograde direction, upto the lower epiphysis of the fractured bone to immobilize and to bring in alignment the fractured bone segments.

c. **Tibia-fibula**

   This bone was approached through medial aspect. An incision was made on the medial aspect on an imaginary line extending from stifle proximally to hock distally. After incising the skin and fascia, muscles were exposed. The bellies of tibialis anterior and flexor digitorum plofundis muscles were retracted cranially and caudally respectively to expose the fracture site. Soft tissue was removed using periosteal elevator on the lateral and medial side of the long bone. The pin was inserted first into the proximal segment and then the fractured segments were lifted and brought in apposition by using traction and counter traction, once the fractured segment were reduced the steinmann pin was inserted into the distal segment of the bone, upto the lower epiphysis of the fractured bone to immobilize and to bring in alignment the fractured bone segments.

### 3.7.4 Technique for intramedullary pinning

On the basis of radiograph suitable size of intramedullary pins were selected. After exposure of fractured site by giving skin incision and separating the muscle bellies using muscle retractor, any soft tissue around the fracture site were removed using periosteal elevator (Plate 06). The intramedullary pin was tightened with the pin chuck using a key, then the pin was inserted in the proximal fracture fragment in retrograde direction with a gradual rotational motion till it pierces the skin and comes out (Plate 07). Thereafter, the chuck was removed from the pin and were tightened on the counter end of the pin using the key, the pin was pulled out from the skin surface in a clockwise and anticlockwise motion till it fully enters inside the
marrow cavity of the proximal fracture fragment. Now the fractured fragments were brought in apposition and held in place by using a bone holding forcep (Plate 08). Thereafter, the pin was further inserted into the distal fracture fragment up to the epiphysis of distal segment in a gradual rotatory motion, now in normograde direction to immobilize and to bring in alignment the fractured bone (Plate 09). Muscle bellies were kept apart from fracture site throughout the surgical procedures using muscle retracting forceps for an uninterrupted procedure (Plate 10). During the procedure the c-arm image intensifier\(^3\) was used to obtain any information regarding the position of pin inside the marrow cavity of fractured bone (Plate 11). The surgical site was closed using standard 3-layer closing technique. Muscle and subcutaneous tissue was closed using chromic catgut no. 1 (Plate 12), followed by suturing of skin incision by using black braided silk no. 1 in interrupted sutures pattern.

3.7.5 Pre and Post operative care

In all the three groups antibiotic amoxycillin sulbactum 10mg/kg b.wt I/M was started 24 hour prior to surgery and was continued up to 7\(^{th}\) post operative day twice daily and injection meloxicam @ 0.2 mg/kg b.wt I/M once daily for 3 days. Post operative dressing of the wound was done by using 5% povidine iodine solution, followed by local application of neomycin sulphate, zinc bacitracin containing ointment and pulv containing polymixin b sulphate, neomycin sulphate, and zinc bacitracin till complete healing of wound. The affected limb was bandaged using Robert- Jones technique immediately after surgery to restrict the movement of animals. The laser irradiated group was given laser therapy for continuous 7 days post operatively. Skin sutures were removed on 12\(^{th}\) post operative day.

3.8 Parameters of study

3.8.1 History

Complete history of the case including age, sex, breed, type of long bone involved, duration of fracture and probable etiological factor was recorded in Teaching Veterinary Clinical Complex (T.V.C.C.), College of Veterinary Science and A.H., Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur from June, 2016 to March, 2017.
3.8.2 Clinical parameters

Temperature (°F), pulse (per minute) and respiration rate (per minute) was recorded prior to surgery (0 hrs) and post operatively at 30 minutes, 24 hours, and 48 hours after the surgery.

3.8.3 Inflammation

Gross appearance of suture line, degree of inflammation (swelling, pain exudation and embedding of sutures) was recorded on 2, 4, 6, 8, 10 and 12 days, post operatively and grading was done as per modified method of Nema (2014).

<table>
<thead>
<tr>
<th>Swelling/pain/exudation/embedding of sutures</th>
<th>Grade</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>++</td>
<td>2</td>
</tr>
<tr>
<td>Marked</td>
<td>+++</td>
<td>3</td>
</tr>
</tbody>
</table>

3.8.4 Weight bearing

Assessment of weight bearing was done on 2, 4, 6, 8, 10 and 12 days post operatively as per the method described by Aithal (1996).

<table>
<thead>
<tr>
<th>Standing</th>
<th>Score</th>
<th>Walking</th>
<th>Score</th>
<th>Running</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrying the limb away from ground</td>
<td>0</td>
<td>Carrying the limb away from ground</td>
<td>0</td>
<td>Carrying the limb away from ground</td>
<td>0</td>
</tr>
<tr>
<td>Touching the toe on the ground</td>
<td>1</td>
<td>Occasional touching the toe on the ground</td>
<td>1</td>
<td>Occasional touching the toe on the ground</td>
<td>1</td>
</tr>
<tr>
<td>Touching the paw on the ground</td>
<td>2</td>
<td>Frequent touching the paw on the ground</td>
<td>2</td>
<td>Frequent touching the paw on the ground</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Touching the paw on the ground on every step</td>
<td>3</td>
<td>Touching the paw on the ground on every step</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Touching the whole paw on the ground on every step</td>
<td>4</td>
<td>Touching the whole paw on the ground on every step</td>
<td>4</td>
</tr>
</tbody>
</table>
3.8.5 Radiographic examinations

Radiograph was taken prior to surgery (0 day) and subsequently on 12, 24, 48, and 72 days post operatively. Radiograph was evaluated as per modified method of Hammer *et al.*, (1985).

<table>
<thead>
<tr>
<th>Callus formation</th>
<th>Fracture line</th>
<th>Stage of union</th>
<th>Grade/ score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No callus</td>
<td>Distinct</td>
<td>Not achieved</td>
<td>0</td>
</tr>
<tr>
<td>Trace: no bridging of fracture line</td>
<td>Distinct</td>
<td>Not achieved</td>
<td>1</td>
</tr>
<tr>
<td>Apparent: bridging of fracture line</td>
<td>Discernible</td>
<td>Uncertain</td>
<td>2</td>
</tr>
<tr>
<td>Massive: bone trabeculae crossing fracture line</td>
<td>Barely discernible</td>
<td>Achieved</td>
<td>3</td>
</tr>
<tr>
<td>Homogenous bone structure</td>
<td>Obliterated</td>
<td>Achieved</td>
<td>4</td>
</tr>
</tbody>
</table>

3.8.6 Haemato-biochemical Estimation

Approximately 5 ml of blood was collected aseptically from the cephalic vein in EDTA containing vaccutainer and without anticoagulant vial.

3.8.6.a Haematological parameters: It include

1) Total erythrocyte count (X10\(^6/\mu l\))
2) Packed cell volume (%)
3) Total leukocyte count (X10\(^3/\mu l\))
4) Differential leucocyte count (%)
5) Haemoglobin (g/dl)
All these parameters were estimated by automatic blood analyser in disease diagnostic laboratory of Teaching Veterinary Clinical Complex, pre operatively (0 hrs.) and subsequently on 6th and 12th post operative days.

3.8.6.b Biochemical parameters:

1) Alkaline phosphate (IU/dl)
2) Total serum protein (g/dl)
3) Serum calcium (mg/dl)
4) Serum phosphorous (mg/dl)

Estimation was done by using blood chemical analyser using their respective commercially available kits. The readings were taken on 0, 12, 24, 48 and 72 days post operatively.

3.8.7 STATISTICAL ANALYSIS:

The quantitative data like rectal temperature, pulse rate, respiration rate, haematological and biochemical parameters were analysed by using hierarchical design as outlined by Snedecor and Cochran (1994), whereas the qualitative data was analyzed by arbitrary score card method (visual analog score).
4. RESULTS

The present study was conducted on eighteen clinical cases of long bone fractures in dogs, which were randomly divided into three equal groups, consisting of 6 animals in each Group. In group I (control group) intramedullary pinning was done alone followed by antiseptic dressing. In group II and III intramedullary pinning along with low level laser therapy and antiseptic dressing was done for seven days. In group II laser therapy was done on one side of fractured bone, whereas, in group III, laser therapy was done on both sides of fractured bone. The results of the study are as follows.

4.1 Anamnesis/ History

4.1.1 Age

In the present study, out of eighteen clinical cases of long bone fractures, eight cases (44.44%) were between 1 to 3 years of age, six cases (33.33%) between 3 to 6 years of age and four cases (22.22%) between 6 to 8 years of age.

Table 01: Age-wise distribution of animals under study

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of animals</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 year</td>
<td>8</td>
<td>44.44</td>
</tr>
<tr>
<td>3-6 year</td>
<td>6</td>
<td>33.33</td>
</tr>
<tr>
<td>6-8 year</td>
<td>4</td>
<td>22.22</td>
</tr>
</tbody>
</table>

4.1.2 Sex

Out of eighteen clinical cases of long bone fractures included in the study, the number of male dogs were 10 (55.55%), while number of female dogs were only 8 (44.44%).

Table 02: Sex-wise distribution of animals under study

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of animals</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10</td>
<td>55.55</td>
</tr>
<tr>
<td>Female</td>
<td>08</td>
<td>44.44</td>
</tr>
</tbody>
</table>
4.1.3 Etiology

The etiology of fracture was fairly variable in all the eighteen cases included in the study. Automobile accident was most common (50%) cause of fracture, followed by dog bite (22.22%), falling from roof (16.66%) and hitting by stick (11.11%).

Table 03: Etiology of fracture in all the three groups

<table>
<thead>
<tr>
<th>Etiology</th>
<th>No. of animals</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automobile accident</td>
<td>9</td>
<td>50.00</td>
</tr>
<tr>
<td>Dog bite</td>
<td>4</td>
<td>22.22</td>
</tr>
<tr>
<td>Falling</td>
<td>3</td>
<td>16.66</td>
</tr>
<tr>
<td>Hitting</td>
<td>2</td>
<td>11.11</td>
</tr>
</tbody>
</table>

4.1.4 Bone involved

In the present study, it was observed that the most common bone involved in fracture was the femur (55.55%), followed by tibia (38.88%) and humerus (5.55%).

Table 04: Bone involved in all the three groups

<table>
<thead>
<tr>
<th>Bone</th>
<th>No. of animals</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
<td>10</td>
<td>55.55</td>
</tr>
<tr>
<td>Tibia</td>
<td>07</td>
<td>38.88</td>
</tr>
<tr>
<td>Humerus</td>
<td>01</td>
<td>05.55</td>
</tr>
</tbody>
</table>

4.1.5 Breed

Out of eighteen cases of long bone fractures fourteen dogs were of non-descript (77.77%), whereas three dogs were Labrador retriever (16.66%) and only one was of German shepherd (5.55%) breed of dog.
Table 05: Breed-wise distribution of animals under study

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. of animals</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-descript</td>
<td>14</td>
<td>77.77</td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>03</td>
<td>16.66</td>
</tr>
<tr>
<td>German shepherd</td>
<td>01</td>
<td>05.55</td>
</tr>
</tbody>
</table>

4.1.6 Duration of fracture

Among all the eighteen dogs referred for the fracture treatment, 7 dogs were presented immediately after injury (38.88%), 8 dogs (44.44%) 1 to 3 days after injury, whereas 3 cases were presented more than 3 days after injury (16.66%).

Table 06: Duration wise distribution of animals under study

<table>
<thead>
<tr>
<th>Duration</th>
<th>No. of animals</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate after injury</td>
<td>7</td>
<td>38.88</td>
</tr>
<tr>
<td>1 to 3 days after injury</td>
<td>8</td>
<td>44.44</td>
</tr>
<tr>
<td>More than 3 days after injury</td>
<td>3</td>
<td>16.66</td>
</tr>
</tbody>
</table>

4.2 Clinical parameters

4.2.1 Rectal Temperature (°F)

Comparison between different time intervals

Rectal temperature showed significant decrease (p<0.05) at 30 minutes interval in all the three groups of dogs and the 0 hour values of 101.60 ±0.25, 101.70 ±0.28 and 101.93 ±0.40 °F, decreased to a minimum values of 95.50 ±0.59, 95.18 ±0.60 and 93.30 ±1.06 °F in group I, II and III respectively. Thereafter, a gradual increase in rectal temperature was observed and the values fluctuated between normal range of rectal temperature, from 24-48 hours time interval (Table 07).

Comparison between different groups

Comparison between the groups showed non-significant difference between all the three groups of dogs.
### Table 07: Mean values (± SE) of rectal temperature (°F) at different time intervals

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour</td>
<td>101.60&lt;sup&gt;a&lt;/sup&gt;±0.25</td>
<td>101.70&lt;sup&gt;a&lt;/sup&gt;±0.28</td>
<td>101.93&lt;sup&gt;a&lt;/sup&gt;±0.40</td>
</tr>
<tr>
<td>30 minutes</td>
<td>95.50&lt;sup&gt;b&lt;/sup&gt;±0.59</td>
<td>95.18&lt;sup&gt;b&lt;/sup&gt;±0.60</td>
<td>93.30&lt;sup&gt;b&lt;/sup&gt;±1.06</td>
</tr>
<tr>
<td>24 hour</td>
<td>100.80&lt;sup&gt;a&lt;/sup&gt;±0.32</td>
<td>101.33&lt;sup&gt;a&lt;/sup&gt;±0.20</td>
<td>101.30&lt;sup&gt;a&lt;/sup&gt;±0.34</td>
</tr>
<tr>
<td>48 hour</td>
<td>101.70&lt;sup&gt;a&lt;/sup&gt;±0.21</td>
<td>101.70&lt;sup&gt;a&lt;/sup&gt;±0.29</td>
<td>101.70&lt;sup&gt;a&lt;/sup&gt;±0.23</td>
</tr>
</tbody>
</table>

Comparison between the groups: 99.90±0.56  99.98±0.60  99.50±0.80

**Group I (control)**: Intramedullary pinning + Dressing of wound

**Group II**: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III**: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both sides of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
4.2.2  Pulse rate (per minute)

Comparison between different time intervals

The dogs of all the three groups showed, significant decrease (p<0.05) in the pulse rate at 30 minutes interval with a minimum values of 60.00±2.59, 48.50±2.71 and 53.33±5.44 per minutes, as compared to the control values (0 hour) of 127.33±5.42, 110.16 ±7.92 and 112.50±9.30 per minute in group I,II and III respectively, followed by increase and at 24 & 48 hours interval the values were near to 0 hour values in all the three groups of dogs( Table 08).

Comparison between different groups

Comparison between three groups of dogs revealed significantly (p<0.05) low values of pulse rate in group II and III, as compared to group I dogs.

4.2.3  Respiration rate (per minute)

Comparison between different time intervals

The control (0 hour) values of respiration rate 52.00±2.30, 35.00±3.42 per minute, decreased significantly (p<0.05) to 15.00±1.39 and 16.16±1.97 per minute, at 30 minute interval in group I and II respectively, followed by increase at 24 and 48 hour intervals. In group III significant decrease was observed from 0 hour to 48 hour interval, with minimum value of 15.83±1.72 per minute at 30 minute from 0 hour value of 61.66±3.03 per minute (Table 09).

Comparison between different groups

Comparison between different groups revealed non-significant difference between them.
Table 08: Mean values (± SE) of pulse rate (per minute) at different time intervals

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour</td>
<td>127.33^Aa±5.42</td>
<td>110.16^Ba±7.92</td>
<td>112.50^Aa±9.30</td>
</tr>
<tr>
<td>30 minutes</td>
<td>60.00^{AB}±2.59</td>
<td>48.50^{Ab}±2.71</td>
<td>53.33^{Ab}±5.44</td>
</tr>
<tr>
<td>24 hour</td>
<td>116.16^{Aa}±6.26</td>
<td>120.00^{Ba}±6.07</td>
<td>113.33^{Aa}±6.56</td>
</tr>
<tr>
<td>48 hour</td>
<td>128.50^{Aa}±9.19</td>
<td>122.33^{Ba}±6.14</td>
<td>126.66^{Aa}±10.86</td>
</tr>
<tr>
<td>Comparison</td>
<td>108.00^{A}±6.57</td>
<td>100.24^{B}±4.56</td>
<td>101.40^{B}±7.07</td>
</tr>
</tbody>
</table>

**Group I (control)**: Intramedullary pinning + Dressing of wound

**Group II**: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III**: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
- Values having different superscripts AB shows significant difference (p<0.05) between groups
Table 09: Mean values (± SE) of respiration rate (per minute) at different time intervals

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour</td>
<td>52.00(^a)±2.30</td>
<td>35.00(^b)±3.42</td>
<td>61.66(^a)±3.08</td>
</tr>
<tr>
<td>30 minutes</td>
<td>15.00(^b)±1.39</td>
<td>16.16(^c)±1.97</td>
<td>15.83(^c)±1.72</td>
</tr>
<tr>
<td>24 hour</td>
<td>54.33(^a)±2.11</td>
<td>48.50(^a)±4.17</td>
<td>42.83(^b)±3.92</td>
</tr>
<tr>
<td>48 hour</td>
<td>51.73(^a)±2.93</td>
<td>48.50(^a)±3.52</td>
<td>39.33(^b)±1.36</td>
</tr>
<tr>
<td><strong>Comparison between the groups</strong></td>
<td>43.26±3.57</td>
<td>37.04±3.18</td>
<td>39.91±3.63</td>
</tr>
</tbody>
</table>

**Group I (control)**: Intramedullary pinning + Dressing of wound

**Group II**: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III**: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
4.2.4 Degree of inflammation (exudation, pain and embedding of suture materials)

Comparison between different time intervals

Significant decrease (p<0.05) in inflammation was noticed in the dogs of group II and III from 4th day postoperatively while from 6th day onwards in group I dogs. It completely abolished from 8th day onwards in group III dogs, while 10th day onwards in group I and II dogs (Table 10).

Comparison between different groups

Comparison between groups revealed significantly less (p<0.05) inflammation in dogs of group III, as compared to dogs of group I and II, with non-significant difference between group I and II.

4.2.5 Weight bearing

4.2.5.1 Weight bearing (Standing)

Comparison between different time intervals

Comparison of weight bearing during standing at different time interval revealed significantly more weight bearing (p<0.05) from 4th day onwards in group III and at 6th day onwards in group II dogs, while it was 8th day onwards in group I dogs (Table 11).

Comparison between different groups

Comparison between groups revealed non-significant difference between them. However, group III and II dogs exhibited more weight bearing as compared to group I.
Table 10: Mean values (± SE) of degree of inflammation (exudation, pain and embedding of suture materials) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>1.83&lt;sup&gt;ABa&lt;/sup&gt;±0.30</td>
<td>1.33&lt;sup&gt;Aa&lt;/sup&gt;±0.33</td>
<td>1.66&lt;sup&gt;Ba&lt;/sup&gt;±0.33</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;Aa&lt;/sup&gt;±0.34</td>
<td>1.16&lt;sup&gt;ABBa&lt;/sup&gt;±0.40</td>
<td>0.83&lt;sup&gt;Bb&lt;/sup&gt;±0.16</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;BB&lt;/sup&gt;±0.34</td>
<td>1.16&lt;sup&gt;Ab&lt;/sup&gt;±0.40</td>
<td>0.33&lt;sup&gt;Bbc&lt;/sup&gt;±0.21</td>
</tr>
<tr>
<td>8&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;Ab&lt;/sup&gt;±0.21</td>
<td>0.33&lt;sup&gt;Ac&lt;/sup&gt;±0.21</td>
<td>0.00&lt;sup&gt;Ac&lt;/sup&gt;±0.00</td>
</tr>
<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;Ab&lt;/sup&gt;±0.00</td>
<td>0.00&lt;sup&gt;Ac&lt;/sup&gt;±0.00</td>
<td>0.00&lt;sup&gt;Ac&lt;/sup&gt;±0.00</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;Ab&lt;/sup&gt;±0.00</td>
<td>0.00&lt;sup&gt;Ac&lt;/sup&gt;±0.00</td>
<td>0.00&lt;sup&gt;Ac&lt;/sup&gt;±0.00</td>
</tr>
<tr>
<td><strong>Comparison between the groups</strong></td>
<td>0.69&lt;sup&gt;AB&lt;/sup&gt;±0.15</td>
<td>0.66&lt;sup&gt;A&lt;/sup&gt;±0.17</td>
<td>0.47&lt;sup&gt;C&lt;/sup&gt;±0.12</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
- Values having different superscripts AB shows significant difference (p<0.05) between groups
Table 11: Mean values (± SE) of weight bearing (standing) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>0.00b±0.00</td>
<td>0.16d±0.16</td>
<td>0.33c±0.21</td>
</tr>
<tr>
<td>4th</td>
<td>0.16b±0.16</td>
<td>0.22d±0.22</td>
<td>1.00b±0.25</td>
</tr>
<tr>
<td>6th</td>
<td>0.43b±0.30</td>
<td>0.66c±0.33</td>
<td>1.33b±0.33</td>
</tr>
<tr>
<td>8th</td>
<td>1.50a±0.22</td>
<td>1.50a±0.22</td>
<td>1.83a±0.16</td>
</tr>
<tr>
<td>10th</td>
<td>1.50a±0.21</td>
<td>1.60a±0.22</td>
<td>1.83a±0.16</td>
</tr>
<tr>
<td>12th</td>
<td>1.83a±0.16</td>
<td>1.83a±0.16</td>
<td>1.83a±0.16</td>
</tr>
</tbody>
</table>

Comparison between the groups

|            | 0.90±0.14 | 0.99±0.13 | 1.35±0.13 |

Group I (control): Intramedullary pinning + Dressing of wound

Group II: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

Group III: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
4.2.5.2 Weight bearing (Walking)

Comparison between different time intervals

Significant increase (p<0.05) in weight bearing during walking, was observed from 4\textsuperscript{th} day onward in group III and 6\textsuperscript{th} day onwards in group II dogs, whereas, it was from 8\textsuperscript{th} day onwards in group I dogs (Table 12).

Comparison between different groups

Comparison between different groups revealed significantly more (p<0.05) weight bearing in group III, as compared to group I and II dogs.

4.2.5.3 Weight bearing (Running)

Comparison between different time intervals

In group I significant increase (p<0.05) in weight bearing during running was observed, from 8\textsuperscript{th} day onwards, while it was from 6\textsuperscript{th} day in group II and 4\textsuperscript{th} day onwards in group III dogs. (Table 13).

Comparison between different groups

Comparison between different groups revealed significantly (p<0.05) more weight bearing in group III, as compared to group I and II (Plate 13).
Table 12: Mean values (± SE) of weight bearing (walking) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00&lt;sup&gt;Bc&lt;/sup&gt;±0.00</td>
<td>0.66&lt;sup&gt;Ac&lt;/sup&gt;±0.21</td>
<td>0.50&lt;sup&gt;Abd&lt;/sup&gt;±0.34</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;Bc&lt;/sup&gt;±0.22</td>
<td>0.83&lt;sup&gt;Abc&lt;/sup&gt;±0.30</td>
<td>1.16&lt;sup&gt;Ac&lt;/sup&gt;±0.30</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.83&lt;sup&gt;Ac&lt;/sup&gt;±0.22</td>
<td>1.50&lt;sup&gt;Ab&lt;/sup&gt;±0.34</td>
<td>1.66&lt;sup&gt;Abc&lt;/sup&gt;±0.33</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;Ab&lt;/sup&gt;±0.21</td>
<td>1.83&lt;sup&gt;Ab&lt;/sup&gt;±0.30</td>
<td>2.16&lt;sup&gt;Ab&lt;/sup&gt;±0.30</td>
</tr>
<tr>
<td>8&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.33&lt;sup&gt;Ab&lt;/sup&gt;±0.21</td>
<td>2.50&lt;sup&gt;Ab&lt;/sup&gt;±0.34</td>
<td>2.83&lt;sup&gt;Ab&lt;/sup&gt;±0.40</td>
</tr>
<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.66&lt;sup&gt;Ab&lt;/sup&gt;±0.21</td>
<td>2.50&lt;sup&gt;Ab&lt;/sup&gt;±0.34</td>
<td>2.83&lt;sup&gt;Ab&lt;/sup&gt;±0.40</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1.33&lt;sup&gt;AB&lt;/sup&gt;±0.17</td>
<td>1.63&lt;sup&gt;A&lt;/sup&gt;±0.16</td>
<td>1.85&lt;sup&gt;B&lt;/sup&gt;±0.19</td>
</tr>
</tbody>
</table>

Comparison between the groups

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
- Values having different superscripts AB shows significant difference (p<0.05) between groups
Table 13: Mean values (± SE) of weight bearing (running) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>2\textsuperscript{nd}</td>
<td>\textsuperscript{Bc}0.00±0.00</td>
<td>\textsuperscript{Abc}0.16±0.16</td>
<td>\textsuperscript{Ac}0.83±0.40</td>
</tr>
<tr>
<td>4\textsuperscript{th}</td>
<td>\textsuperscript{Bc}0.16±0.16</td>
<td>\textsuperscript{Bc}0.33±0.33</td>
<td>\textsuperscript{Ab}1.33±0.33</td>
</tr>
<tr>
<td>6\textsuperscript{th}</td>
<td>\textsuperscript{Bc}0.83±0.30</td>
<td>\textsuperscript{Bb}1.16±0.40</td>
<td>\textsuperscript{Ab}2.50±0.34</td>
</tr>
<tr>
<td>8\textsuperscript{th}</td>
<td>\textsuperscript{Bb}1.50±0.34</td>
<td>\textsuperscript{Bab}1.66±0.49</td>
<td>\textsuperscript{Aab}2.66±0.33</td>
</tr>
<tr>
<td>10\textsuperscript{th}</td>
<td>\textsuperscript{Bab}2.00±0.25</td>
<td>\textsuperscript{Ba}2.16±0.40</td>
<td>\textsuperscript{Aa}3.33±0.33</td>
</tr>
<tr>
<td>12\textsuperscript{th}</td>
<td>\textsuperscript{Ba}2.33±0.42</td>
<td>\textsuperscript{Ba}2.33±0.33</td>
<td>\textsuperscript{Aa}3.50±0.22</td>
</tr>
</tbody>
</table>

Comparison between the groups

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textsuperscript{B}1.13±0.18</td>
<td>\textsuperscript{B}1.30±0.19</td>
<td>\textsuperscript{A}2.36±0.20</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
- Values having different superscripts AB shows significant difference (p<0.05) between groups
4.3 Radiographic examination

Fracture healing was assessed by evaluating radiographs, which were taken prior to surgery and subsequently on 12\textsuperscript{th}, 24\textsuperscript{th}, 48\textsuperscript{th} and 72\textsuperscript{th} post operative days of immobilization.

Comparison between different time intervals

4.3.1 Group I (control)

0 day- Radiograph was taken prior to surgery revealed improper alignment of fractured fragments and a distinct fracture line was visible in all the dogs of this. After immobilization, proper alignment and fixation of pin was observed in all the dogs of this group.

12\textsuperscript{th} day- Radiograph of 12\textsuperscript{th} post-operative day revealed proper alignment of fractured fragments and perfect fixation of intramedullary pins in all the dogs of this group. Initiation of periosteal reaction was evident on both the segments adjoining the fracture line. The score for callus formation achieved by the dogs of this group at this stage was 1.00±0.00 and was graded as nil to trace.

24\textsuperscript{th} day- Radiographic interpretation on 24\textsuperscript{th} post-operative day, showed a mild radiolucent area, indicative of primary callus at the fractured site. In all the dogs of this group bridging of the fracture line was incomplete and the score for callus formation at this stage was 1.66±0.16 and it was graded as trace.

48\textsuperscript{th} day- Radiograph of 48th day revealed, increase in callus formation in the most of the dogs of this group. The radiolucent area of the callus designated as primary callus had started to cover itself into radiopaque structure, while fracture line was still distinct and the callus formation was graded as apparent and the score achieved by this group at this interval was 2.16±0.16 (Plate).

72\textsuperscript{th} day- Radiographic interpretation of all the dogs of this group exhibited radio dense area at the fracture site, indicative of extensive periosteal and endosteal callus. Fracture line was completely obliterated. An initiation of
homogenisity was well appreciated in most of the cases, which were indicative of the start of remodeling. The score achieved by this group at this time interval was $3.00 \pm 0.16$, which was graded as apparent to massive (Plate 14 a-b).

4.3.2 Group II (One side LLLT+IM Pinning)

0 day- Pre operative radiograph revealed improper alignment of fractured fragments and a distinct fracture line in all the six dogs of this group and radiograph taken after immobilization showed proper alignment of fractured segments and placement of intramedullary pin.

12th day- Radiographic evaluation of the dogs with one sided LLLT on 12th postoperative day, exhibited, proper fixation of the implant with slight increase in the soft tissue density. Periosteal callus was visible in both the segments adjoining the fracture line, while fracture line was distinct and stage of union was graded as $1.10 \pm 0.00$.

24th day- Radiographic interpretation on 24th post-operative day showed distinct callus, bridging of fracture line was almost complete in all the dogs of this group. Radiographic score achieved in this group, at this stage was $1.83\pm0.16$ and it was graded as apparent.

48th day- Radiograph of 48th day revealed, massive callus formation in the majority the dogs of this group. The radiopaque area of the callus could be observed in all the dogs of this group. Bone trabeculae were found to be crossing the fracture line. Stage of union was ascertained as apparent to massive and the radiographic score at this interval was $2.33\pm0.21$.

72th day- Radiographic interpretation of all the dogs of this group exhibited complete union with radiopaque callus between the two fractured segments of the bone. The periosteal as well as intercortical callus organized and bridged across the fracture line, which were indicative of the start of remodeling. The score achieved by this group at this time interval was $3.16\pm0.30$, which was graded as massive to homogenous (Plate 15 a-b).
4.3.3 Group III (both side LLLT+IM Pinning)

0 day- Pre operative radiograph of all the six dogs of this group (LLLT on both side) revealed improper alignment of fractured fragments with distinct fracture line, however after the immobilization with intramedullary pin the fractured segments were visualized in alignment with proper placement of intramedullary pin.

12th day- Radiographic evaluation of the dogs of both side LLLT group on 12th postoperative day, depicted proper apposition of fracture segments with stable implant and moderate increase in the soft tissue density. Development of callus around the cortex and the fracture gap was visible. Radiographic score for callus formation could be graded as 1.16±0.16, which was considered as apparent.

24th day- Radiographic interpretation on 24th post-operative day showed high density of radiopaque area. Both endosteal as well as periosteal callus were well appreciated with bone trabeculae crossing the fracture line. Radiographic score achieved in this group, at this stage was 1.86±0.16, which was graded as apparent to massive.

48th day- Radiograph of 48th post-operative day revealed, massive and dense callus formation without any evidence of fracture line, almost in all the dogs of this group. The newly formed periosteal as well as intercortical callus became more organized with bridging across the fracture line. Stage of union was ascertained as massive with bone trabeculae crossing the fracture line. A slight evidence of homogenesity of the callus was indicative of initiation of remodeling stage. The radiographic score at this interval achieved was 2.63 ± 0.21.

72nday- Radiographic interpretation at 72 post-operative day exhibited complete union with marked reduction in callus, suggestive of remodeling and formation of cortical bone with prominent bone marrow. Stage of union was achieved with complete obliteration of fracture line. The score achieved by this group at this time interval was 3.33±0.33, which was graded as homogenous (Plate 16 a-b).
Comparison between different groups

0day – Radiographic interpretation before the surgery revealed improper alignment of fractured ends in all the dogs of group I, II and III and proper fixation of intramedullary pin and alignment of fractured segment after surgical procedure.

12th day- Radiographic score on 12th day showed traces of callus formation in group I dogs, while it was apparent to distinct in group II and III respectively. The radiographic score was maximum in group III (1.16±0.16) followed by group II (1.10±0.00) and I (1.00±0.00).

24th day- Radiograph of 24th day revealed maximum callus formation in group III (1.86±0.16) followed by group II (1.83±0.16), while it was minimum in group I dogs (1.66±0.16). Score of callus formation on 24th day depicted the initiation of bridging of fracture line which was slightly increase in group III followed by group II dogs, whereas, it was trace in group I.

48th day- Radiographic score achieved at this stage by the group III, II and I were 2.63±0.21, 2.33± 0.21 and 2.16±0.16 respectively. Massive to homogeneous callus was observed in dogs of group III and II while it was apparent to massive in group I dogs.

72th day- Mean score of callus formation on 72th post operative days showed massive to homogenous callus formation in group III (3.33±0.33) followed by group II (3.16±0.30)and group I (3.00±0.00). Massive to homogenous callus formation was an indicative of start of remodeling process and it was more pronounced in group III followed by group II and I.
### Table 14: Mean (± SE) of radiographic score at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;d&lt;/sup&gt;±0.00</td>
<td>0.00&lt;sup&gt;e&lt;/sup&gt;±0.00</td>
<td>0.00&lt;sup&gt;e&lt;/sup&gt;±0.00</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;c&lt;/sup&gt;±0.00</td>
<td>1.10&lt;sup&gt;d&lt;/sup&gt;±0.00</td>
<td>1.16&lt;sup&gt;d&lt;/sup&gt;±0.16</td>
</tr>
<tr>
<td>24&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;b&lt;/sup&gt;±0.16</td>
<td>1.83&lt;sup&gt;c&lt;/sup&gt;±0.16</td>
<td>1.86&lt;sup&gt;c&lt;/sup&gt;±0.16</td>
</tr>
<tr>
<td>48&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.16&lt;sup&gt;b&lt;/sup&gt;±0.16</td>
<td>2.33&lt;sup&gt;b&lt;/sup&gt;±0.21</td>
<td>2.63&lt;sup&gt;b&lt;/sup&gt;±0.21</td>
</tr>
<tr>
<td>72&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3.00&lt;sup&gt;a&lt;/sup&gt;±0.00</td>
<td>3.16&lt;sup&gt;a&lt;/sup&gt;±0.30</td>
<td>3.33&lt;sup&gt;a&lt;/sup&gt;±0.33</td>
</tr>
<tr>
<td><strong>Comparison between the groups</strong></td>
<td>1.56 ±0.19</td>
<td>1.68±0.22</td>
<td>1.79±0.22</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
4.4 Hematological parameters

4.4.1 Total Erythrocyte count (million/µl)

Comparison between different time intervals

Total erythrocyte count showed non-significant decrease on 6\textsuperscript{th} post operative day, followed by increase on 12\textsuperscript{th} post-operative day, with a maximum values of 6.11±0.51 and 6.67±0.45 million/µl on 12\textsuperscript{th} post operative day as compared to control values of 5.92±0.40 and 6.47±0.29 million/µl in dogs of group I and II respectively. Whereas, Dogs of group III showed non-significant decrease on day 6\textsuperscript{th}, followed by significant increase (p<0.05) with a maximum value of 7.66±0.28 million/µl on 12\textsuperscript{th} post operative day as compared to the control value of 6.08±0.18 million/µl (Table 15).

Comparison between different groups

Significantly high (p<0.05) total erythrocyte was observed in group II and III as compared to group I dogs.

4.4.2 Total Leucocyte count (thousand/µl)

Comparison between different time intervals

Total leucocyte count showed non-significant increase on 6\textsuperscript{th} day interval in group I and II with maximum values of 22.79±3.43 and 27.35±2.23 thousand/µl respectively, thereafter non-significant decrease was observed in group I and significant decrease(p<0.05) in group II with values of 20.49±0.37 and 17.74±1.13 thousand/µl respectively. However, the dogs of group III showed a significant increase (p<0.05) on 6\textsuperscript{th} day interval followed by a significant decrease (p<0.05) on 12\textsuperscript{th} day interval with maximum value of 25.61±2.44 thousand/µl on day 6\textsuperscript{th} and minimum value of 17.43±0.83 thousand/µl on day 12\textsuperscript{th} (Table 16).

Comparison between different groups

Comparison between groups revealed non-significant difference between them.
Table 15: Mean values (± SE) of total erythrocyte count (million/µl) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt;</td>
<td>5.92&lt;sup&gt;A&lt;/sup&gt;±0.40</td>
<td>6.47&lt;sup&gt;A&lt;/sup&gt;±0.29</td>
<td>6.08&lt;sup&gt;Ab&lt;/sup&gt;±0.18</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>5.29&lt;sup&gt;B&lt;/sup&gt;±0.37</td>
<td>6.33&lt;sup&gt;A&lt;/sup&gt;±0.27</td>
<td>5.75&lt;sup&gt;Bb&lt;/sup&gt;±0.33</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>6.11&lt;sup&gt;B&lt;/sup&gt;±0.51</td>
<td>6.67&lt;sup&gt;B&lt;/sup&gt;±0.45</td>
<td>7.66&lt;sup&gt;Aa&lt;/sup&gt;±0.28</td>
</tr>
<tr>
<td>Comparison between the groups</td>
<td>5.77&lt;sup&gt;B&lt;/sup&gt;±0.25</td>
<td>6.49&lt;sup&gt;A&lt;/sup&gt;±0.19</td>
<td>6.50&lt;sup&gt;A&lt;/sup&gt;±0.25</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
- Values having different superscripts AB shows significant difference (p<0.05) between groups
Table 16: Mean values (± SE) of total leucocyte count (thousand/µl) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt;</td>
<td>21.53±3.42</td>
<td>24.45&lt;sup&gt;a&lt;/sup&gt;±0.64</td>
<td>19.58&lt;sup&gt;b&lt;/sup&gt;±1.24</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>22.79±3.43</td>
<td>27.35&lt;sup&gt;a&lt;/sup&gt;±2.23</td>
<td>25.61&lt;sup&gt;a&lt;/sup&gt;±2.44</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>20.49±0.37</td>
<td>17.74&lt;sup&gt;b&lt;/sup&gt;±1.13</td>
<td>17.43&lt;sup&gt;b&lt;/sup&gt;±0.83</td>
</tr>
<tr>
<td><strong>Comparison between the groups</strong></td>
<td>21.60±1.63</td>
<td>23.18±1.01</td>
<td>20.72±1.22</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
4.4.3 Haemoglobin (g/dl)

Comparison between different time intervals

Hemoglobin showed a non-significant difference at different time intervals in the dogs of all the three groups and the values of haemoglobin fluctuated between 14.03±1.44 to 14.87±1.22, 12.80±1.02 to 12.87±0.64 and from 11.32±1.13 to 12.20±0.84 g/dl in group I, II and III respectively (Table 17).

Comparison between different groups

Comparison between all the three groups revealed non-significant difference between them.

4.4.4 Packed cell volume (Per cent)

Comparison between different time intervals

Packed cell volume showed non-significant decrease on 6th day interval with a minimum values of 34.87±1.40 and 33.80±2.19 per cent in group II and III respectively, followed by its increase with maximum values of 40.42±0.68 and 36.10±1.73 in group II and III respectively on 12th postoperative day. However, in dogs of group I significant decrease (p<0.05) with minimum value of 33.56±2.70 percent was observed on 6th day followed by increase at 12th day interval (38.18±0.53) (Table 18).

Comparison between different groups

Comparison between all the groups revealed non-significant difference between them.
Table 17: Mean values (± SE) of haemoglobin (g/dl) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th</td>
<td>14.87±1.22</td>
<td>12.85±1.64</td>
</tr>
<tr>
<td></td>
<td>6th</td>
<td>14.03±1.44</td>
<td>12.80±1.02</td>
</tr>
<tr>
<td></td>
<td>12th</td>
<td>14.69±0.86</td>
<td>12.87±0.64</td>
</tr>
<tr>
<td></td>
<td>Comparison between the groups</td>
<td>14.53±0.65</td>
<td>12.84±0.63</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment
**Table 18: Mean values (± SE) of packed cell volume (per cent) at different time intervals**

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th</td>
<td>38.62±1.20</td>
<td>36.80±0.54</td>
<td>34.96±1.99</td>
</tr>
<tr>
<td>6th</td>
<td>33.56±2.70</td>
<td>34.87±1.40</td>
<td>33.80±2.19</td>
</tr>
<tr>
<td>12th</td>
<td>38.18±0.53</td>
<td>40.42±0.68</td>
<td>36.10±1.73</td>
</tr>
</tbody>
</table>

**Comparison between the groups**

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36.78±1.09</td>
<td>37.36±0.76</td>
<td>34.95±1.09</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
4.4.5 Differential leukocyte count (Per cent)

4.4.5.1 Neutrophil count

**Comparison between different time intervals**

Neutrophil count showed non-significant increase in all the groups of dogs on 6th day interval followed by a significant decrease (p<0.05) on 12th day interval with a maximum values of 70.36±4.51, 73.16±1.07 and 64.50±1.89 per cent on 6th day interval, while minimum values of 54.26±6.76, 55.10±5.21 and 52.60±2.39 percent on 12th day intervals as compared to the control values of 63.08±2.08, 70.23±3.90 and 62.33±7.88 in group I, II and III respectively (Table 19).

**Comparison between different groups**

Comparison between three groups showed significantly high neutrophil count in group II and significantly low value in group III, as compared to group I dogs.

4.4.5.2 Lymphocyte count

**Comparison between different time intervals**

Lymphocyte count showed non-significant decrease in all the groups of dogs with a minimum value of 26.22±4.66, 11.70±1.54 and 33.00±2.71 per cent on 6th day interval. Thereafter, a significant increase (p<0.05) in the values were observed on 12th day interval in group I, II and III with a maximum value of 36.86±5.88, 23.85±4.96 and 41.20±1.97 per cent as compared to control values of 27.50±2.72, 17.21±4.38 and 36.51±7.78 per cent respectively (Table 20).

**Comparison between different groups**

Comparison between three groups of dogs showed significantly low value of lymphocyte count in group II dogs, as compared to group I and III dogs.
Table 19: Mean values (± SE) of neutrophil count (per cent) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(^{th})</td>
<td>63.08(^{Bab})±2.08</td>
<td>70.23(^{Aab})±3.90</td>
<td>62.33(^{Bab})±7.88</td>
</tr>
<tr>
<td>6(^{th})</td>
<td>70.36(^{Ba})±4.51</td>
<td>73.16(^{Aa})±1.07</td>
<td>64.50(^{Ba})±1.89</td>
</tr>
<tr>
<td>12(^{th})</td>
<td>54.26(^{Bb})±6.76</td>
<td>55.10(^{Ab})±5.21</td>
<td>52.60(^{Bb})±2.39</td>
</tr>
<tr>
<td>Comparison between the groups</td>
<td>62.56(^{B})±3.06</td>
<td>66.16(^{A})±2.43</td>
<td>59.81(^{C})±2.92</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
- Values having different superscripts AB shows significant difference (p<0.05) between groups
Table 20: Mean values (± SE) of lymphocyte count (per cent) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th</td>
<td>27.50^{Ab}±2.72</td>
<td>17.21^{Bab}±4.38</td>
<td>36.51^{Aa}±7.78</td>
</tr>
<tr>
<td>6th</td>
<td>26.22^{Ab}±4.66</td>
<td>11.70^{Bb}±1.54</td>
<td>33.00^{Aa}±2.71</td>
</tr>
<tr>
<td>12th</td>
<td>36.86^{Aa}±5.88</td>
<td>23.85^{Ba}±4.96</td>
<td>41.20^{Ab}±1.97</td>
</tr>
<tr>
<td></td>
<td>Comparison between the groups</td>
<td>30.19^{A}±2.74</td>
<td>17.58^{B}±2.44</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
- Values having different superscripts AB shows significant difference (p<0.05) between groups
4.4.5.3 Eosinophil count

Comparison between different time intervals

Eosinophil count showed a non-significant difference at different time interval in all the three groups of dogs and the values fluctuated between 0.66±0.33 to 3.46±0.92, 2.50±0.65 to 3.50±1.23 and 2.40±0.79 to 3.60±1.28 per cent in group I, II and III respectively (Table 21).

Comparison between different groups

Comparison between groups revealed non-significant difference between them.

4.4.5.4 Basophil count

Comparison between different time intervals

Basophil count revealed non-significant difference between all the three groups of dogs at different time intervals (table 22).

Comparison between different groups

Comparison between all the three groups revealed non-significant difference between them.
Table 21: Mean values (± SE) of eosinophil count (per cent) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.66±1.11</td>
<td>3.16±1.35</td>
<td>2.80±0.70</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.66±0.33</td>
<td>3.50±1.23</td>
<td>2.40±0.79</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3.46±0.92</td>
<td>2.50±0.65</td>
<td>3.60±1.28</td>
</tr>
<tr>
<td>Comparison between the groups</td>
<td>2.26±0.54</td>
<td>3.05±0.61</td>
<td>2.93±0.53</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment
Table 22: Mean values (± SE) of basophil count (per cent) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.16±0.16</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.16±0.16</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Comparison between the groups: 0.11±0.07 0.00±0.00 0.00±0.00

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment
4.4.5.5 Monocyte count

**Comparison between different time intervals**

Monocyte count showed non-significant difference at different time interval and the values fluctuated between 2.16±1.13 to 5.45±1.72, 2.35±0.59 to 2.73±0.47 and 2.30±1.21 to 3.40±0.61 per cent in group I, II and III respectively (Table 23).

**Comparison between different groups**

Comparison between all the three groups revealed non-significant difference between them.

4.5 Biochemical Parameter

4.5.1 Alkaline phosphatase (IU/L)

**Comparison between different time intervals**

Alkaline phosphatase showed significant increase (p<0.05) from 12th post-operative day in all the three groups of dogs, with maximum values of 131.67±18.00 IU/L at 24th day in group I and 141.44±7.35 and 150.86±6.97 IU/L at 12th day in group II and III as compared to the control values of 86.62 ±19.00, 93.93±17.79 and 105.05±22.36 IU/L in group I, II and III respectively. Thereafter, significant decreasing trend was noticed (p<0.05) with minimum value of 74.63±11.64, 76.07±21.88 and 80.24±8.95 IU/L on 72th day in group I, II and III respectively (Table 24).

**Comparison between different groups**

Comparison between all the three groups revealed non-significant difference between them.
Table 23: Mean values (± SE) of monocyte count (per cent) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt;</td>
<td>5.45±1.72</td>
<td>2.35±0.47</td>
<td>2.30±1.21</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.82±0.59</td>
<td>2.35±0.59</td>
<td>3.40±0.61</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>2.16±1.13</td>
<td>2.73±0.47</td>
<td>3.00±0.68</td>
</tr>
</tbody>
</table>

Comparison between the groups

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.47±0.75</td>
<td>2.46±0.28</td>
<td>2.92±0.48</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment
Table 24: Mean values (± SE) of alkaline phosphatase (unit/liter) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th</td>
<td>86.62b±19.00</td>
<td>93.93b±17.79</td>
<td>105.05bc±22.36</td>
</tr>
<tr>
<td>12th</td>
<td>123.37a±17.32</td>
<td>141.44a±7.35</td>
<td>150.86a±6.97</td>
</tr>
<tr>
<td>24th</td>
<td>131.67a±18.00</td>
<td>111.10ab±10.39</td>
<td>130.88ab±17.56</td>
</tr>
<tr>
<td>48th</td>
<td>124.64a±16.26</td>
<td>77.00b±14.38</td>
<td>101.70bc±13.53</td>
</tr>
<tr>
<td>72th</td>
<td>74.63b±11.64</td>
<td>76.07b±21.88</td>
<td>80.24c±8.95</td>
</tr>
</tbody>
</table>

Comparison between the groups

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>108.18±8.11</td>
<td>99.92±7.77</td>
<td>113.75±7.69</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
4.5.2 Total protein (g/dl)

Comparison between different time intervals

The dogs of group I showed non significant changes in total protein and the values fluctuated between 4.78±0.27 to 6.41±0.29 g/dl. Whereas, in group II significant increase (p<0.05) was observed on 24th day with the maximum values of 7.87±0.62 g/dl compared to control values of 5.00±0.65 g/dl. However, in dogs of group III significant increase was observed from 12th day onwards with maximum value of 6.95±0.21 g/dl from control value of 4.27±0.42 g/dl (Table 25).

Comparison between different groups

Comparison between all the three groups revealed non-significant difference between them.

4.5.3 Serum calcium (mg/dl)

Comparison between different time intervals

Serum calcium showed non-significant decrease in all the three groups of dogs with minimum value of 10.73±1.23 mg/dl at 48th day in group I and 10.07±0.38 and 9.93±0.55 mg/dl in group II and III dogs at 24th day interval as compared to control value of 11.41± 1.54, 11.73± 0.79 and 11.19± 1.50 mg/dl in group I, II and III respectively (Table 26).

Comparison between different groups

Comparison between all the three groups revealed non-significant difference between them.
Table 25: Mean values (± SE) of total protein (g/dl) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;th&lt;/sup&gt;</td>
<td>5.10&lt;sup&gt;ab&lt;/sup&gt;±0.51</td>
<td>5.00&lt;sup&gt;b&lt;/sup&gt;±0.65</td>
<td>4.27&lt;sup&gt;c&lt;/sup&gt;±0.42</td>
</tr>
<tr>
<td>12&lt;sup&gt;th&lt;/sup&gt;</td>
<td>4.78&lt;sup&gt;b&lt;/sup&gt;±0.27</td>
<td>6.03&lt;sup&gt;b&lt;/sup&gt;±0.67</td>
<td>5.40&lt;sup&gt;b&lt;/sup&gt;±0.35</td>
</tr>
<tr>
<td>24&lt;sup&gt;th&lt;/sup&gt;</td>
<td>5.47&lt;sup&gt;ab&lt;/sup&gt;±0.31</td>
<td>7.87&lt;sup&gt;a&lt;/sup&gt;±0.62</td>
<td>5.58&lt;sup&gt;b&lt;/sup&gt;±0.41</td>
</tr>
<tr>
<td>48&lt;sup&gt;th&lt;/sup&gt;</td>
<td>6.16&lt;sup&gt;a&lt;/sup&gt;±0.19</td>
<td>6.58&lt;sup&gt;b&lt;/sup&gt;±0.19</td>
<td>6.95&lt;sup&gt;a&lt;/sup&gt;±0.21</td>
</tr>
<tr>
<td>72&lt;sup&gt;th&lt;/sup&gt;</td>
<td>6.41&lt;sup&gt;ab&lt;/sup&gt;±0.29</td>
<td>6.16&lt;sup&gt;b&lt;/sup&gt;±0.15</td>
<td>6.27&lt;sup&gt;ab&lt;/sup&gt;±0.38</td>
</tr>
<tr>
<td>Comparison between the groups</td>
<td>5.58±0.16</td>
<td>6.32±0.28</td>
<td>5.69±0.22</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment

- Values having different superscripts ab shows significant difference at 5% level between intervals
Table 26: Mean values (± SE) of serum calcium (mg/dl) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th</td>
<td>11.41±1.54</td>
<td>11.73±0.79</td>
<td>11.19±1.50</td>
</tr>
<tr>
<td>12th</td>
<td>11.30±1.42</td>
<td>10.53±0.56</td>
<td>10.61±1.02</td>
</tr>
<tr>
<td>24th</td>
<td>10.76±1.28</td>
<td>10.07±0.38</td>
<td>9.93±0.55</td>
</tr>
<tr>
<td>48th</td>
<td>10.73±1.23</td>
<td>10.44±0.83</td>
<td>10.76±0.68</td>
</tr>
<tr>
<td>72th</td>
<td>11.44±0.86</td>
<td>10.71±0.63</td>
<td>10.88±0.59</td>
</tr>
<tr>
<td>Comparison between the groups</td>
<td>11.12±0.53</td>
<td>10.69±0.26</td>
<td>10.67±0.40</td>
</tr>
</tbody>
</table>

**Group I (control):** Intramedullary pinning + Dressing of wound

**Group II:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 6 minutes on one side of fracture fragment

**Group III:** Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4 joules for 3 minutes each on both side of fracture fragment
4.5.4 Serum phosphorous (mg/dl)

Comparison between different time intervals

Non-significant decreasing trend in serum phosphorous was observed from 12\textsuperscript{th} day onward in all the three groups of dogs with minimum value of 5.15±1.10, 5.44±0.58 and 5.73±1.16 mg/dl at 72\textsuperscript{th} day interval from control values of 7.82±0.92, 7.03±0.87 and 6.68±1.29 mg/dl in group I, II and III respectively (Table 27).

Comparison between different groups

Comparison between all the three groups revealed non-significant difference between them.

Table 27: Mean values (± SE) of serum phosphorous (mg/dl) at different time intervals

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0\textsuperscript{th}</td>
<td>7.82±0.92</td>
<td>7.03±0.87</td>
<td>6.68±1.29</td>
</tr>
<tr>
<td>12\textsuperscript{th}</td>
<td>7.26±0.89</td>
<td>6.95±0.77</td>
<td>6.84±0.92</td>
</tr>
<tr>
<td>24\textsuperscript{th}</td>
<td>6.40±1.04</td>
<td>6.31±0.74</td>
<td>6.78±1.02</td>
</tr>
<tr>
<td>48\textsuperscript{th}</td>
<td>5.61±1.12</td>
<td>6.45±0.71</td>
<td>6.19±1.10</td>
</tr>
<tr>
<td>72\textsuperscript{th}</td>
<td>5.15±1.10</td>
<td>5.44±0.58</td>
<td>5.73±1.16</td>
</tr>
<tr>
<td>Comparison between the groups</td>
<td>6.44±0.46</td>
<td>6.44±0.32</td>
<td>6.45±0.46</td>
</tr>
</tbody>
</table>

Group I (control): Intramedullary pinning + Dressing of wound

Group II: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 6 minutes on one side of fracture fragment

Group III: Intramedullary pinning + Dressing of wound + Low level laser therapy (LLLT) @ 10 Hz, 2.4joules for 3 minutes each on both side of fracture fragment
4.6 Complications

In one dog of group I slight exudation was observed from 4\textsuperscript{th} day onwards which were treated accordingly whereas, the another dog of same group showed traumatic wound at the fracture site at 48\textsuperscript{th} postoperative day. The dog was re-operated, intramedullary pin was removed and Robert jones bandage was done along with restriction of the movement.

The pin migration was noticed in one dog of group I on 72\textsuperscript{th} day post operatively, however the fracture was partially healed at that time (Plate 17).
5. DISCUSSION

5.1 Anamnesis/ History

5.1.1. Age

In the present study, maximum number of long bone fractures were recorded from 1 to 3 year of age followed by 3 to 6 year of age and minimum were between 6 to 8 year of age. These may be because of activeness or playing habit of the puppies or young dogs which makes them more prone for fracture either due to automobile accident or failing from the height.

Similar finding were also reported by Simon (2011), De’ Souza (2012), Rajhans (2013) and Singh et al. (2015), who reported highest incidence of fracture of long bone in young dogs.

Sran et al. (2016) also conducted a retrospective study on frequency of long bone fracture in dogs and concluded that the dogs of younger age between 6-12 months of age showed the highest occurrence (21.89%) of fracture.

5.1.2 Sex

In the present study long bone fractures were reported more in the male dogs (55.55%) while number of female dogs were only 8 (44.44%).

Higher incidence in male can be attributed to the fact that, males are more aggressive and tend to wander more than female, which predisposes them to the factors responsible for causing the fracture.

Aithal et al. (1999), Simon (2011), De’ Souza (2012), Rajhans (2013) and Singh et al. (2015) also reported higher incidence of fracture in male than female.

5.1.3 Etiology

Automobile accident was most common (50%) cause of fracture followed by dog bite (22.22%), falling from roof (16.66%) and hitting (11.11%).
The finding attributed to the fact that most of the dogs of the present study were non-descript and usually let loose to roam outside freely, thus gets more prone for the accidental trauma and dog bite.

Similar findings were observed by De’ Souza (2012), Minar et al. (2013), Rajhans (2013) and Singh et al. (2015) who also reported that automobile accident was the major cause of fracture followed by falling from height and other causes.

5.1.4 Bone involved in fracture

In the present study it was observed that the most common bone involved in fracture was the femur (55.55%), followed by tibia (38.88%), whereas the incidence of humerus fracture was least i.e. 5.55% of all the cases.

Higher incidence of fracture of femur may be attributed to its position which is inclined in position or anatomically placed in oblique direction forward and downward. Whenever, the animal jumps or run this bone receives tangential force opposite to each other leading to fracture and most of the fracture were caused by automobile accidents, where the animals are most likely hit from backside. However similar tangential force is too acted upon humerus, but the fracture case of humerus are less, because the force in case of femur is more due to weight of the hind part of the body. Tibia bone is less supported by musculature and is just subcutaneous on the medial surface, so more prone for accidental trauma.

The present findings are in accordance with the findings of Aithal et al. (1999), who also reported highest number of fractures in femur (38.56%) followed by tibia-fibula (17.16%) and humerus (7.71%) among four long bones. Similar observation were also recorded by Minar et al. (2013), Singh et al. (2015) and Sran et al. (2016) in dogs.

5.1.5 Breed

Out of eighteen cases of long bone fractures fourteen dogs were non-descript (77.77%), whereas three dogs were Labrador retriever (16.66%) and only one dog was of German shepherd (5.55%) breed.
Higher number of non-descript might be due to their higher population in and around Jabalpur.

Simon *et al.* (2011) discussed that pelvic limb fracture are more common in non-descript dogs (47.48%) followed by Spitz (20.08%), German shepherd (7.11%) and Labrador (5.85 %). However Ben-Ali (2013) observed German shepherd (19.32%) was the most common breed affected with the fracture, followed by Wolf (17.05%), Mongrels (15.90%) and other six breed. This different breed finding may be due to prevalence of different breeds in different countries.

Similar observation was also reported by Jani *et al.* (2014), who conducted a retrospective study of five years on occurrence of fracture in dogs and reported that the non-descript breed are mostly affected.

**5.1.5 Duration of fracture**

Among all the eighteen animals referred for the fracture treatment, 7 animals were presented immediately after injury (38.88%), 8 between 0 to 3 days after injury (44.44%), whereas 3 cases were presented more than 3 days after injury (16.66%).

This observation showed the awareness of the owner for his/her pet, the distance may also be another important factor. Socio-economic status of the dog owner is also important factor for presenting the pet for treatment at hospital.

Similar findings were also observed by De’ Souza (2012), Rajhans (2013), and Singh *et al.* (2015) in dogs.

**5.2 Clinical parameters**

**5.2.1 Rectal Temperature**

Rectal temperature showed significant decrease at 30 minute after surgery in all the three groups. Thereafter, a gradual increase was observed and it returns to the normal value from 24-48 hour interval. Comparison between the groups showed non-significant difference between them.
The post-operative decrease in rectal temperature may be attributed to effect of pre anesthetics and analgesics used during surgical procedure.

These findings are in accordance with the findings of Tembhurne et al. (2010), De’ Souza (2012), Rajhans (2013), and Singh et al. (2015) in dogs. They also reported fluctuation of body temperature within normal physiological limit at different post-operative intervals.

5.2.2 Pulse rate

All the three groups showed a significant decrease in the pulse rate at 30 minutes interval followed by increase at 24 and 48 hours interval. Comparison between all the three groups of dogs revealed significant difference between them.

Decrease in the value of pulse rate at 30 minutes after surgery might be due to general anesthesia used for intramedullary pinning, which initially causes increase in heart rate, followed by decrease.

These observations are in accordance with the findings of Agnihotri (2007), Tembhurne et al. (2010), De’ Souza (2012) and Rajhans (2013), as they also reported an initial increase, followed by decrease in pulse rate during their study on internal fixation of fracture in dogs.

5.2.3 Respiration rate

The respiration rate decreases significantly at 30 minute interval in the three groups. Thereafter, a gradual increase in the respiration rate were observed in the animals of group I and II, whereas animals of group III showed decreasing trend in respiration rate from the control value. Comparison between different groups revealed non-significant difference between them.

The post-operative decrease in respiration rate may be attributed to effect of pre anesthetics and analgesics, used during surgical procedure.

Temburne et al. (2010), De’ Souza (2012) and Rajhans (2013) also reported fluctuation of respiration rate after internal fixation of fracture in dogs.
5.2.4 Degree of inflammation (exudation, pain and embedding of suture materials)

The postoperative inflammation decreased from 4th day onwards in laser irradiated groups i.e. group II and III, while from 6th day onwards in group I (without laser). Comparison between groups revealed significantly less inflammation in the dogs of group III, those were subjected to LLLT on both side of fractured bone followed by group II and I. However, low inflammation was observed in dogs of group II who were treated laser on one side, as compared to group I.

Reduction in inflammation at a greater rate in the group II and III dogs treated with laser therapy may be attributed to the fact that laser might have enhanced the reabsorption of tissue debris by the macrophage and proliferation of fibroblast and angiogenesis.

Anti-inflammatory effect of laser therapy has also been reported by many workers as Zhevago and Samoilova (2006) discussed that one of the major mechanism responsible for anti-inflammatory effect of LLLT is the, decrease in the plasma level of pro-inflammatory cytokines (TNF-α, IL-6 and IL-2).

Singh et al. (2011) and Bhomick and Bhargava (2015) also reported decreased inflammation and exudation at wound site treated with low level laser therapy in the dogs and calves respectively.

Nema et al., (2016) also observed minimum inflammation , exudation and embedding of sutures on 5th day of LLLT in the dogs suffering from aural haematoma.

5.2.5 Weight bearing

5.2.5.1 Weight bearing (Standing)

The weight bearing score during standing improved significantly from 4th day onwards in the dogs of group III subjected with LLLT on both sides and at 6th day onwards in group II dogs (one side LLLT). This might be due to faster reduction of inflammation as observed in the previous study and alleviation of pain at these time intervals in laser treated groups. Contrary to this weight bearing was observed from 8th day onwards in group I dogs.
Comparison between groups also revealed more weight bearing in laser treated group III and II dogs as compared to group I dogs, indicative of therapeutic effect of laser, that reduces inflammation and pain thereby earlier weight bearing (Honmura et al., 1992).

Partial weight bearing from 7th to 10th day was too reported by Dubey et al. (1992), Raghunath and Singh (2003), Ganesh et al. (2004), Yuvraj et al. (2007) Gupta et al. (2008) and Singh et al. (2015).

5.2.5.2 Weight bearing (Walking)

The weight bearing score showed more weight bearing during walking from 4th day onward in group III and 6th day onwards in group II dogs, whereas, it was from 8th day onwards in group I dogs.

Comparison between different groups revealed significantly more weight bearing in group III, as compared to group I and II dogs.

The improvement in weight bearing during walking must have been depended upon the weight bearing score while standing at different time interval, as the dogs of group III and II showing early weight bearing can be predicted to express an early weight bearing at further interval.

Gradual increase in weight bearing during walking was too observed by Singh (2015) in canine.

5.2.5.3 Weight bearing (Running)

Weight bearing while running was also observed, in the same pattern as weight bearing while standing and walking, with significantly more weight bearing in group III dogs, followed by II and I.

This finding is also based on the fact that early weight bearing is indicative of an early return of limb function with full range of motion.

Khadra et al., (2004) reported that the LLLT enhances the functional attachment of implant to bone and promotes bone healing and mineralization.

Similar findings were also observed by Agnihotri (2007), Tembhurne et al. (2010), De’ Souza (2012), Rajhans (2013) and Singh (2015) in canine.
5.3 Radiographic examination

5.3.1 0 day

Radiograph taken just after immobilization showed proper alignment of fracture segments and placement of intramedullary pins in dogs of all the three groups.

5.3.2 12th day

Radiograph of 12th post-operative day depicted, the initiation of periosteal reaction with traces of callus in the dogs of group I. A slight increase in the soft tissue density at fracture site with apparent callus was observed in dogs of group II while, high soft tissue density with moderate callus was observed in the group III dogs.

This may be due to effect of the LLLT in the laser treated groups (II and III), which might have stimulated the non-differentiated mesenchymal cells in to the osteoblasts, which converted in to the osteocytes in the initial phase of the fracture healing, results in early initiation of healing process in these two groups of animals.

The above findings correlate with the findings of Penheiro and Frame (1992), who also reported that, the LLLT may enhance the osteogenic potential of the bone regenerating cells.

Raghunath and Singh (2008), Agarwal (2007) and De’ Souza (2012), also observed periosteal reaction on 12th postoperative day after internal fixation of fracture in dogs.

5.3.3 24th day

Interpretation of radiograph on 24th post-operative day revealed mild radiolucent area of primary callus at fracture site in the dogs of group I, whereas the distinct callus formation was observed in the dogs of group II. In the dogs of group III, the high density endosteal as well as periosteal callus was observed on 24th post-operative day.

Formation of high density periosteal and endosteal callus in group III and high density callus in group II dogs might be due to faster proliferation of osteoblast due to LLLT in these two group of dogs, as compared to group I dogs.
Khadra et al., (2005), also observed growth and proliferation of bone forming cells around the implant. They concluded that biostimulation by LLLT may have enhanced the osteogenic potential of these cells.

Ribeiro and Matsumoto (2008) and Pereira et al., (2009), evaluated the effect of LLLT on bone repair on rats and concluded that laser therapy improves bone repair because of an up regulation of sacculo-oxygenase to expression in bone cells.

**5.3.3 48th day**

Radiograph of 48th day in group I, showed that primary callus had started to cover itself into radiopaque structure, while fracture line was still distinct. The massive callus formation with obliteration of bone trabeculae were observed in the dogs of group II, whereas evidence of homogenesity without any evidence of fracture line and dense callus formation was seen in the group III dogs.

Formation of dense callus in group III and massive callus in group II, might be due to osteoinductive property of LLLT, which enhances the process of fracture healing and proliferation of fibroblast of trabecular osteoid as also observed in the dogs of laser treated group II and III.

Similarly Ozawa et al., (1998), reported the stimulatory effect of low power laser irradiation on bone formation in vitro and reported that LLLT increases cellular proliferation and bone nodule formation.

Takeda (1988) evaluated the effect of low energy laser on extraction socket healing and observed fibroblast formation of trabecular osteoids more prominent after laser treatment.

Rajhans (2013) and Singh (2015), also reported bridging of callus between intercortical and periosteal adjoining area on 45th post-operative day in dogs, after internal fixation of long bone fracture.

**5.3.4 72th day**

Radiographic interpretation of all the dogs of group I exhibited extensive periosteal and endosteal callus, indicative of initiation of remodeling
while, dogs of group II showed bridging of periosteal as well as intercortical callus with homogenisity. The dogs of group III exhibited complete union with formation of cortical bone with prominent bone marrow.

Organization of callus and complete union of fractured bone in the dogs of group III and II might be the beneficial effects of LLLT, which produces osteointegration along with intramedullary pin and provide early fracture healing than the intramedullary pin alone as in the dogs of group I.

Luger et al. (1998) evaluated the effect of LLLT in tibial fracture healing in rats. They removed the tibia after irradiation of the fracture for four weeks and tested for its tension strength and reported significantly increased tibial stiffness in laser irradiated tibial fracture.

Bouvet-Gerbettaz et al. (2009) evaluated the effect of LLLT on proliferation and differentiation of murine bone marrow cells. They used laser thrice a week on murine bone marrow cells containing osteoblast progenitors and concluded that laser does not alter murine bone cell proliferation and differentiation.

Petri et al. (2010) investigated the effect of LLLT on human osteoblastic cell grown on titanium and observed that LLLT affected cell responses in a complex way and concluded that LLLT might have beneficial effects on implant osteointegration despite a transient effect immediately after laser irradiation.

5.4 Haematological parameters

5.4.1 Total Erythrocyte count (million/µl)

Total erythrocyte count showed non-significant decrease on 6th post-operative day, followed by increase on 12th post-operative day, in dogs of group I and II respectively, whereas, dogs of group III showed non-significant decrease on day 6th, followed by significant increase. Significantly high total erythrocyte was observed in group II and III as compared to group I dogs.

The transient fall in total erythrocyte count may be attributed to mild hemorrhages during surgical procedure or sequestration of RBC to spleen as opinioned by Lobo et al. (2013).
The above observations correlated with the findings of Aithal et al., (1998), Agnihotri (2007), Tembhurne et al. (2010), De’ Souza (2012) and Rajhans (2013), they also reported non-significant changes in total erythrocyte count after internal fixation of fracture in canine.

5.4.2 Total Leucocyte count (thousand/µl)

Total leucocyte count showed non-significant increase on 6th day interval in group I and II, followed by decrease. However, the dogs of group III, showed a significant increase on 6th day interval, followed by significant decrease at 12th day interval. Comparison between groups revealed non-significant difference between them.

This increase in TLC may be due to more inflammation due to trauma resulting in fracture. In the present study the fluctuation of total leukocyte count at different time intervals in all three groups of dogs, as well as between the groups, suggestive of no influence of laser therapy on total leukocyte count.

Singh et al. (2011) in dogs and Bhowmick et al. (2013) in calves also reported increase in total erythrocyte count in the laser treated groups.

De’ Souza (2012) reported a non-significant increase in total leucocyte count on 7th post-operative day, after plating and cross pinning for fracture repair in dogs.

Toth et al., (2014) also observed an elevated leucocyte count in early postoperative period in beagle dogs while conducting study on unilateral implantation of polytetrauroethylene vascular graft in their femoral artery.

5.4.3 Hemoglobin (g/dl)

Hemoglobin showed a non-significant difference at different time intervals in the dogs of all the three groups. Comparison between all the three groups revealed non-significant difference between them.

These fluctuation in hemoglobin level may be attributed to the physical stress at the time of fracture, as well as haemodilution and anesthesia during internal fixation procedure.
Agnihothri (2006), Tembhrune et al. (2010), De’ Souza (2012) Rajhans (2013) and Singh (2015), also reported non-significant changes in hemoglobin after internal fixation of fracture in canine.

5.4.4 Packed cell volume (Per cent)

Packed cell volume showed non-significant decrease in group II and III and significant decrease in dogs of group I at 6th day interval, followed by increase at 12th post-operative day. This might be attributed to hemorrhage during internal fixation.

The above findings were in accordance with the findings of Aithal et al., (1998) , Agnihotri (2007), De’ Souza (2012) and Rajhans (2013), who also reported initial decrease and followed by increase in PCV after internal fixation of fracture in canine.

5.4.5 Differential leukocyte count (Per cent)

5.4.5.1 Neutrophil count

Neutrophil count showed non-significant increase in all the groups of dogs followed by a significant decrease. Comparison between three groups showed significantly high neutrophil count in group II and significantly low value in group III, as compared to group I dogs.

Neutrophilia followed by its decrease in postoperative period is a normal response of the body and may be attributed to extent of trauma leading to inflammation and any other infection.

Similarly Saravanan (2002), De’ Souza (2012), Rajhans (2013) and Singh (2015) also reported higher values of neutrophils after fracture fixation in dogs.

5.4.5.2 Lymphocyte count

Lymphocyte count showed non-significant decrease in all the groups of dogs. Comparison between three groups of dogs showed significantly low value of lymphocyte count in group II dogs, as compared to I and III dogs.
This can be attributed to relative variation in neutrophil count which increases initially after surgical intervention and further activates the production of immune regulatory cytokines by macrophages and monocyte. Cytokines are responsible for activation of adrenal axes and increase production of glucocorticoids, which might be responsible for lyses of lymphoid tissue and circulating lymphocytes (Kaneko, 1997).

Zama et al., (1999) and Tembhurne et al., (2010) also reported lymphocytopenia on 10th post-operative days after horn peg fixation of a femoral fracture in canines.

### 5.4.5.3 Eosinophil count

Eosinophil count showed a non-significant difference at different time interval in all the three groups of dogs. Comparison between groups revealed non-significant difference between them.

Similar observations were also recorded by Saravanan (2002), De’ Souza (2012), Rajhans (2013) and Singh (2015).

### 5.4.5.4 Basophil count

Basophil count also revealed non-significant difference between all the three groups of dogs at different time intervals. Comparison between the three groups also showed non-significant difference between them.

Similar observation were recorded De’ Souza (2012) and Singh (2015), during fracture healing.

### 5.4.5.5 Monocyte count

Monocyte count showed non-significant difference at different time interval. Similarly non-significant difference was observed between the groups.

Similar observation were also reported by Zama et al., (1999), De’ Souza (2012) and Singh (2015), who reported non-significant difference in monocyte during fracture healing.
5.5 Biochemical Parameter

5.5.1 Alkaline phosphatase

The mean values of enzyme serum alkaline phosphatase showed significant increase from 12\textsuperscript{th} post-operative day, followed by significant decreased in all the three groups of dogs.

Comparison between all the three groups revealed non-significant difference between them. However the laser treated group III showed higher alkaline phosphatase activity as compared to the group I and II.

In the present study, increase in the level of alkaline phosphatase, from 12\textsuperscript{th} post-operative day might be due to increased osteoblastic activity. Osteoblast secretes large quantities of alkaline phosphatase, which is involved in the process of matrix formation and its mineralization. Alkaline phosphatase is believed to either increase the concentration of local inorganic phosphate or inorganic pyrophosphate, which is an inhibitor of hydroxyapatite crystal that is necessary for fracture healing (Volpin \textit{et al.}, 1998).

Significant decreasing trend in the value of alkaline phosphatase in the 72 day might be indicative of cessation of osteoblastic activity and receding of the values towards its base value due to ossification and consolidation of fractured bone.

The findings observed in laser treated groups, well collaborated with the findings of Pinheiro and Frame (1992), who observed that LLLT biomodulated positively to osteoblasts non-differentiated mesenchymal cells, that would more rapidly change to osteocytes.

Similar observation was also reported by Ozawa \textit{et al.}, (1998), who studied the stimulatory effect of low power laser irradiation on bone formation in vitro and reported that LLLT increases cellular proliferation, bone nodule formation and alkaline phosphatase activity.

Guzzardella \textit{et al.}, (2002) also observed significantly higher level of alkaline phosphatase in the laser treated bone defect healing in femur of rat and concluded that low level laser therapy stimulation could accelerate bone healing.
5.5.2 Total protein

The dogs of group I showed non-significant changes in total protein whereas, in group II significant increase was observed from 24th day and 12th day onwards in group III. However non-significant difference was observed between them.

Singh (2015) in dogs observed protein level towards lower reference range during initial phase of fracture healing and suggested that it might be due to inflammatory condition.

In contrary to this significant increase in total protein level at 12th and 24th postoperative day in group III and II, might be due to effect of laser, which may have enhanced the protein synthesis.

5.5.3 Serum calcium

Serum calcium showed non-significant decrease at 48th day in group I whereas, 24th day in group II and III dogs. However comparison between all the three groups revealed non-significant difference between them.

The gradual decrease in serum calcium level could be due to deposition of the excessive calcium at the fracture site and further increase in its value on 72 postoperative day attributed to remodeling phase. Newton and Nunamaker (1985) were of opinion that acid phosphatase released by osteoclast first cause demineralization, which may be responsible for high level of calcium in serum and then removal of organic matrix.

The above findings are in accordance with the finding of Komnenou et al., (2005), De' Souza (2012), Rajhans (2013) and Singh (2015), who reported initial decrease in mean concentration of serum calcium followed by increase and returned to normal value.

5.5.4 Serum phosphorous

Non-significant decreasing trend in serum phosphorous was observed from 12th day onward in all the three groups. Comparison between all the three groups revealed non-significant difference between them.
This might be attributed to osteoclastic activity leading to resorption of dead bone resulting in gradual decrease in serum phosphorus as observed in present study.

The above findings are in accordance with the finding of De’ Souza (2012), Rajhans (2013) and Kumar (2016), they reported initial decrease in concentration of serum phosphorus followed by increase and returned to normal value.

5.6 Complications

The dogs of the group I showed slight exudation on 4th day, traumatic wound 48th post operative day and pin migration on 72th post operative day, however the dogs of the group II and III, did not revealed any post operative complications and the fracture healing was completed with in 72th post operative day.

This might be because of mismanagement of the dog owner and the temperament of the dog or might be due to the dog was allowed to run freely in the field after the achieving the weight bearing.

The present findings are correlated with the findings of Hunt et al., (1980) reported 24 percent complication in the fracture of femur and observed that complication of internal fixation are caused by errors of technique, such as choice of inappropriate method of fixation, the use of an implant of inadequate size and strength to stabilize the fracture, errors in application of the implant and excessive damage to the tissues during surgery.

Hansda et al., (2012) also conducted a study on comparative evaluation of steinmann pin, kuntscher nail and interlocking nail for femur fracture repair in 18 dogs and observed complications of seroma formation, and pin migration in group I whereas rotational malposition was observed in group II.
6. SUMMARY, CONCLUSION AND SUGGESTIONS FOR THE FURTHER WORK

6.1 Summary

The present study was conducted on 18 dogs, aged between 1-8 years brought to T.V.C.C, Jabalpur for the treatment of long bones fracture. The dogs were selected irrespective of their sex, breed and body weight. These dogs were randomly divided into three equal groups, consisting of 6 animals in each group.

In group I (control group) intramedullary pinning was done alone followed by antiseptic dressing. In group II and III intramedullary pinning along with low level laser therapy and antiseptic dressing was done for seven days. In group II laser therapy was done on one side of fractured bone, whereas, in group III, laser therapy was done on both sides of fractured bone.

In the present study, out of eighteen clinical cases of long bone fractures, eight cases were of 1 to 3 years of age, six were 3 to 6 years of age and four of 6-8 years of age. The fracture were common in male than female dogs. Automobile accident was the most common cause of fracture, followed by dog bite, falling from roof and hitting by stick. Maximum non-descript dogs were affected with fracture followed by Labrador retriever and German shepherd breed. Out of the 18 dogs, the 7 dogs (38.88%) were presented immediately after injury whereas, 8 dogs (44.44%) ,3 days after injury and 3 dogs presented more than 3 days after injury.

Rectal temperature, respiration rate and pulse rate recorded in all groups revealed significant decrease for short period. In laser treated groups postoperative inflammation was less as compare to control group. The weight bearing score during standing, walking and running improved significantly from 4th day onwards in the dogs of group III subjected with both side LLLT and at 6th day onwards in group II dogs (one side LLLT). Slight weight bearing was observed from 8th day onwards in group I dogs.
Fracture healing was assessed by evaluating radiographs, which were taken prior to surgery and subsequently on 12th, 24th, 48th and 72th post operative days of immobilization. In the dogs of group I, radiograph score of 12th post-operative day depicted perfect fixation of intramedullary pins and initiation of periosteal reaction and development of primary callus on 24th post-operative day. While radiopaque structure on 48th post-operative day and extensive periosteal and endosteal callus on 72th post-operative day were indicative of the start of remodeling.

Radiographic evaluation of group II dogs or dogs with one sided LLLT on 12th postoperative day, exhibited proper fixation of the implant with slight increase in the soft tissue density and distinct callus on 24th post-operative day. Massive callus formation on 48th day and organized periosteal as well as intercortical callus was observed on 72th post-operative day.

Radiographic interpretation of the dogs of both side LLLT group on 12th postoperative day showed moderate increase in the soft tissue density and high density callus on 24th post-operative day. Massive and dense callus with homogenesity on 48th day and marked reduction in callus with prominent bone marrow, on 72 post-operative day exhibited complete union of fractured bone.

In all the three groups, the total erythrocyte count showed non-significant decrease on 6th day as compared to day 0. However, this count increased significantly in group III and non-significantly in group I and II, on 12th day postoperatively. Whereas, hemoglobin concentration showed non-significant fluctuations in all the three groups. The packed cell volume decreased non-significantly in group II and III and significantly in group I on 6th day post operatively.

Total leucocyte count showed non-significant increase on 6th day interval in group I and II followed by decrease. However, the dogs of group III showed a significant increase on 6th day interval followed by a significant decrease at 12th day interval.
Neutrophil count showed non-significant increase in all the groups of dogs followed by a significant decrease, however significantly high neutrophil count in group II and significantly low value in group III, were noticed as compared to group I dogs. Lymphocyte count showed non-significant decrease in all the groups of dogs. The eosinophil, basophil and monocyte count showed non-significant differences between the groups.

The mean values of enzyme serum alkaline phosphatase showed significant increase from 12th post-operative day, followed by significant decreasing trend in all the three groups of dogs. Total protein showed non-significant changes in dogs of group I whereas, in group II and III significant increase was observed from 24th and 12th day post-operatively. Serum calcium showed non-significant decrease at 48th day in group I whereas, 24th day in group II and III dogs. Non-significant decreasing trend in serum phosphorous was observed from 12th day onward in all the three groups and comparison between all the three groups revealed non-significant difference between them.

Therefore on the basis of above findings it can be concluded that fracture healing was more in laser treated groups II and III as compared to group I which was subjected to Intramedullary pin alone without laser therapy.
6.2 Conclusion

On the basis of present study following conclusions can be drawn:

- Low level laser therapy alleviates pain, inflammation and exudation at the fracture site and increases the pin–bone contact, therefore it nullify the pin migration.

- Low level laser therapy promotes the fracture healing by stimulating the osteosynthesis at fracture site.

- Remodeling of the bone at an earlier phase is governed by LLLT.
6.3 Suggestion for further work

1. The study may be conducted for fracture healing in large animals.

2. Study may be conducted on application of LLLT for osteoarthritis in older dogs.

3. Clinical application of laser therapy in acute inflammatory conditions of joints in dogs.

4. Histological study can be done to support the fracture healing at cellular level.
7. REFERENCES


