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A comparative biochemical study of ultrasound and interferential therapy for bone healing in rabbits

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Abstract
In present study 18 New Zealand White rabbit were randomly divided into 3 equal groups. For comparing the therapeutic efficacy we developed a rabbit model with 2mm bone defect in mid-shaft of right radius. The animals in the group A did not receive any physiotherapy and kept as control, whereas, groups B and C animals were treated with low intensity pulsed ultrasound (0.5 watt/cm^2) and bipolar interferential therapy (100 KHz), respectively, for 10 min on alternate days up to 2 weeks. In all the animals initially there was decrease in serum calcium level up to 7th to 15th day followed by an increase. However, Group B animals exhibited significantly low calcium at 15 days as compared to other groups of animal. In animals of groups B and C, phosphorus level significantly increased from 15th day to last of the observation period, whereas, in group A animals showed these significant increased level on 30th and 60th day. In group B, also exhibited significant higher alkaline phosphatase levels and low lipid per oxidation level as compared to interferential and control group animal. In group B animals cortisol level did not vary considerably at different time intervals. However in group c animal's significantly higher cortisol level was observed.
Thus ultrasound therapy resulted in faster bone healing and early resolution of physiological stress due to creation of bone defect in comparison to interferential therapy.

Keywords: ultrasound, interferential therapy, bone healing, physiological stress

Introduction
Fractures healing are one of the clinical problems in humans and animals too. Most of the fractures do not heal well. With the aim of reducing the substantial incapacity associated with bone fracture and the high socioeconomic costs, a variety of interventions have been studied, including the use of low-intensity pulsed ultrasound (LIPUS) (Kristiansen *et al.,* 1997) [1]. LIPUS has a strong positive influence on inflammation, repair, and remodeling of bone, because it can enhance bone repair by angiogenic, chondrogenic, osteogenic activity and by promoting bone resorption in the osteotomy area (Rubin *et al*., 2001) [2]. Cortical bridging was earlier following LIPUS therapy in fresh fractures (Bashardoust *et al*., 2012) [3]. Another form is Interferential therapy which produces a range of effect within the tissues. Interferential therapy is now effectively used by physiotherapist to produce rapid inhibition or blocking of pain impulses within the central nervous system, stimulate muscle contraction, increase blood flow, reduce swelling, and stimulate healing. It was used to stimulate mandibular bone fracture without surgical intervention. The current was applied through the soft tissue around the bone as well as through the bone (Ganne *et al.,* 1979) [4].

Material and Methods
The animals used in this experiment consisted of 18 New Zealand White rabbit of either sex, 6-8 months old, clinically healthy, average weight 1-1.3 kg. Animals were kept off fed for 8 hours but water was not withheld before surgery. Animals were anesthetized using xylazine (6 mg/kg body weight) followed, 10 minutes later by ketamine (60 mg/kg body weight) by intramuscular route (Amarpal *et al.,* 2010) [5]. Approximately 2mm bone defect was created mid shaft of right radius. Irrigation with saline was used to avoid overheating. Adequate stability was achieved by leaving the ulna intact without any fixation of the radius. The muscle and subcutaneous tissue were closed over as a deep layer using 4/0 absorbable catgut sutures and skin was sutured using 2-0 nylon with horizontal mattress suture pattern. Postoperatively all the animals were treated with cefotaxim @ 50 mg/kg and meloxicam @ 0.5mg/kg body weight once in a day by intramuscular route for 3 days. The animals were randomly divided in three equal groups. The animals in the group A did not receive any physiotherapy and kept as control group. Group B and C, animals treated with the ultrasound therapy and interferential

therapy respectively on alternate days up to 2 wks. To assess the values of serum calcium phosphorus and alkaline phosphatase blood samples were collected from animals on the day of surgery at day 0 and 7th, 15th, 30th and 60th day. Serum calcium, phosphorus and alkaline phosphatase level was assayed using the commercial research kit produced by span diagnostic Ltd. India. Data were subjected to statistical analysis by using one way analysis of variance (ANOVA) followed by Duncan test, using SPSS 17.0 version. The level of statical significance for all compression was established at $P<0.05$.

Result and Discussion

a) Serum calcium: In all the animals initially there was non significant decrease in serum Ca level up to 7th day followed by nonsignificant increase except in group B animals, which showed further significant decreased level of serum Ca level on 15th day thereafter showed nonsignificant increase throughout the observation period. These significant decreased levels of serum Ca could be due to increased urinary excretion after traumatic bone injury as stated by Kumar *et al.* (1992) [6]. Our findings are in agreement with the earlier workers viz., Komnenou *et al.* (2005) [7] and Paskalev *et al.* (2005) [8].

b) Serum inorganic phosphorus: In animals of groups A, phosphorus level showed increasing trend throughout the study period and this increase was significant on 30th and 60th day. In animals of group B and C, phosphporus level significanty higher from 15th day to the 60th day as compared to the base value. Thereafter, the level began decreasing towards the base value however it did not reach the base value till 60th day but remained significantly higher than the base value. The early rise in serum phosphorus level could be due to the necrotic disintegration of cells at the site of fracture (Singh *et al.,* 1976) [9]. All the values were within the normal physiological limits. These results are in agreement with the findings of Rani *et al.* (2012) [10].

c) Serum alkaline phosphatase: In group A, the mean value of ALP showed nonsignificantly increasing trend up to 30th day, thereafter, it decreased significantly on 60th day. In groups B, ALP level showed a significantly increasing trend up to the 15th day. Thereafter, it decreased but did not reach the base value and remained significantly higher than the base value. In the animals of group C, the mean value of ALP showed a nonsignificant increase on 7th day and then it decreased nonsignificantly throughout the observation period. Low intensity pulsed ultrasound has been highly efficacious in the treatment of fresh fracture healing. The increase in level of alkaline phosphatase following bone injury was attributed to exuberant proliferation of fibrous tissue at fracture site, proliferating osteogenic cells maturing and active osteoblast and the formation of bone matrix (Umashankar and Ranganath, 2008) [11]. These finding are in agreement with the earlier findings viz. Komnenou *et al.* (2005) [12] and Shivprakash (1995) [13] noticed increased acitivity of alkaline phosphatase during early period of fracture healing in dogs and goats.

d) Plasma Cortisol: In the animals of groups A and C, cortisol value increased significantly up to the 15th day, then decreased nonsignificantly up to the 60th day. However, could not reach to the base value. Surgical procedure in the form of creation and fixation of tibial fracture under the general anaesthesic induced a significant hypercortisolaemic response indicative of great surgical stress in control group of dogs (Chastain *et al*., 1986) [14]. This significant increase might be due to increased release of cortico releasing factor in response to trauma will lead to increased secretion of adreno-corticotropin hormone (ACTH) from adenohypophysis (Bhardwaj *et al.,* 2011) [15]. In group B, Cortisol level did not differ significantly at different time interval from the respective base value. In group C, high level of cortisol might be due to release of beta-endorphin and ACTH from the pituitary. ACTH induced the release of cortisol from the adrenal glands, which increase the blood cortisol level (De Domenico G. 1982) [16]

e) Plasma lipid peroxidation: Bone fractures are associated with inflammation and ischemia, stimulating free radical oxidation (Petrovich *et al.,* 2004) [17]. In group C, LPO level showed significantly higher value on 7th day and declined towards the base value up to the 60th day but remained significantly higher in comparison to the base value up to 30th day. Prasad *et al.* (2003) [18] they revealed that oxidant level rose by the 2nd and 3rd weeks, perhaps due to callus formation and angiogenesis and also opined that oxidative stress is proportional to the number of bone fractured. Our findings are in agreement with that of Yeler *et al.* (2005) [19] and Turgut *et al.* (1999) [20].

Table 1: Mean ± SE values of serum Ca (mg/dl) in different groups at different time intervals

Group	0	7	15	30	60
A	11.24±0.65	10.77±0.93	10.94±0.41	11.87±0.34	12.96±0.81
B	12.23^{bc}±0.86	10.18^{ab}±0.84	9.49^{a}±0.89	13.27^{c}±0.24	14.29^{b}±0.62
C	12.42^{ab}±0.63	10.56^{a}±0.55	11.19^{a}±0.49	13.49^{b}±0.56	11.91^{ab}±0.80

Values in the same row without a common superscript letter a- e are significantly different ($P<0.05$)

Table 2: Mean ± SE values of phosphorus (mg/dl) in different groups at different time intervals

Group	0	7	15	30	60
A	5.35^{aA}±0.35	5.6^{aA}±0.33	6.29^{abA}±0.32	6.80^{bA}±0.13	6.83^{bA}±0.14
B	6.40^{aB}±0.24	7.12^{abB}±0.17	8.05^{bcBC}±0.36	7.93^{bcAB}±0.38	8.16^{cB}±0.31
C	6.32^{aB}±0.31	6.76^{abB}±0.08	7.81^{bB}±0.21	7.35^{bAB}±0.50	7.48^{bAB}±0.25

Values in the same row without a common superscript letter a- e are significantly different ($P<0.05$)
Values in the same column without a common superscript letter A- E are significantly different ($P<0.05$)

Table 3: Mean ± SE values of alkaline phosphatase (U/L) in different groups at different time intervals

Group	0	7	15	30	60
A	112.74^{b}±14.38	125.55^{bA}±10.55	127.24^{bA}±18.43	145.81^{bA}±14.86	64.99^{aA}±12.15
B	126.19^{a}±5.73	179.15^{bAB}±7.65	224.09^{cC}±4.07	218.4^{cBC}±9.80	157.41^{bAB}±14.99
C	108.24±23.0	156.81^{AB}±32.08	156.15^{AB}±28.59	149.62^{A}±34.98	133.21^{AB}±56.07

Values in the same row without a common superscript letter a- e are significantly different (P<0.05)
Values in the same column without a common superscript letter A- E are significantly different (P<0.05)

Table 4: Mean ± SE values of Cortisol (µg/dl) in different groups at different time intervals

Group	0	7	15	30	60
A	1.18^{a}±0.08	1.65^{bcB}±0.07	1.99^{cB}±0.10	1.56^{ab}±0.22	1.38^{ab}±0.09
B	1.16±0.11	1.48^{AB}±0.06	1.50^{A}±0.11	1.41±0.12	1.39±0.28
C	1.31^{a}±0.15	1.78^{bB}±0.08	1.80^{bB}±0.12	1.58^{ab}±0.16	1.39^{ab}±0.15

Values in the same row without a common superscript letter a- e are significantly different (P<0.05)
Values in the same column without a common superscript letter A- E are significantly different (P<0.05)

Table 5: Mean ± SE values of lipid peroxidasion (nmol MDA/g Hb) in different groups at different time intervals

Group	0	7	15	30	60
A	1.65^{a}±0.08	3.27^{b}±0.54	3.05^{b}±0.25	2.45^{ab}±0.57	2.11^{ab}±0.16
B	1.56^{a}±0.08	2.91^{b}±0.28	2.84^{b}±0.37	2.51^{ab}±0.31	2.30^{ab}±0.62
C	1.66^{a}±0.19	3.04^{c}±0.18	2.64^{bc}±0.19	2.39^{bc}±0.20	2.16^{ab}±0.34

Values in the same row without a common superscript letter a- e are significantly different (P<0.05)

Conclusion

Animals which are exposed to low intensity ultrasound therapy shown higher Ca and ALP level and low phosphorus, Cortisol and lipid peroxidation level which revealed better bone defect healing and reduced physiological stress occurred due to creation of bone defects, as compared to interferential therapy treated animals

Healing of bone defect was faster with ultrasound therapy followed by interferential therapy as compared to control group animals.

Physiological stress, induced due to creation of bone defect, was less in ultrasound treated animal followed by interferential therapy and untreated animal

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