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**Taziyun Imtiyaz**
Division of Veterinary Surgery and Radiology, FVSc and AH, SKUAST-K, Shuhama Alusteng, Jammu and Kashmir, India

**Firdous AD**
Division of Veterinary Anatomy, FVSc and AH, SKUAST-K, Shuhama Alusteng, Jammu and Kashmir, India

**Neloofer N**
Division of Veterinary Anatomy and Histology, SKUAST-J, Jammu and Kashmir, India

**Corresponding Author:**
**Taziyun Imtiyaz**
Division of Veterinary Surgery and Radiology, FVSc and AH, SKUAST-K, Shuhama Alusteng, Jammu and Kashmir, India

# Effect of static magnetic field, transcutaneous electrical nerve stimulation and combined therapies on fracture healing in rabbits: A histomorphological study

**Taziyun Imtiyaz, Firdous AD and Neloofer N**

**Abstract**
The present study was aimed to evaluate the SMF (Static Magnetic Field Therapy) and TENS (Transcutaneous Electrical Nerve stimulation Therapy) and their combined therapies on fracture healing in rabbits (N=24) divided into 4 equal groups. In all the animals fracture was induced using electrical bone saw with two blades creating bone gap of 1.65-2.93 mm between fracture fragments. Post-operatively, all the animals were given analgesic for 3 days and antibiotic for 7 days in addition daily antiseptic dressing of wound for 2 weeks. In group 1, no specific treatment was given and it acted as control whereas group 2 the rabbits were treated with SMF therapy for 6 hours daily. In group 3 the TENS was employed for 10 minutes on daily basis and in group 4 both the therapies were used together for 6 hours and 10 minutes on daily basis respectively. Efficacy of the treatment was monitored by histomorphological evaluation. After completion of the study the animals were slaughtered and the test bone was subjected to Histomorphological study using H and E stain.

**Keywords:** Rabbits, SMF, TENS

## Introduction
Histomorphological study evaluates the effect of electrical stimulation on experimentally created fracture (Sharifi *et al.,* 2006) [20]. The underlined tissue get effect by centripetal electrical field which is controlled by electromagnetic stimulation to accelerate bone healing in more regular fashion (Otter *et al.,* 1998) [18]. Electrical stimulation leads synthesis and secretion of growth factors, lead to differentiation of tissue such as Bmp-4,-Bmp2, which supports the histomorphological findings (Gohot and Bose *et al.*, 1988) [10].
Electrical stimulation causes acceleration of cellular differentiation and potency of osteogenesis leads to shortening of healing period (Sharifi *et al.,* 2006) [20]. The electromagnetic fields induce endothelial cell proliferation and capillary formation (Yen-Patton, 1988) [22], stimulation of matrix formation (Aaron, 1989) [2] and calcification (Norton *et al.,* 1988) [16] by augmenting signals. The theory that led to the application of exogenous electromagnetic energy to stimulate osteogenesis was based on the observation of naturally occurring stress-generated and bioelectric potentials in bone. These electric potentials are believed to drive the bone remodeling process (Friedenberg *et al.*, 1973) [9]. Externally applied electric energy could modify the behavior of bone cells (Bassett *et al.,* 1989) [3]. The electromagnetic stimulation increases the osteoblast proliferation (DeMattei *et al.,* 1999) [7], enhances the osteoblast differentiation (Hartig *et al.,* 2000) [11] and increases bone formation (Aaron and Ciombor, 1995) [1]. Electromagnetic stimulation of bone leads to changes in the intracellular $Ca^{2+}$ concentration and consequent alteration in the transport of ions (Cho *et al.,* 1999) [5].

## Material and Methods
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 apparently 24 healthy adult rabbits of either sex of 9-15 months' age with their body weight ranging between 1 - 2.5 kg. The animals were purchased from Mountain Research station for sheep and goat, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K, Shuhama, Srinagar, Kashmir. The animals were tagged and housed individually in their cages. All the animals were reared under identical managemental conditions for one week before starting the actual study.

**Surgical procedure**

The animals were pre fasted and denied access to water 6 hours before the start of the surgical procedure. The animals were weighed and subjected to a thorough physical and clinical examination. Whole of the forelimb either right or left was shaved and scrubbed with antiseptics prior to the actual start of the study. 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.

The rabbits were positioned in a lateral recumbency. The forelimb was scrubbed 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 electrical bone cutting saw was placed horizontally over the mid shaft area and unilateral, complete, transverse fracture of the radius was created.

Post-operatively, animals of both the groups were given analgesic and antibiotic for 3 and 5 days respectively. The sutures were removed on 12$^{th}$ post-operative day. The animals received therapeutic protocol as detailed in table 1.

At the end of the study period (10 weeks), the animals were slaughtered. The operated forelimb of all the animals was recovered. The limb was deskinned and preserved in 10% formalin solution for histological evaluation. The radial bone obtained was first decalcified using 85% Formic Acid solution followed by routine processing for paraffin embedding. Six micron sections were taken and stained using Harris H&E Stain staining for microscopy

**Table 1:** Treatment provided/Magnetic therapy/Transcutaneous electrical nerve stimulation therapy

| S. No. | 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 |

**Result and Discussion**

Histomorphological examination exhibited thickening of periosteum and presence of necrotic debris was seen at the fracture site in group 1 (plate no. 1a), however hyperplasia of osteogenic cell layer, deposition of peripheral fibrin and more fibroblastic activity was present at the fracture site (plate no. 1b)

Group 2 showed progressive development and differentiation with recognizable spicules of bone. fibrocellular connective tissue surrounded the developing bone. Osteoblasts became entrapped and indicated high presence of bony matrix and presence of osteogenic activity within high vascular tissue (plate no. 2a and 2b),

Group 3 indicated presence of cartilaginous matrix with chondrocyte proliferation, osteoblastic activity within the bone (plate no.3 a) there is presence of predominant soft callus with fibroplasia at the peripherial region (plate no. 3b), and deposition of collagen with neovascularization.

Group 4 animals (plate no. 4 a) revealed presence of ossifying centers with increased bone formation, increased mineralization and extracellular matrix with formation of increased marrow cavities (plate no. 4 b)and remodeling of bone has occurred (plate no. 4 c). As evidence from radiographic studies, by 10$^{th}$ postoperative week union was complete in all treated groups, with presence of remodeling in some animals of group 4. However histomorphology revealed complete healing only in group 4 animals. Previous studies have shown accelerated fracture healing in presence of SMF (Saifzadeh *et al.*, 2007, Bruce *et al.*, 1987, Darendeliler *et al.*, 1997) [4, 6, 19] or transcutaneous electrical nerve stimulation (Sharifi *et al*, 2006, Friedenberg and Brighton, 1971) [9, 20] used separately.

SMF can stimulate bone healing by promoting osteoblastic differentiation (Kotani *et al.*, 2002, Yamamoto *et al.*, 2003) [13, 21]. This is apparently due to the effect of increased blood supply due to magnetic field and thus pooling of oxygen and nutrients (Man *et al.*, 1997) [14] resulting into increased metabolic activity (Kobluk *et al.*, 1994) [12] at fracture site. Increased blood flow also brings natural healer to the site (Null, 1998) [17]. It has also been shown in the *in- vitro* studies that magnetic field increased collagen production in rabbit marrow fibroblasts (Bruce *et al.*, 1987) [4]. Magnetic field is also believed to increase adherence of calcium ion to the blood clot at fracture site, thus allowing proper formation of callus needed for fracture healing (Messonnier, 2001) [15].

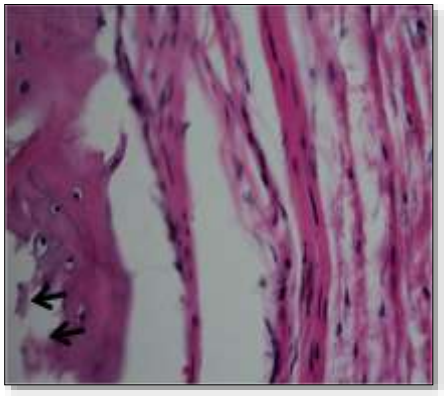
Transcutaneous electrical nerve stimulation results in faster healing due to stimulation of osteogenesis and synthesis of growth factor that lead to differentiation of tissue such as Bmp-4 and Bmp-2 (Gohat *et al.*, 1988).

**Summary and Conclusion**

In all the previous studies magnetic field and electric stimulation have been used separately and have not been compared with each other. In the present study these treatments have been compared and it was found that SMF resulted in more stimulation of fracture healing than TENS. This is evident radiologically as well as histomorphologically. In the present study the two treatments have been used alone as well as in combination and it was noted that when used in combination the two treatments have additive effect. Complete union with remodeling was seen in group 4 animals only.

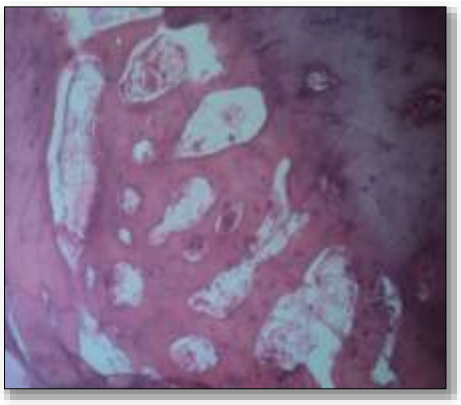
**Group 1 (Plate 1)**

**(a) L.S. of radius of rabbit. H.E. x 10**
Revealing necrotic debris of the bone (arrow), thickening of the periosteum



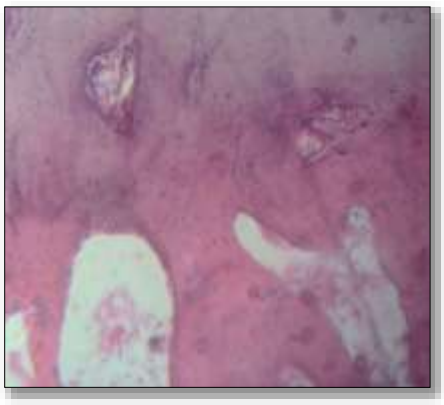
**(b) L.S. of radius of rabbit. H.E. x 40**
Hyperplasia of osteogenic layer, peripheral fibrin deposition and fibroblastic activity

**Group 2 (Plate 2)**



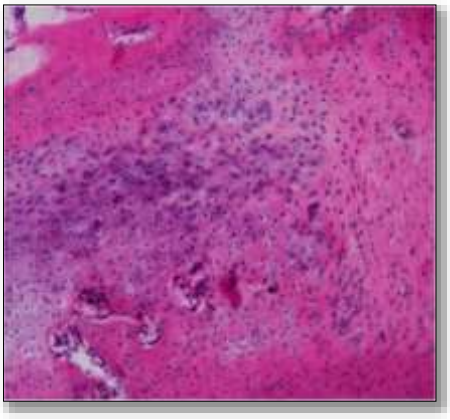
**(a) L.S. of radius of rabbit. H.E. X 10**

1. There is a clear cut bone formation with high presence of bony spicules.
2. Fibrocatilagenous connective tissue
3. Osteoplastic activity with deposition of dense matrix at the fracture site

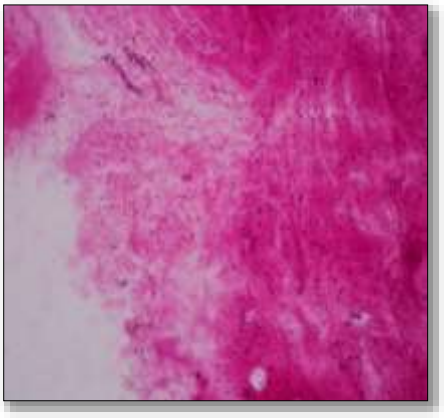


**(b) L.S. of radius of rabbit. H.E. X 40**
Entrapped osteoblasts

**Group 3 (Plate 3)**

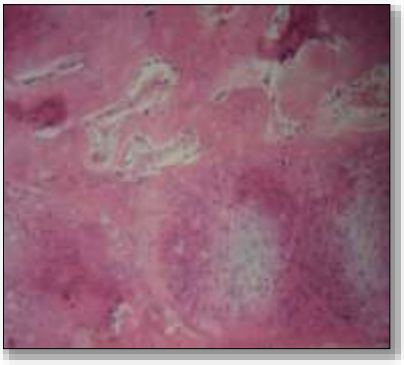


**(a) L.S. of radius of rabbit. H.E. x 10.**
Deposition of cartilagenous matrix and Chondrocyte proliferation
Osteoblasts within the bone

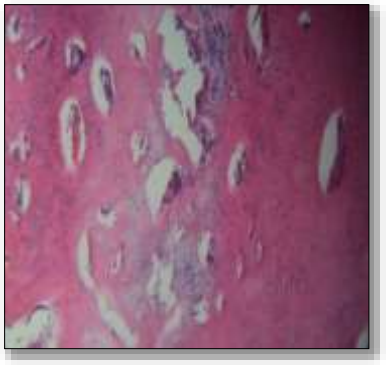


**(b) L.S. of radius of rabbit. H.E. x 10**
Soft callus predominated by fibroplasia at the peripherial section

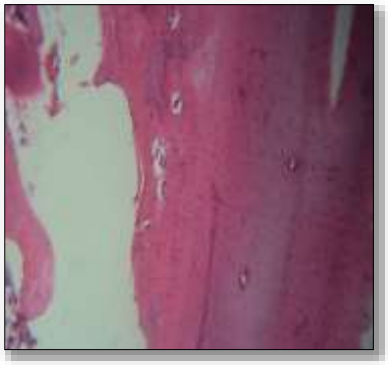
**Group 4 (Plate 4)**



**(a) L.S. of radius of rabbit. H.E. x 10**
1. Increased bone within ossification centre
2. Interosseous spaces



**(b) L.S. of radius of rabbit. H.E. x 10**
Mineralized extracellular matrix and formation of marrow cavities



**(c) L.S. of radius of rabbit. H.E. x 10**
Remodelling of bone

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