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# Evaluation of the Effect of Nebivolol in Bone Defect Healing in An Experimental Rat Model

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## ABSTRACT

***Objective:*** to evaluate the beneficial role of Nebivolol on bone formation in Experimental model of rat.
***Study Design:*** Laboratory-based experimental study
***Place and Duration of Study:*** Department of Pharmacology, Army Medical College, Rawalpindi Pakistan, from Jun-Aug 2020
***Methodology:*** Fourteen (14) rats of the Sprague Dawley variety were obtained for the study. The bone defect was made in each rat's upper quadrant of the right femur shaft by surgical intervention.1-2mm bone defect was created using a Dental Engine motor, avoiding any injury to muscles or other areas of bone. Rats were divided into two equal groups. The Disease Control Group was labelled as Group-A, which was restricted to normal drinking water and feed for three weeks after surgery. Nebivolol-Group was tagged as Group-B, given Nebivolol (2.5mg/kg) once daily (OD) for three weeks starting post-op Day 1. Rats included in the study were sacrificed at the end of the three weeks, and their right femurs were collected in sample jars for radiography and histopathology.
***Results:*** The radiographic scores, as well as the histopathology scores, were analyzed. Results depicted noteworthy differences between Group-A and Group-B and found to be highly significant ($p$-value <0.01).
***Conclusion:*** Nebivolol efficaciously enhances bone regeneration in bone defects induced in a rat model.

**Keywords:** Anti-oxidant effect, Bone regrowth, Nebivolol, Nitric oxide.

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## INTRODUCTION

Hypertension is the leading cause of cardiovascular diseases and a major factor cultivating various complications.[1] Recent studies report that β-blocker usage remains constant and may increase in older age groups.[2,3] Over the years, clinical studies suggest that stimulation of the adrenergic beta receptors damages bone regeneration and remodelling.[4] Thus, blocking of beta receptors positively reinforces bone homeostasis.[5] Bone healing is an intricate process and requires adequate tissue perfusion and decreased free radicals.[6] Numerous factors that influence the bone healing process possess their significance.[6]

Nebivolol is a highly selective beta-1 blocker commonly prescribed in clinics for treating hypertension and non-cardiovascular conditions. It releases nitric oxide from the vascular endothelium. This happens due to endothelial Nitric oxide synthase activation via calcium mobilization.[7,8] Current research has publicized that when β-receptors are stimulated, they adversely affect bone remodelling.[9] Additionally, we already acknowledge the presence of β-receptors in osteoplastic cells and osteoclasts. Therefore, β-receptor's activation using agonists inhibits alkaline phosphatase (ALP) activity. This substantiates that the upsurges of catecholamines produced due to bone damage trigger adrenergic β-receptors. It negatively impacts the metabolism of bone, inhibiting bone healing. Consequently, it is concluded that beta-adrenergic receptors are a prospective therapeutic target to improve the treatments of bone injury.[10]

Therefore, large bone defect cases beyond a critical size lack regrowth ability, which results in bone malunion. Hence, bone defects of larger size are of major concern. The development of newer biomaterials that facilitate bone repair is necessary to enhance the reformation of fractured bone.

## METHODOLOGY

The study was carried out at the National Institute of Health (NIH) animal lab in coordination with the Pharmacology Department, Army Medical College (AMC), Rawalpindi from June to August 2020 Ethical Review was undertaken by The Centre for Research in Experimental and Applied Medicine (CREAM), AMC, Rawalpindi (ERC/ID/94).

**Inclusion Criteria:** Sprague Dawley rats of either gender (male and non-pregnant females) aged about

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08-10 weeks, and weight around 300-400 grams were included.

**Exclusion Criteria:** Apparently-sick animals were excluded. Mice with any obvious injury and disease were excluded.

Preliminary fourteen (14) adult rats were selected for the study. Animals were kept in the animal house of NIH Islamabad during the whole duration of the study. Rats of each Group were kept in metal cages and labelled. The lab temperature was maintained at 25±5˚C with 40±5% humidity, considering a 12-hour day-night cycle. During the entire tenure of the study, animals were provided free access to clean drinking water and a standard rodent diet *adlibitum*. All rats were anaesthetized with an intraperitoneal cocktail injection containing (50mg/kg) Ketamine with (10mg/kg) Xylazine Hydrochloride. This dose was re-confirmed in the light of the pilot project conducted. Complete anaesthesia was achieved in 3-5mins. Following the anaesthesia, the right hind limb of each rat was shaved and prepared for aseptic surgery. The skin was disinfected with Pyodine, and using an aseptic technique, a longitudinal incision was made on the lateral aspect of the right hind limb. A bone defect was created on the mid-shaft of the femur using a 1.0 mm diamond needle attached to the dental motor engine. A fine bone defect was created measuring 1-2mm in size. It was thoroughly washed with distilled water to prevent any bone residues and attain a clear picture of bone defect in that area. Then, the wound was closed using the following sutures (Kat gut and polypropylene, each 4/0 in size). The rats were left without cast immobilization for three weeks. At the end of the study period, sampling was done. Inhaled chloroform in a closed desiccator was used to sacrifice the animals. Femurs were surgically removed from the rats under aseptic measures after euthanizing them and kept in normal saline containers for transportation to the Radiology Department of a private institute. Radiography was done using a digital X-ray machine operated at 220 V with 0.3 seconds of exposure time. Anterior-posterior X-ray films were taken. Each radiograph was accurately labelled according to the groups. Radiological evaluation was done with the help of an orthopaedic surgeon. Post radiography, the right femurs were cut using a 5.5″ angled bone cutter. Isolated bone tissue was fixed, decalcified and embedded for slide preparation.

Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Chi-square test was applied to explore the inferential statistics. The *p*-value of ≤0.05 was considered statistically significant.

## RESULTS

After three weeks of study, the samples of 14 Sprague Dawley adult rats were collected for radiological and histological examinations. Histologically, Group-B (Nebivolol treated) rats showed prominent bone formation and thickening in the area of induced defect.Radiographs of Disease Control Group (Circular defects) are shown in Figure-1.

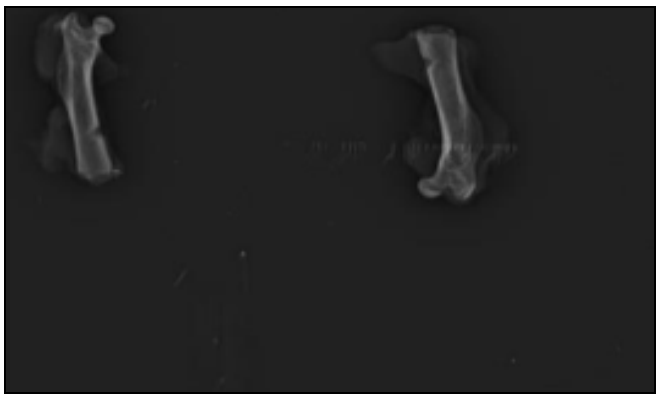

**Figure-1: Radiographs of Disease Control group (Circular defects) (n=14)**

On radiological evaluation, in Group-B (Nebivolol treated), six radiographs out of 7 showed complete union and were graded as Class-2. A well-formed medullary canal and compact and continuous cortical bone were appreciated on radiological assessment (Figure-2).

**Figure-2: Radiograph showing Nebivolol treated femurs (n=14)**

One radiograph also showed healed bone but lacked normal cortex thickening and hence was graded as Class-1. In Group-A (disease control), radiographs

of all seven rats showed mild union (Class-1) and no complete union of bone defect was seen, as graded according to the Fracture Healing scale. The cortical bone and medial cortex formed was thin on histology findings of rats contained in Group-A. The lens of X400 was used for histopathology examination, and all slides were scored based on histological changes that have occurred according to the Bone Healing scale. The findings of both groups were compared, *p*-value of <0.001 showed significant comparable results. Osteocytes embedded in lamellar bone were well appreciated, as seen in Figure-3. Six out of 7 in Group-B scored ten on the histological scale for bone healing and showed mature bone formation.

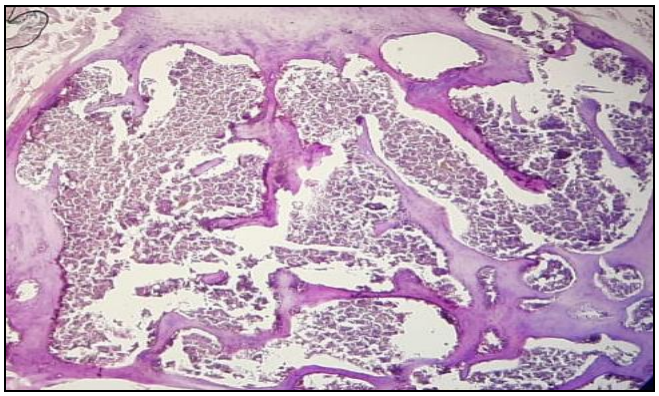

**Figure-3: Histology slide of Nebivolol (Treated Bone) (n=14)**

## DISCUSSION

Bone healing is a complex process and requires adequate tissue perfusion & decreased free radicals.[11] Numerous factors that influence the bone healing process possess their significance.[12,13] Clinically, the present study emphasizes the ortho-protective role of Nebivolol, which is a widely prescribed anti-hypertensive agent. Nebivolol treated Group in the current study showed statistically significant results for all the parameters. A similar research was conducted in 2017 to prove the beneficial effects of Nebivolol against interleukin-1β (IL-1β)-induced type II collagen destruction.[14] Nebivolol selectively binds to β1adrenoceptor and possesses vasodilating properties. Nitric oxide is produced from vascular endothelium by activating endothelial Nitric Oxide synthase.[15] Another study, claimed the modulatory effects of Nebivolol on osteoporosis in rat animal models in 2018. The antioxidant activity of Nebivolol causes a reduction in the release of reactive oxygen species by inhibiting the NADPH oxidase system.[16] In our study, radiographs of the Nebivolol Group showed completely healed bones and histological scores showed mature bone formation in more than 90 % of the rats. Histologically, trabecular bone thickening and enhanced cortical bone formation in this Group were significant. At the cellular level, abundant Osteocytes embedded in lamellar bone were evident. Side by side, another study supported that Nebivolol enhances fracture healing by positively influencing an organized hematoma into a bony callus. Its anti-oxidant effect through NO release influences the endochondral and intra-membranous ossification stages. Recent advocated that Nebivolol-treated patients showed beneficial effects on human bone metabolism.[17] Similar to our study, it signified the beneficial effect of beta-blockers on bone cells, bone metabolism regulation, and long-term remodelling. Very much like our work, a recent study was conducted that confirms the presence of β-adrenergic receptors on the surface of the osteoblasts and osteoclasts. The sympathetic nervous system can help regulate bone metabolism, and so is the need to unfold the role of beta-blockers on bone metabolism.[18,19] There was a significant comparison amongst radiographic and histologic scores of Nebivolol and the Disease Control group. Statistical results depicted that Nebivolol enhances bone repair and healing efficaciously.

Although several treatment options are available for osteoporosis, bone defects and fractures, an inexpensive and relatively safe therapeutic agent remains the focus. Meanwhile, β- adrenergic blockers like Nebivolol are widely used for multiple diseases like HTN, congestive cardiac failure and other cardiac issues. Our study depicts the significant beneficial emerging role of Nebivolol on bone defect healing that needs to be probed clinically and, more specifically, in humans.

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## DISCLOSURE

We are highly obliged to the National University of Medical Sciences, Rawalpindi, for funding our research.

## CONCLUSION

As compared to the Control Group, Nebivolol possesses ortho-protective effects. Nebivolol exhibits a beneficial effect on bone metabolism by enhancing bone healing in drill-induced bone defects. Further proposed human studies must be carried out to validate the outcomes of beta-blockers on bone.

**Conflict of Interest:** None.

**Author's Contribution**

Following authors have made substantial contributions to the manuscript as under:

QUAH & SA: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

MWAK & KF: Study design, drafting the manuscript, critical review, approval of the final version to be published.

NSB & NA: Critical review, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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