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Effect of static magnetic field, transcutaneous electrical nerve stimulation on fracture healing in rabbits

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Abstract
In the present study the static magnetic field and transcutaneous electrical nerve stimulation therapy, alone and in combination on fracture healing in rabbits was studied. In the animals fracture was induced under general anesthesia using electrical bone saw with two blades creating bone gap of 1.65-2.93mm between fracture fragments. Complete union (100% reduction) was observed by 5th week post-surgery only in the animals of group treated with both the therapies, complete union (100% reduction) in the animals with single therapy was observed by 9th week while in the animals of group with no therapy, 97.31% of reduction in gap was recorded by the end of the study period.

Keywords: Rabbits, Static Magnetic Field, Transcutaneous Electrical Nerve Stimulation, Fracture, Callus

1. Introduction
Fractures are common in domestic animals. Under stressful or continuous compressive conditions, the ability of the bone tissue to tolerate strength decreases and results in fracture. Fracture healing involves a series of events including hematoma formation, inflammation, soft cartilaginous callus formation, neovascularization, soft callus mineralization, hard callus formation and osteoclastic remodeling of the hard callus to differentiate the callus to the lamellar bone [1]. Fracture healing shows much similarity with soft tissue healing, but its ability to be completed without formation of scar tissue is unique [2].
A variety of therapeutic modalities have been developed to enhance the healing response and fill the bone defects [3]. Electromagnetic stimulation of bone leads to changes in the intracellular Ca^{2+} concentration and consequent alteration in the transport of ions [4]. Recently, however interest in magnetic field therapy has revived and a variety of products are available for treatment of fractures in humans and horses [5]. Biomagnetics is an interdisciplinary field where magnetism biology and medicine overlap [6]. In canine practice, static magnets has been implanted into the bandaging at the fracture site and reportedly has been resulting a decrease in the healing time of fractures by 40-50% [7]. It has been reported, that SMF enhanced blood flow to the site of surgery, thus pooling oxygen and nutrients thereby speeding the overall healing process [8]. Transcutaneous electrical nerve stimulation has ability to stimulate physiology and growth factors at injured site which has a positive effect on skin wound healing and accelerates the callus formation [9]. Electrical stimulation accelerates bone healing by centripetal electrical field effect [10]. Electric current stimulates the fracture healing by stimulating the synthesis and secretion of growth factor and factors differentiating the tissues like Bmp-4 and Bmp-2 [11]. Magnetic and transcutaneous electrical nerve stimulation therapies have been used separately for orthopedic conditions, wound combined use of magnetic and TENS therapies for fracture healings.

2. Materials and Methods
2.1 Selection of animals
The present study was conducted at the Division of Veterinary Surgery & Radiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K, Shuhama, Srinagar, Kashmir. The study was conducted on 24 apparently healthy adult rabbits of either sex (9-15 months) with their body weight ranging between 1–2.5 kg. The animals were tagged and housed

individually in their cages at Mountain Research station for sheep and goat, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K, Shuhama, Srinagar, Kashmir. All the animals were reared under identical managemental conditions for one week before starting the actual study.

2.2 Preparation
The animals were pre-fasted and denied access to water six hours before the start ohe surgical procedure. The animals were weighed and subjected to a thorough physical and clinical examination. Either right or left, whole of the forelimb was shaved and scrubbed with antiseptics prior to the actual start of the study.

2.3 Anesthesia
The animals were administered xylazine @ 10 mg/kg I/M, then left alone in a calm environment for 5 minutes followed by administration of ketamine hydrochloride @ 50 mg/kg I/M.

2.4 Surgical Procedure
The rabbits were positioned in a lateral recumbency and forelimb was scrubbed once again using 7.5% Povidone-Iodine cleansing solution and draped. A longitudinal incision along the cranial aspect of the limb overlying the radius was given. Care was taken not to damage or severe the cephalic vein, proximal radial artery and the cutaneous nerve, which lie close to the site of incision. Skin was retracted to expose the underlying muscles. With the help of Iris scissors, the muscles extensor carpi radialis (anteriorily), extensor digitorum communis, abductor digiti I longus, extensor digitorum lateralis (laterally) and pronator teres (medially) were separated. The muscles were then retracted by Allis tissue forceps to expose the underlying radius. The electrical bone cutting saw was placed horizontally over the mid shaft area and unilateral, complete, transverse fracture of the radius was created. Care was taken not to damage any of the muscles. The fractured bone and the site was flushed with sterile normal saline solution to remove any debris present at the site. The forceps were removed and the muscles were allowed to come back to their original anatomical position. The skin incision was sutured with fine Nylon. Povidone-Iodine ointment (5%) was applied over the surgical site followed by proper bandaging of the area. Post-operatively, animals of both the groups were given analgesic and antibiotic for 3rd and 5th days respectively. The sutures were removed on 12th post-operative day. All the animals were managed for a period of 10 weeks under identical conditions.

2.4 Experimental Design
The animals received therapeutic protocol as detailed under.
Treatment provided/Magnetic Therapy/Transcutaneous electrical nerve stimulation therapy

Groups	No. of animals	Therapy provided
1	6	No therapy (Control group)
2	6	Static magnetic field (SMF) for 6 hours on daily basis
3	6	Trans cutaneous electrical nerve stimulation (TENS) for 10 minutes on daily basis
4	6	Both TENS and SMF for 10minutes and 6 hours respectively on daily basis

2.6 Radiography
Orthogonal cranio-caudal and medio-lateral radiographs of the fracture site were taken just before the start of the surgical procedure, 0 hr (immediately after surgery) followed by weekly intervals for a period of 10 weeks using. The factors used during this study varied from 45-55kVp with constant 10 mAs and 90 cm film focal spot distance. The radiographs were evaluated quantitatively as well as qualitatively. The limbs of each experimental animal were radiographed preoperatively for ensuring the correctness of exposure factors and normality of the bone. No radiograph of any animal depicted any developmental or acquired abnormality warranting its expulsion from the experiments.

3. Results and Discussion
Features of fracture healing are evaluated quantitatively as well as qualitatively. Progression of fracture healing is quantified using a radiographic bone callus index based on Milles index [12]. Veriner's Caliper was used to measure the maximum length and width of bony callus at four points on the lateral radiographs; cranial, caudal aspects of the proximal radius, the cranial, caudal aspects of the distal radius. By 1st week, postoperative fracture gap was found widened with no evidence of periosteal reaction and callus formation in any animal of any group. Periosteal reaction was discernable only in 3 animals of group 4 by 2nd week postoperatively. However, by 3rd week postoperatively, periosteal reaction was seen in the animals of other treated groups too, while it was still lacking in the control group. Interpretation of 3rd week, postoperative radiographs depicted discernable bridging callus only in 4 animals of group 4. Callus formation started in the animals of group 2 and 3 by 4th week postoperatively. Fracture gap had got obliterated by the callus formation, completely in the animals of group 4, partially in animals of group 2 and scantly in animals of group 3 by 4th week postoperatively.

Even periosteal reaction was not describable by this period of study in group 1.

By 5th week, callus formation was discernable, significantly in the animals of group 4 and moderately in the animals of group 3 and 2. The periosteal reaction was first time observed in the animals of group 1 by 6th week postoperatively. By this time the fracture gap had got completely bridged leading to primary union in the animals of all treated groups. Callus formation was discernible in control group from week 7 – 10 postoperative without sign of any kind of union at the fracture site. By 10th week postoperatively, complete healing was seen in all the treated groups. The callus was dense, significantly in group 2 and 4 animals, and moderate in group 3 animals. External callus was not observed on any radiograph of any animal except one animal of group 3. Remodeling was noticed only in group 4 animals by the end of the study period (Plate 1- Plate 12).

Radiographic bone callus index based on Milles has been commonly used by the researchers for assessing fracture healing [13]. Qualitative assessment of fracture healing includes the relative amount of periosteal reaction and bony callus, overall callus quantity and degree of bridging callus at the osteotomy site. The subjective evaluation of fracture healing is a routine process [14]. In this study quantitative evaluation of fracture healing was measured in terms of gap reduction with the help of caliper. The gap in the fracture was traced on cellphone paper and then measured by caliper. This quantitative assessment of fracture healing proved very simple and ready to use. Mean±S.E values of gap (mm) between fracture fragments in all the groups at different observational intervals are presented in Table 1 and Table 2. An overall mean gap of 1.60mm to 2.93mm distance was observable between fracture fragments at the start of the study period. Postoperatively, a slight increase in gap was seen by week 1 in all the groups. The fracture gap started to reduce from week 2 postoperatively in all the groups. The reduction in gap was progressive in all the groups but faster in group 4 animals followed by groups 2, 3 and 1 respectively. In the animals of group 4 complete union (100% reduction) was observed by 5th week post-surgery. There was still 0.77mm, 0.57 and 1.17mm gap in the animals of group 1, 2 and 3 respectively by 5th week. Complete union (100% reduction) in the animals of group 2 and 3 was observed by 9th week while in the animals of group 1 only 97.31% of reduction in gap could be achieved by the end of the study period. Comparison among the groups revealed that reduction in gap was significantly ($p<0.05$) higher in the animals of groups 2 and 4 by 4th week post-operatively as compared to those of groups 1 and 3 at the corresponding interval. The reduction in gap in group 4 animals was significantly higher by 5th week postoperatively as compared to other groups at the corresponding intervals. The results obtained during this study indicate that the therapies employed have beneficial effect on fracture healing in rabbits. These findings are in concurrence with that of Sharifi et *al.* who reported that in rabbits the effect of TENS on fracture site in treated groups was discernible and in control group there was blurring of fracture gap [8]. The study also is in consonance with that of Saifzadeh *et al.* [13] who reported that in dogs, exposure of fracture site to magnetic field resulted in periosteal reaction earlier than the control group animals. Combined therapies viz application of SMF together with TENS showed a cumulative or additive effect on fracture healing resulted in any early fracture healing and clinical ambulation without side effects than application of SMF and TENS alone. Histomorphology revealed complete healing only in group 4 animals. Previous studies have shown accelerated fracture healing in presence of SMF [15, 16] or transcutaneous electrical nerve stimulation used separately [17, 18, 19]. SMF can stimulate bone healing by promoting osteoblastic differentiation [20, 21].

4. Conclusion

Application of Static Magnetic Field (SMF) and Transcutaneous Electrical Nerve stimulation (TENS) therapies at the fracture site enhances the rate of fracture healing that is radio graphically visible after 4 weeks. The animals receiving both the therapies exhibit comparatively more callus formation and early fracture union. The results are further verified histologically with animals of Group 4 exhibiting increased osteogenic proliferation and increased vascularization at the fracture site. In the light of the above results, it is only proper to conclude that TENS and SMF application do indeed enhance the rate of fracture healing without causing any damage to the physiological, hematological or biochemical parameters of the body.

5. Acknowledgment

We are highly thankful to Division of Veterinary Surgery & Radiology and Mountain Research Centre for Sheep and Goat for providing all the necessary help.

Table 1: The Mean ± SE values of Gap between the fracture fragments of different groups at different observational intervals

Groups	**Weeks**										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	1.60 $\pm0.21^{aA}$	1.70 $\pm0.21^{aA}$	1.42 $\pm0.22^{abA}$	1.15 $\pm0.18^{bcA}$	1.03 $\pm0.17^{bcdA}$	0.77 $\pm0.07^{cdeA}$	0.63 $\pm0.06^{defA}$	0.53 $\pm0.06^{efA}$	0.43 $\pm0.04^{efgA}$	0.25 $\pm0.02^{fgA}$	0.02 $\pm0.02^{g}$
2	1.45 $\pm0.16^{aA}$	1.50 $\pm0.16^{aA}$	1.40 $\pm0.15^{aA}$	1.17 $\pm0.11^{bA}$	0.68 $\pm0.05^{bB}$	0.57 $\pm0.05^{bB}$	0.33 $\pm0.09^{cdB}$	0.27 $\pm0.09^{cdeB}$	0.03 $\pm0.02^{eB}$	0.00 $\pm0.00^{eB}$	0.00 $\pm0.00^{e}$
3	2.93 $\pm0.17^{aB}$	3.00 $\pm0.17^{aB}$	2.68 $\pm0.12^{aB}$	2.27 $\pm0.10^{bB}$	2.00 $\pm0.06^{bC}$	1.17 $\pm0.11^{cC}$	0.95 $\pm0.05^{cC}$	0.53 $\pm0.06^{dA}$	0.25 $\pm0.03^{eC}$	0.00 $\pm0.00^{eB}$	0.00 $\pm0.00^{e}$
4	1.85 $\pm0.18^{aA}$	1.95 $\pm0.18^{aA}$	1.50 $\pm0.18^{aA}$	1.57 $\pm0.19^{aC}$	0.82 $\pm0.07^{bA}$	0.00 $\pm0.00^{cD}$	0.00 $\pm0.00^{cD}$	0.00 $\pm0.00^{cC}$	0.00 $\pm0.00^{cB}$	0.00 $\pm0.00^{cB}$	0.00 $\pm0.00^{c}$

Different small superscript indicate significant difference ($p<0.05$) within the group
Different capital superscript indicate significant difference ($p<0.05$) among the groups

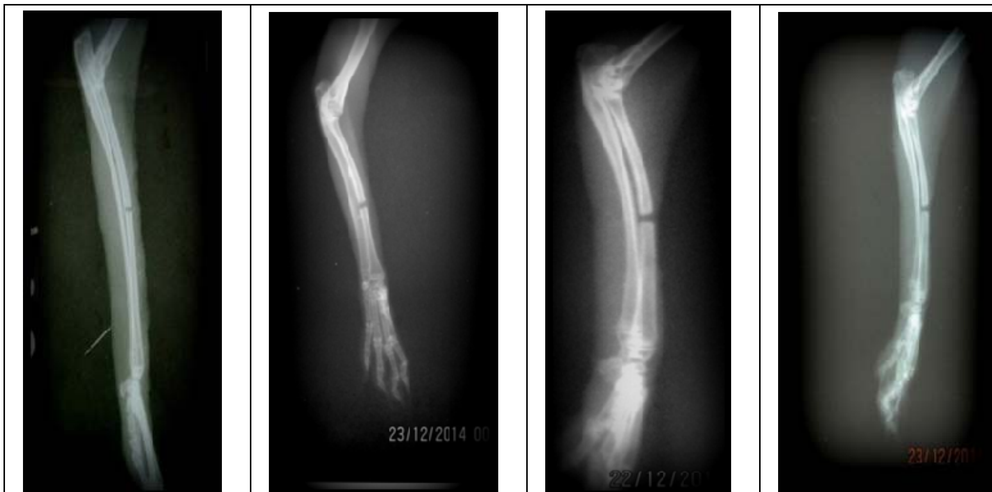
Table 2: The Mean ± SE values of Percentage Gap Reduction of different groups at different observational intervals

Groups	**Weeks**										
	0	1	2	3	4	5	6	7	8	9	10
1	0 $\pm0^{aA}$	9.17 $\pm9.17^{ab}$	23.23 $\pm11.27^{bcA}$	31.22 $\pm10.37^{cdA}$	47.40 $\pm7.81^{deA}$	56.37 $\pm7.56^{efA}$	63.37 $\pm6.14^{efgA}$	72.13 $\pm5.11^{fgA}$	82.55 $\pm3.13^{ghA}$	97.31 $\pm1.87^{hA}$	0 $\pm0^{aA}$
2	0 ± 0^{aA}	9.88 $\pm1.20^{ab}$	15.07 $\pm8.48^{bA}$	50.63 $\pm4.96^{cB}$	59.03 $\pm4.36^{cA}$	77.22 $\pm5.31^{dB}$	82.43 $\pm5.40^{dB}$	97.22 $\pm1.81^{eB}$	100 $\pm0^{eB}$	100.00 $\pm0^{eA}$	0 ± 0^{aA}
3	0 $\pm0^{aA}$	7.93 $\pm3.18^{b}$	2.20 $\pm2.39^{cA}$	31.10 $\pm2.85^{dA}$	59.93 $\pm3.29^{eA}$	66.97 $\pm2.83^{fAB}$	81.78 $\pm1.47^{gB}$	91.55 $\pm0.81^{hB}$	100.00 $\pm0^{iB}$	100.00 $\pm0^{iA}$	0 $\pm0^{aA}$
4	0 $\pm0^{aA}$	10.11 $\pm1.0^{b}$	15.18 $\pm5.51^{bA}$	55.27 ±2.67cB	100.00 $\pm0^{dB}$	100.00 $\pm0^{dC}$	100.00 $\pm0^{dC}$	100.00 $\pm0^{dB}$	100.00 $\pm0^{dB}$	100.00 $\pm0^{dA}$	0 $\pm0^{aA}$

Different small superscript indicate significant difference ($p<0.05$) within the group
Different capital superscript indicate significant difference ($p<0.05$) among the groups

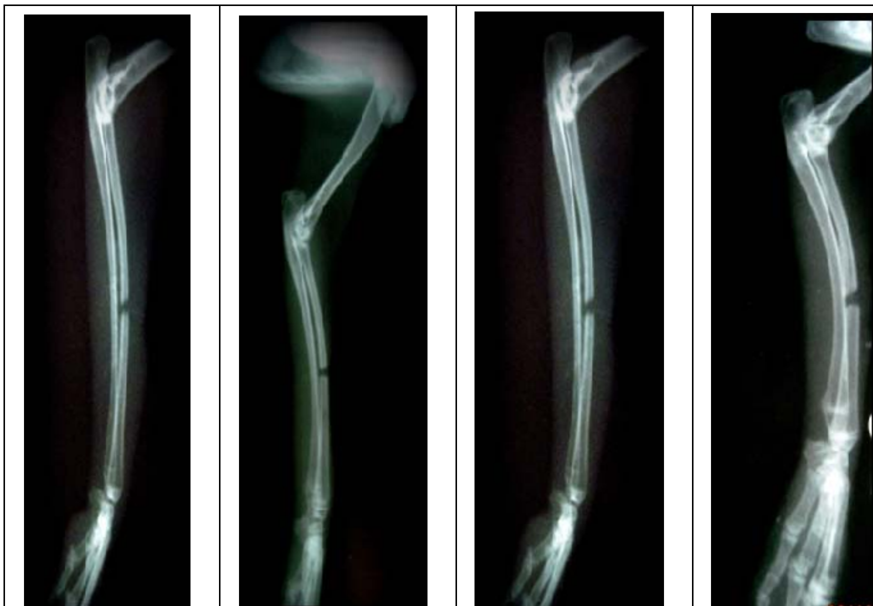
6. Images

Plate 1: Survey Radiograph



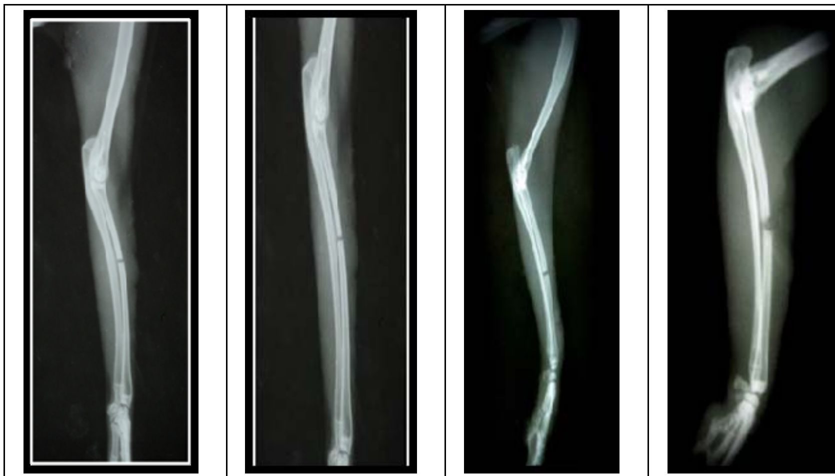
Group 1	Group 2	Group 3	Group 4

Plate 2: Radiographs taken immediately after surgery



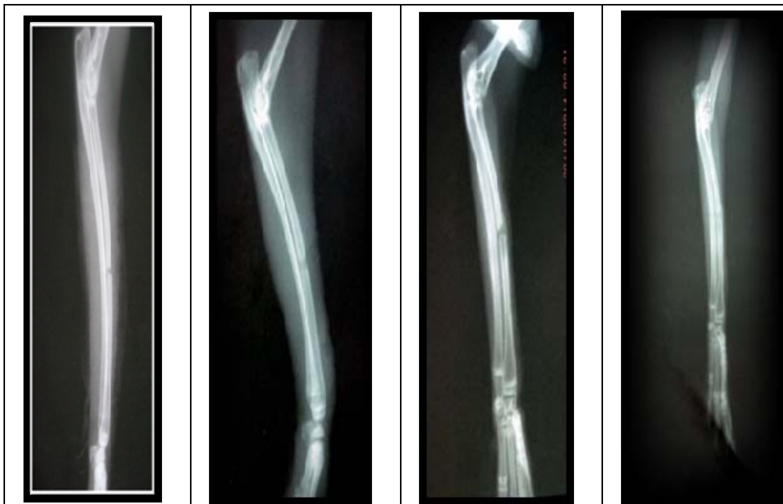
Group 1	Group 2	Group 3	Group 4

Plate 3: 1st week Post operative radiographs



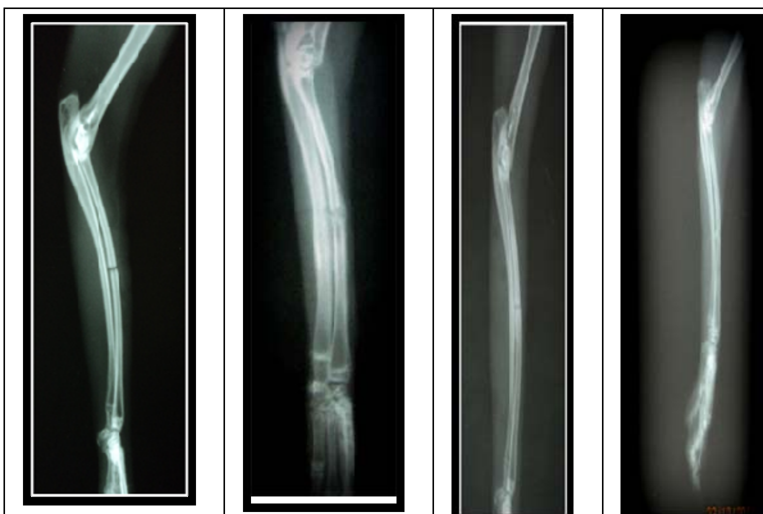
Group 1	Group 2	Group 3	Group 4

Plate 4: 2nd week Post-operative radiographs



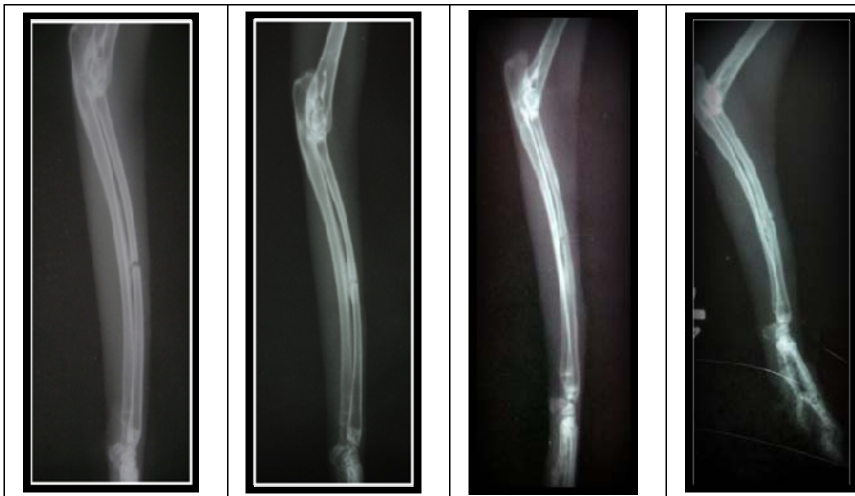
Group 1	Group 2	Group 3	Group 4

Plate 5: 3rd week Post-operative radiographs



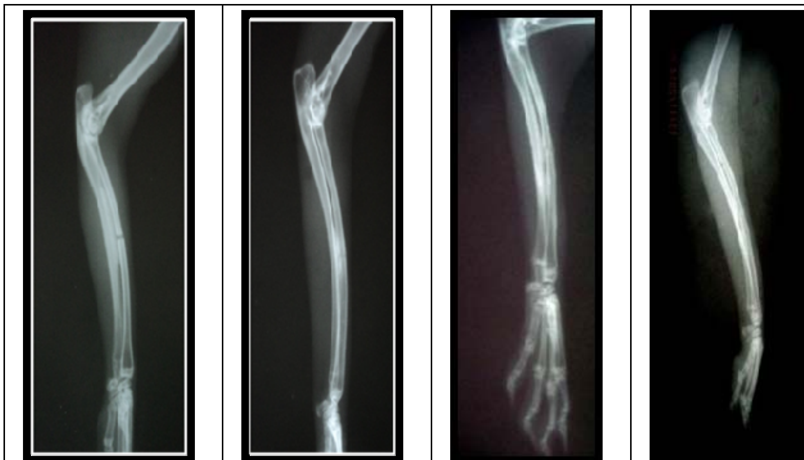
Group 1	Group 2	Group 3	Group 4

Plate 6: 4th week Post operative radiographs



Group 1	Group 2	Group 3	Group 4

Plate 7: 5th week Post operative radiographs



Group 1	Group 2	Group 3	Group 4

Plate 8: 6th week Post operative radiographs

Group 1	Group 2	Group 3	Group 4

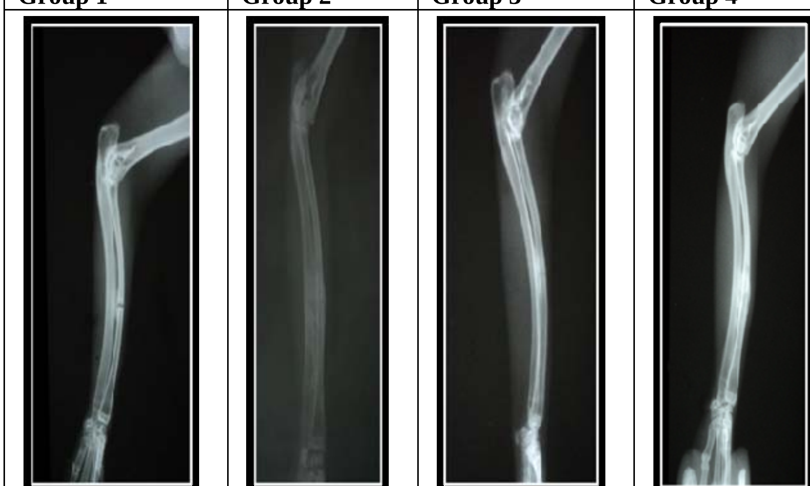


Plate 9: 7th week Post operative radiographs

Group 1	Group 2	Group 3	Group 4
			

Plate 10: 8th weekPost operative radiographs

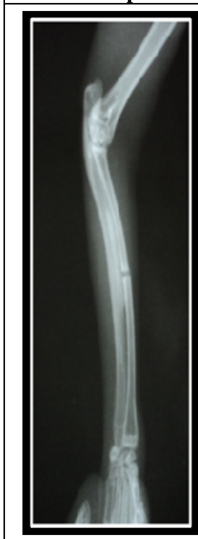
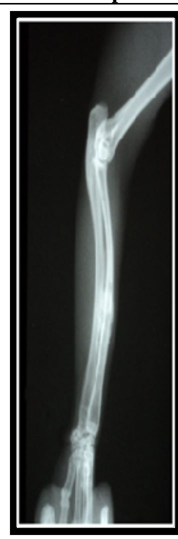
Group 1	Group 2	Group 3	Group 4
			

Plate 11: 9th week Post operative radiographs

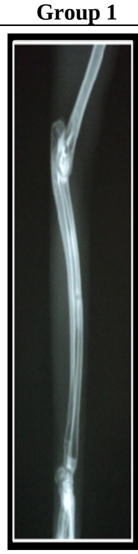
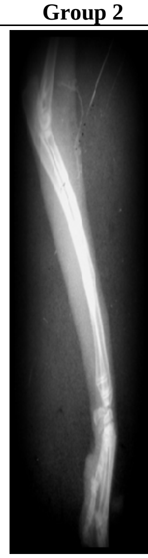
Group 1	Group 2	Group 3	Group 4
			

Plate 12: 10th week Post operative radiographs

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