Original Article

# Effect of Therapeutic Ultrasound on Histomorphometeric Features of Tibia of Rats



**Mahnoor Khursheed[1], Syed Nudrat Nawaid Shah[2], Amna Amir Khan[1], Rehan Ahmed Siddiqui[3], Sumaira Imran Farooqui[1] and Kevin Joseph Jerome Borges[2]**

## ABSTRACT

**Objective:** To evaluate the effects of duration of its exposure on healthy bone.
**Study Design:** Experimental study.
**Place and Duration of Study:** This study was conducted at the Department of Physiotherapy Ziauddin University, Karachi from April 2021 to June 2021 for a period of 8 weeks.
**Materials and Methods:** Total of 18 rats were enrolled in this study (6 rats in each group). Group A was the control group, whereas groups B and C were given 10 minutes and 20 minutes of ultrasound at 1.5 MHz frequency respectively for 14 days. All rats were sacrificed next day using standard protocols and data was analyzed using SPSS version 25.
**Results:** Our study showed statically significant decrease (p-value 0.003) in mean number of osteon per hpf ( 6 in both group A and B while 2.3 in group C) and in mean number of lamellae (13.1 in group A, 12.8 in group B and 3.3 in group C )as the duration of ultrasound increased. Area of haversion canal and area of osteon showed statically significant increase (p-value 0.027 and 0.002 respectively) as the duration of therapeutic ultrasound increased. Mean area of haversion canal in group A, B and C were 37.6 $\mu m^2$, 37.3 $\mu m^2$ and 59.3 $\mu m^2$ respectively. Mean osteon area in group A, B and C were 311.2 $\mu m^2$, 266.9 $\mu m^2$ and 230 $\mu m^2$ respectively.
**Conclusion:** Use of therapeutic ultrasound on healthy bone showed damage to bone histology by decreasing the number of osteons, osteon area and number of lamellae and increase in area of haversion canal.
**Key Words:** Therapeutic Ultrasound, Bone, Osteon

**Citation of article: Khursheed M, Shah SNN, Khan AA, Siddiqui RA, Farooqui SI, Borges KJJ. Effect of Therapeutic Ultrasound on Histomorphometeric Features of Tibia of Rats. Med Forum 2022;33(6):82-86.**

## INTRODUCTION

Therapeutic ultrasound (TUS) is a modality used by physical therapists all over the world for pain management in musculoskeletal injuries as well as in soft tissue lesions such as sprains, tendinitis and bursitis[1] in the clinical setups mostly 1 to 3 MHz frequency of therapeutic ultrasound is used. Although it has a frequency range between 0.75-3.00 MHz but the frequency of 1 MHz is the starting point utilized in the clinical setting for deep tissue injuries due to its penetration (3-5 cm in tissue). On the other hand, 3 MHz frequency is used for superficial injuries as it can go up to 1-2 cm depth.[2]

Therapeutic ultrasound has both thermal and non-thermal effects where non-thermal effects are related to cavitation. Whereas increased metabolism and blood flow are the thermal effects including analgesic effect on nerves[3].

Evidences present in the current literature shows both benefits as well as potential risks of using the therapeutic ultrasound in the clinical settings especially after fractures[4]. Results obtained on rabbits showed damage to the femur bone when applied to the thighs resulting in necrosis and osteocyte damage[5]. On the other hand, therapeutic ultrasound increased osteoblastic activity and accelerated the healing process when applied on tibia of rats[6]. In a similar study on Wistar rats it was reported that application of therapeutic ultrasound on the site of the fracture showed rapid ossification in the early stages of healing[7] It was reported by Miller et al that therapeutic ultrasound treatment showed damage to the femur bone when applied to the thighs of the rabbits including necrosis and osteocyte damage and on the other hand it shows good results when applied to an injured bone.[8] This study aims to evaluate the effects of TUS on histomorphomerty of healthy bone.

## MATERIALS AND METHODS

The study was conducted in the Ziauddin University, Karachi, Pakistan, following ethical committee

[1.] Department of Physiotherapy / Anatomy[2] / Molecular Medicine[3], Ziauddin University, Karachi.

Correspondence: Mahnoor Khursheed, Lecturer of Physiotherapy, Ziauddin University, Karachi.
Contact No: 0320-2422645
Email: mahnoorkhursheed@yahoo.com

Received: January, 2022
Accepted: February, 2022
Printed: June, 2022

approval of Ziauddin University Ethical review committee.

The sample was collected by simple random sampling. The subjects were 18 healthy young male Sprague Dawly Rats (SD) weighing 200-300gms, divided into three groups; 6 were assigned to the control group A with no TUS exposure, 6 to the 10 minutes ultrasound experimental group B and 6 to the 20 minutes ultrasound experimental group C. Both groups B and C were given 1 Hz frequency of ultrasound waves for two weeks. They were housed in individual cages and fed a standard laboratory stock feed and water. Adequate temperature and day night cycles were maintained.

All rats of all three groups were dissected anesthetically and their tibia was harvested to study under the microscope on the 15$^{th}$ day, after which bilateral tibia of all the rats were harvested and the transverse section at the level of mid-shaft of tibia was removed from the bone for histological examination. The harvested bone was kept in formaldehyde solution for few hours and then the process of bone demineralization and dehydration using isopropyl alcohol was followed. After the removal of alcohol using xyelene solution, sample fixation and overnight paraffin embedding was done. Finally, 5 micrometer size sectioning of samples was done. The samples were then stained with hematoxylin and eosin dye. 10 slides were made per mouse tibia. Once the slides were prepared,

Nikon Ts2R-FL Inverted Research Microscope was used to capture images and analysis which included determination of the number of osteons per hpf, number of lamellae, area of haversion canal and osteon area and Nikon NIS Elements-D Software was used For image processing - Adobe Photoshop Version CS2.

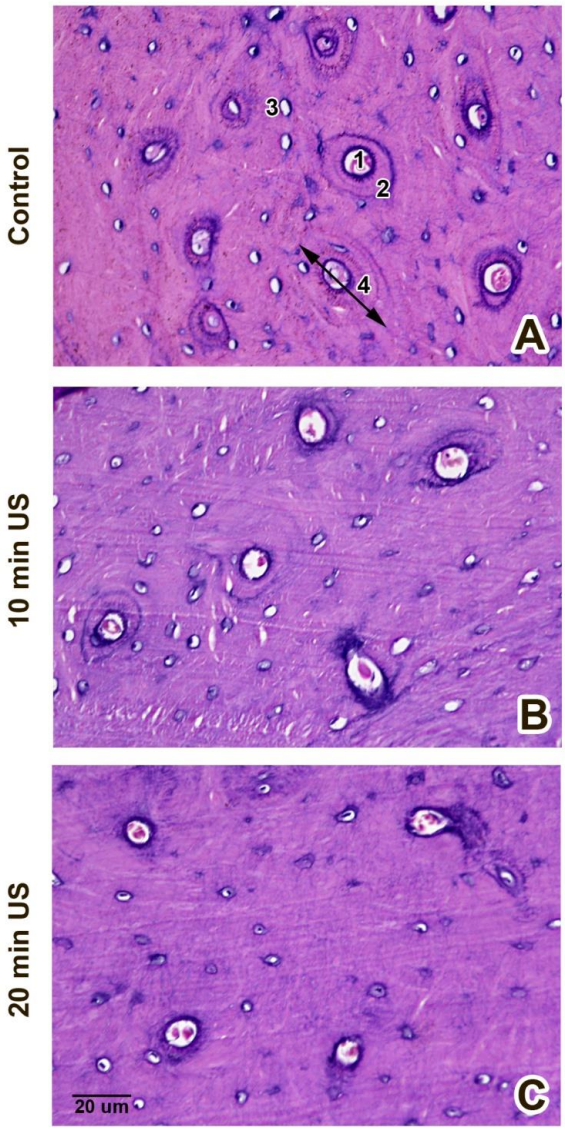
Data was analyzed using SPSS version 25. Shapiro Wilk test was run to analyze the distribution of data, it was found that two variables i.e. No. of osteons and no. of lamellae showed significant p-value <0.05, rejecting the normal distribution, however rest of two variables i.e., osteon area and area of haversion canal showed normal distribution, p-value >0.05. The differences in between the group analyses in normally distributed variables was done using the parametric test ANOVA, however in not normally distributed variables, Kruskal Wallis H test was applied.

## RESULTS

After the preparation of the microscopic slides, all groups were studied under microscope to assess the TUS intervention outcomes in experimental and control groups including number of osteons/ Hpf, Osteon area, number of lamellae per osteon and area of Haversian canal. All these morphological differences are shown in in Figure 1 which showed H&E-stained photo-micrographs showing sections of bone.

1A represents control group without any TUS intervention, 1B represents 10 minutes TUS intervention and 1C represents 20 minutes TUS intervention. 1,2,3,4 represent Haversian canal, lamellae, lacune and osteon respectively.

Figure 1 A shows normal bone histology, 1B represents reduced number of lamealle and reduced area of osteon as a result of 10 minutes TUS exposure. 1C represents reduced number of osteons and lamealle, reduced area of osteon and increased area of haversion canal at magnification, 200 X.




**Legend: Figure 1:** H&E-stained photomicrographs showing sections of bone. **A** control group without any TUS intervention, **B** 10 minutes TUS intervention and **C** 20 minutes TUS intervention. **1** Haversion canal, **2** lamealle, **3** lacune and **4** Osteon. **A** showing normal bone histology, **B** showing reduced number of lamealle and reduced area of osteon as a result of 10 minutes TUS exposure. **C** showing reduced number of osteons and Lamealle, reduced area of osteon and increased area of Haversion canal. Magnification, 200 X.

**Comparison of number of osteons, number of lamellae, area of haversion canal and osteon area in between the groups:** Comparison of number of osteons and number of lamellae in between the three groups was done using Kruskal Wallis H test and it showed significant difference among the groups in context to number of osteons and lamellae, with the p-value 0.003 and 0.003 respectively. (Table 1)

**Table No.1: Comparison of Number of Osteons, Number of Lamellae, Area of Haversion Canal and Osteon area**

| Variables | Group | Mean ± SD | P-Value |
|---|---|---|---|
| Number of Osteons per hpf* | A | 6±0.89 | 0.003 |
| | B | 6±0.89 | |
| | C | 2.3±0.81 | |
| Number of Lamellae per hpf* | A | 13.1±2.3 | 0.003 |
| | B | 12.8±1.8 | |
| | C | 3.3±1.96 | |
| Area of Haversion Canal $\mu m^2$** | A | 37.6±10.45 | 0.027 |
| | B | 37.3±11.49 | |
| | C | 59.3±19.3 | |
| Osteon area $\mu m^2$** | A | 311.2±31.4 | 0.002 |
| | B | 266.9±32.6 | |
| | C | 230±30.4 | |

*Kruskal Wallis H test for comparison between the groups.

**ANOVA used for comparison between the groups

In our study mean number of osteons in groups A, B and C were found to be 6±0.89, 6±0.89, and 2.3±0.81. This shows a statistically significant (p value 0.003) decrease in number of osteons as the duration of exposure of was increase. Similar results were obtained when numbers of lamellae were compared between the control and therapeutic ultrasound groups. Mean number of lamellae in groups A, B and C were found to be 13.1±2.3, 12.8±1.8, and. 3.3±1.96. This shows statistically significant (p value 0.003) decrease in the number of lamellae as the duration of exposure of therapeutic ultrasound was increase.

The area of haversion canal and osteon area were compared between the groups using ANOVA test. Detailed description is mentioned in (Table 1). The mean area of haversion canal A, B and C were found to be 37.6±10.45 $\mu m^2$, 37.3±11.49 $\mu m^2$ and 59.3±19.3 $\mu m^2$. Which shows a statistically significant increase in the area of haversion canal (p value 0.027) as the duration of exposure of was increase. When Osteon Area was compared between the control and therapeutic ultrasound groups the mean Osteon Area in groups A, B and C were found to be 311.2±31.4 $\mu m^2$, 266.9±32.6 $\mu m^2$ and 230±30.4 $\mu m^2$. It shows a statistically significant decrease in the Osteon Area (p-value 0.002) as the duration of exposure of therapeutic ultrasound was increase.

**Comparison of Variables Using Post HOC:** Variable was compared with each of the group using post hoc test, it was found that there is no difference in any of two variables (number of osteons and number of lamella) in between the Group A (Control) and Group B (10 min ultrasound) with the p-value >0.005.Whereas, there is significant difference in both variables between Group A (Control) and Group C (20 min ultrasound) with the p-value 0.009 in Number of osteons and 0.007 in number of lamellae.

Similarly, there is significant difference in both variables between Group B (10 min ultrasound) and Group C (20 min ultrasound) with the p-value 0.009 in Number of osteons and 0.013 in number of lamellae. Comparison with respect to each of the group is mentioned in Table 2.

**Table No.2: Comparison of variable with each of the group**

| **Difference in Between the Group A & B** | | | |
|---|---|---|---|
| **Variables** | **Std. Error** | | **P-Value** |
| Number of osteons | 3.02 | | 1.0 |
| Number of Lamella | 3.04 | | 1.0 |
| **Difference in Between the Group A & C** | | | |
| Number of osteons | 3.02 | | 0.009 |
| Number of Lamella | 3.04 | | 0.007 |
| **Difference in Between the Group B & C** | | | |
| Number of osteons | 3.02 | | 0.009 |
| Number of Lamella | 3.04 | | 0.013 |
| **Comparison of area of haversion canal and osteon area with each of group** | | | |
| **Difference between Group A & B using Post Hoc Dunnett's test:** | | | |
| **Variables** | **Mean Difference** | **Std. Error** | **P-Value** |
| Area of HC ($\mu m^2$) | -0.03 | 8.26 | 0.99 |
| Osteon area ($\mu m^2$) | -44.3 | 18.2 | 0.05 |
| **Difference between Group A & C using Post Hoc Dunnett's test:** | | | |
| Area of HC ($\mu m^2$) | 21.6 | 8.2 | 0.035 |
| Osteon area ($\mu m^2$) | -81.19 | 18.21 | 0.001 |

Area of haversion canal and osteon area was compared with each of the group using post hoc Dunnet's test, When Group A is compared to Group C, significant result was found in many of the variables including area of haversion canal with the p-value 0.03 and a mean difference 21.6 and osteon area with the p-value 0.001 and a mean difference -81.19 The comparison with respect to each of the group is mentioned in Table 2.

In our study the number of osteons indicated mean±SD in Group A & B is 6±0.89 with the compared to the Group C who were given 20 minutes of ultrasound, their mean±SD is decreased 2.3±0.81. The Osteon area also showed significant difference in between the three Groups A, B &, 0.002. The mean±SD in Group A is 311.2±31.4 $\mu m^2$, 266.9±32.6 $\mu m^2$ in Group B & 230±30.4 $\mu m^2$ in Group C. The number of lamellae also

showed significant difference, the mean±SD is 13.1 ±2.3 in Group A, and 12.8±1.8 in Group B whereas 3.3±1.96 in Group C. The area of haversion canal showed significant difference in between the three Groups A, B & C, the mean±SD in Group A is 37.6±10.45 μm$^2$, 37.3±11.49 μm$^2$ in Group B & 59.3±19.3 μm$^2$ in Group C, shown in figure 2.

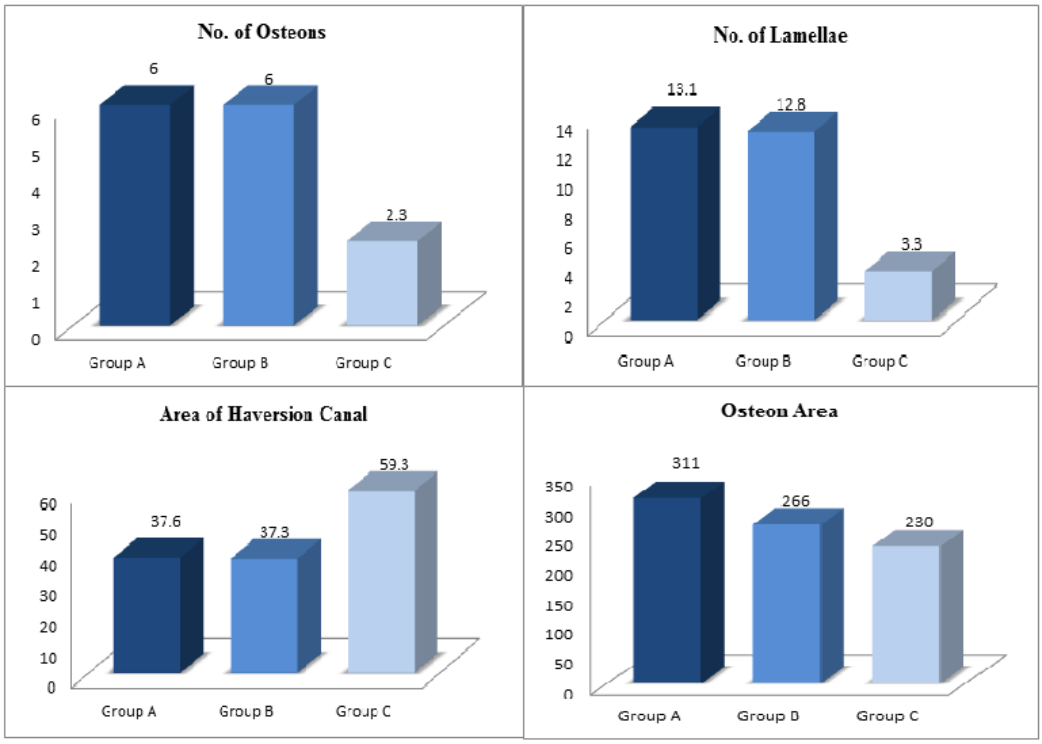



**Figure No.2: Representation of No of osteons, No. of lamellae, Area of Haversion Canal and Area of osteons in three groups.**

## DISCUSSION

All over the world low-intensity pulsed ultrasound has been used to treat the fractured bones. TUS shows promising effect on bone fracture healing[9]. Several studies have found that low intensity ultrasound increases the rate of tissue repair after injuries, especially those related with bone fracture[10,11]. The present study was aimed to analyze the outcomes of exposure of therapeutic ultrasound to the healthy bones. Number of osteons in Group A & B showed no significant difference as compared to the Group C who were given 20 minutes of ultrasound, it significantly reduced the number of the osteon, Area of osteon also significantly decreased with the increased exposure of the Therapeutic ultrasound. These findings are supported by those of Izadifar which highlighted osteon damage and necrosis, characterized by pyknotic cells and empty lacunae, occurred within the ablation area extending through the bone after the exposure to the therapeutic ultrasound[12]. Palanisamy also in their studies documented that heating in the biological tissues including bone was caused by the higher ultrasound intensity as the therapeutic ultrasound increases the heat in the body tissues[13]. Another important observation was significant decreased number of lamellae per osteon after exposure to the therapeutic ultrasound. It has been implicated by studies carried out previously that tensile strength of bones is directly proportional to the number of lamellae.[14 15] The lamellar structure may contribute to bone toughness by acting as delamination barriers, causing crack deflections. Decreased number of lamellae represents weak bone and porosity [16].

In our study evidence of porosity after TUS exposure was also supported by area of haversion canal, which showed significant difference in between the three Groups A, B & C. In 10 minutes TUS group Area of haversion canal decreased slightly and in 20 minutes TUS group area of haversion canal significantly increased, which again show pores in cortical bone. These findings are supported by those of Miszkiewicz[17] that increase in the area of haversion canal represents porosity in a bone.

There are many studies on the effect of ultrasound on the bone after fracture with and without other therapies in combination[18] but very little evidence is present on the effect of therapeutic ultrasound on healthy bone tissues and different studies shows different results on healthy and injured bone.

# CONCLUSION

In our study therapeutic ultrasound on healthy bone showed damage to the bone by decreasing the number of osteons, area of osteon and number of lamellae. It suggests that Therapeutic ultrasound is harmful for healthy bone tissues with more than 10 minutes exposure. It was also observed that exposure to the therapeutic ultrasound increases area of the haversion canal which represents porosity in bone.

**Author's Contribution:**

| | |
|---|---|
| Concept & Design of Study: | Mahnoor Khursheed, Syed Nudrat Nawaid Shah |
| Drafting: | Amna Amir Khan, Rehan Ahmed Siddiqui |
| Data Analysis: | Sumaira Imran Farooqui, Kevin Joseph Jerome Borges |
| Revisiting Critically: | Mahnoor Khursheed, Syed Nudrat Nawaid Shah |
| Final Approval of version: | Mahnoor Khursheed, Syed Nudrat Nawaid Shah |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

# REFERENCES


1. Gomes GB, Matheo D, Lobianco L, Omena TP, Abdala T, Kruger MA, Albuquerque Pereira WC. A Comparison Between the Heating Process of the Therapeutic Ultrasound and Infrared Radiation Applied in Physical Therapy Using PVCP Phantoms. InXXVI Brazilian Congress on Biomedical Engineering. Springer, Singapore; 2019.p.715-720
2. Ekechukwu NI, Ekechukwu EN. Effects of Ultrasound Therapy on Pain Intensity of Patients with Knee Osteoarthritis-A systematic Review of Evidence. Afr J Health Sciences Technol 2019; 1(2):61-8.
3. Rich KT. Characterization of cavitation effects in therapeutic ultrasound: Sonophoresis experiments and quantitative emission measurements (Doctoral dissertation, University of Cincinnati).
4. Harrison A, Lin S, Pounder N, Mikuni-Takagaki Y. Mode & mechanism of low intensity pulsed ultrasound (LIPUS) in fracture repair. Ultrasonics 2016;70:45-52.
5. Smith NB, Temkin JM, Shapiro F, Hynynen K. Thermal effects of focused ultrasound energy on bone tissue. Ultrasound Medicine Biol 2001; 27(10):1427-33.
6. Fontes-Pereira AJ, Teixeira RD, Oliveira AJ, Pontes RW, Barros RS, Negrão JN. The effect of low-intensity therapeutic ultrasound in induced fracture of rat tibiae. Acta Ortopédica Brasileira 2013;21(1):18-22.
7. Cheung WH, Chow SK, Sun MH, Qin L, Leung KS. Low-intensity pulsed ultrasound accelerated callus formation, angiogenesis and callus remodeling in osteoporotic fracture healing. Ultrasound Med Biol 2011;37(2):231-8.
8. Miller DL, Smith NB, Bailey MR, Czarnota GJ, Hynynen K, Makin IR, Bio effects Committee of the American Institute of Ultrasound in Medicine. Overview of therapeutic ultrasound applications and safety considerations. J Ultrasound Med 2012; 31(4):623-34.
9. Nicholson JA, Tsang STJ, MacGillivray TJ, Perks F, Simpson AHRW. What is the role of ultrasound in fracture management? Diagnosis and therapeutic potential for fractures, delayed unions, and fracture-related infection. Bone Joint Res 2019; 8(7):304-312.
10. Dijkman BG, Sprague S, Bhandari M. Low-intensity pulsed ultrasound: Nonunions. Ind J Orthop 2009;43:141–148.
11. Mundi R, Petis S, Kaloty R, Shetty V, Bhandari M. Low-intensity pulsed ultrasound: Fracture healing. Indian J Orthop 2009;43:132–140.
12. Izadifar Z, Babyn P, Chapman D. Mechanical and biological effects of ultrasound: A review of present knowledge. Ultrasound Med Biol 2017;43(6):1085-1104.
13. Palanisamy P, Alam M, Li S, Chow SK, Zheng YP. Low-Intensity Pulsed Ultrasound Stimulation for Bone Fractures Healing: A Review. J Ultrasound Med 2022;41(3):547-563.
14. Fonseca H, Moreira-Gonçalves D, Coriolano HJ, Duarte JA. Bone quality: the determinants of bone strength and fragility. Sports Med 2014;44(1): 37-53.
15. Osterhoff G, Morgan EF, Shefelbine SJ, Karim L, McNamara LM, Augat P. Bone mechanical properties and changes with osteoporosis. Injury 2016;47:S11-20.
16. Muñoz A, Docaj A, Ugarteburu M, Carriero A. Poor bone matrix quality: What can be done about it? Current Osteoporosis Reports 2021;19(5): 510-531.
17. Miszkiewicz JJ. Investigating histomorphometric relationships at the human femoral midshaft in a biomechanical context. J Bone Mineral Metabolism 2016;34(2):179-92.
18. Wei FY, Leung KS, Li G, Qin J, Chow SK, Huang S, et al. Low intensity pulsed ultrasound enhanced mesenchymal stem cell recruitment through stromal derived factor-1 signaling in fracture healing. PLoS One 2014;9(9):e106722.