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YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF ORAL AND MAXILLOFACIAL SURGERY

EVALUATION OF THE EFFECT OF LOCAL AND SYSTEMIC ERYTHROPOIETIN ADMINISTRATION ON ALVEOLAR BONE HEALING AFTER TOOTH EXTRACTION IN RATS

DOCTOR OF PHILOSOPHY THESIS

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İstanbul- 2022

THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Programme : Oral and Maxillofacial Surgery

Title of the Thesis : Evaluation of the Effect of Local and Systemic Erythropoietin Administration on Alveolar Bone Healing After Tooth Extraction in Rats

Owner of the Thesis : Çınar KULLE

Examination Date : 08.06.2022

This study have approved as a Master/Doctorate Thesis in regard to content and quality by the Jury.

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

07.07.2022

Çınar KULLE

DEDICATION

I dedicate this thesis to my parents and my grandparents for always being there for me and caring for me.

ACKNOWLEDGEMENTS

I would like to offer my sincerest gratitude to my supervisor and Head of Department Prof. Dr. Ceyda ÖZÇAKIR TOMRUK who gave me the opportunity to learn from her experiences, expertise and allowed me to have an environment in which I was free to learn and practice remarkable cases.

I want to express by an appreciation for the learning opportunities provided by Prof. Dr. Nurhan GÜLER, Prof. Dr. Ahmet Hamdi ARSLAN, Assoc. Prof. Dr. Ediz DENİZ, Assoc. Prof. Dr. Fatih CABBAR, Assoc. Prof. Dr. Çağrı BURDURLU and Lecturer Dr. Volkan DAĞAŞAN.

I would like to offer my sincerest appreciation to Assoc. Prof. Dr. Selahattin Yiğit ŞİRİN for his guidance and help throughout the entire thesis process.

I would like to thank Assoc. Prof. Merva SOLUK TEKKEŞİN for her guidance and aid along the histologic and histomorphometric analysis of my thesis

The finalization of my thesis could not have been possible without the participation of Dr. Elif Çiğdem KELEŞ and Veterinarian Engin SÜMER together with the members of YUDETAM. Their inputs were deeply appreciated.

I would like to thank my colleagues Dr. Faisal ALABEIDI, Dt. Fikri Can AYIK, Dt. Oğuzhan TUNÇ, and Dt. Can KARAKURT.

I would also like to sincerely thank the nurses, dental assistants, and other members of our faculty who helped me during my Ph.D. period.

I would like to express my gratitude to my amazing parents, Hamdi KULLE and Canan KINIK KULLE, and my grandmother, İclal KINIK who put a lot of effort to support me and always believe in me.

Last but not least, I would like to thank my dearest friend and colleague Dt. Akanay ÇOPUROĞLU in her never-ending support and love.

My heartfelt thanks to everyone, I would not have been successful without you.

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LIST OF SYMBOLS AND ABBREVIATIONS

BMD	Bone Mineral Density
BMP	Bone Morphogenic Proteins
BFU-E	Bust-Forming Unit-Erythroid
CKD	Chronic Kidney Disease
CFU-E	Colony Forming Unit - Erythroid
CFU- GEMM	Colony Forming Units Generating Granulocytes, Erythrocytes, Monocytes, Megakaryocytes
EGF	Epidermal Growth Factor
EPO	Erythropoietin
EpoR	Erythropoietin Receptor
ECM	Extracellular Matrix
FGF	Fibroblastic Growth Factor
FDA	Food and Drug Administration
g	Gram
GM-CSF	Granulocyte/Macrophage-Colony Stimulating Factor
GH	Growth Hormone
H&E	Hematoxylin and Eosin
HCT	Hematocrit
Hb	Hemoglobin
HPT	Hypothalamic-Pituitary-Thyroid Axis
HIF	Hypoxia-inducible Transcription Factor
HRE	Hypoxia-Response Element
IGF	Insulin-like Growth Factors
IL-1	Interleukin 1
IL-6	Interleukin 6
IL-11	Interleukin 11
INN	International Non-proprietary Drug Name
IU	International Unit
IM	Intramuscular
IP	Intraperitoneal
IV	Intravenous
kDA	Kilodalton

kg	Kilogram
M-CSF	Macrophage-Colony Stimulating Factor
MSC	Mesenchymal Stem Cell
mg	Miligram
mL	Milileter
mm	Milimeter
NBF	Neutral Buffered Formalin
NF-kB	Nuclear Factor kB
PTH	Parathyroid Hormone
PBS	Phosphate Buffered Saline
PLT	Platelet
PDGF	Platelet-derived Growth Factor
PPGE2	Prostaglandin E_2
rhEPO	Recombinant Human Erythropoietin
RBC	Red Blood Cell
SS	Saline Solution
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Packages of Social Sciences
SC	Subcutaneous
SHP-1	Src Homology Phosphotase -1
TGF	Transforming Growth Factor
TNF	Tumor Necrosis Factor
U	Unit
VEGF	Vascular Endothelial Growth Factor
VDR	Vitamin D Receptor
WBC	White Blood Cell
YUDHEK	Yeditepe University Experimental Animals Ethics Committee
YUDETAM	Yeditepe University Experimental Animals Study Center
<	Less than
µ	Micron
>	More than
%	Percent
®	Registered Trademark

ABSTRACT

Kulle, Ç. (2021). Evaluation of the effect of local and systemic erythropoietin administration on alveolar bone healing after tooth extraction in rats. Yeditepe University, Institute of Health Sciences, Department of Oral and Maxillofacial Surgery, Ph.D, Thesis. Istanbul. The aim of this experimental study is to examine the effect of local and systemic erythropoietin administration on alveolar bone healing after tooth extraction in rats. In the current study, thirty male Sprague-Dawley rats were randomly divided into five groups. The day the experimental animals were received was accepted as the first day of the study (day 1). On day 1, the left maxillary first molar of the rats in all study groups was extracted. After tooth extraction, an intraperitoneal (IP) saline solution (SS) (0.1 mg/ml) injection was made to the rats in Group 1. A single dose of 450 IU/kg and 1350 IU/kg erythropoietin (EPO) (BINOCRIT 4000 IU/0.4 ml, Sandoz, Austria) was injected subcutaneously (SC) into the rats in Group 2, and Group 3 respectively. While Geletamp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) was placed alone in the extraction sockets of the rats in Group 4, Geletamp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) was locally placed together with EPO (20 IU/kg) into the extraction sockets of the rats in Group 5. At the end of the study period, which is the 8th day, 2.5 ml of blood samples were taken from the jugular vein of all study animals before sacrification. A hemogram test was performed on each of the blood samples. All study animals were sacrificed on the same day by the decapitation method. Samples taken from all rats were evaluated histopathologically in terms of inflammation, fibrosis, and foreign body reaction. The new bone formation in the extraction socket was evaluated histomorphometrically. White blood cell (WBC), platelet (PLT), hemoglobin (Hb) and hematocrit (HCT) mean values were evaluated hematologically. When all study groups were evaluated histopathologically in terms of inflammation, fibrosis and foreign body reaction; no statistically significant difference was found between the groups in all parameters ($p>0.05$). When the new bone formation was evaluated histomorphometrically, a statistically significant difference was found between the mean values of the amount of new bone formation according to the groups ($p<0.05$). In the pairwise comparisons between the groups, a statistically significant difference was found between Group 3, Group 5 and Group 1 ($p<0.05$). When compared with the control group , the group which was administered locally with EPO had the least amount of new bone formation. When the mean values of white blood cells and platelets

were evaluated, no statistically significant difference was found ($p>0.05$). When the mean hemoglobin and hematocrit values were evaluated, a statistically significant difference was found between the groups ($p<0.05$). A statistically significant difference was found between the pairwise comparisons of Group 2 and Group 4 ($p<0.05$). In this experimental study model, it was concluded that high-dose systemically administered erythropoietin after tooth extraction had a positive and significant effect on bone healing and did not increase the hematocrit value. In conclusion, it can be said that erythropoietin has a positive effect on bone healing.

Keywords: Erythropoietin, Tooth extraction, Rat

ÖZET

Kulle, Ç. (2021). Sıçanlarda Diş Çekimi Sonrası Lokal ve Sistemik Eritropoietin Uygulamasının Alveolar Kemik İyileşmesi Üzerine Etkisinin Değerlendirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Ağız, Diş ve Çene Cerrahisi Anabilim Dalı, Doktora Tezi, İstanbul. Bu deneysel çalışmanın amacı diş çekimi yapıldıktan sonra çekim soketinde tedavi amacıyla kullanılan eritropoietin'in (EPO) yeni kemik alanı oluşumu üzerine olan etkisinin incelenmesidir. Çalışmamızda otuz tane erkek Sprague-Dawley sıçan randomize olarak beş gruba ayrıldı. Deney hayvanlarının teslim alındığı gün çalışmanın ilk günü olarak kabul edilmiştir (gün 1). 1. günde tüm çalışma gruplarındaki sıçanların sol üst 1. moları çekildi. Diş çekiminden sonra Grup 1'de yer alan sıçanlara intraperitonal (IP) serum fizyolojik (0,1 mg/ml) enjeksiyonu, Grup 2 ve 3'te yer alan sıçanlara subkutan (SC) olarak sırasıyla tek doz 450 IU/kg ve 1350 IU/kg eritropoietin (EPO) (BINOCRIT 4000 IU/0.4 ml, Sandoz, Avusturya) enjeksiyonu yapıldı. Grup 4'te yer alan sıçanların çekim soketlerine Geletamp® (Roeko Geletamp, Coltene/Whaledent GmbH, Almanya) tek başına yerleştirilirken Grup 5'te yer alan sıçanların çekim soketlerinin içerisine Geletamp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) lokal olarak EPO (20 IU/kg) ile beraber yerleştirildi. Çalışma süreci sonunda, yani 8. günde tüm deney hayvanlarından sakrifikasyon öncesi 2,5 ml jugular venden kan örneği alındı. Alınan her kan örneği için hemogram testi yapıldı. Tüm deney hayvanları aynı gün dekapitasyon yöntemi ile sakrifiye edildi. Tüm sıçanlardan alınan örnekler enflamasyon, fibrosis ve yabancı cisim reaksiyonu açısından değerlendirilmiştir. Diş çekim soketindeki yeni kemik alanı histomorfometrik olarak değerlendirilmiştir. Beyaz kan hücresi, trombosit, hemoglobin ve hematokrit ortalama değerleri hematolojik açıdan değerlendirilmiştir. Tüm çalışma grupları enflamasyon, fibrosis ve yabancı isim reaksiyonu açısından histopatolojik olarak değerlendirildiklerinde tüm parametrelerde gruplar arası istatistiksel olarak anlamlı bir fark bulunmamıştır ($p>0.05$). Yeni kemik oluşumu histomorfometrik açıdan değerlendirildiğinde gruplara göre yeni kemik oluşum miktarı ortalama değerleri arasında istatistiksel olarak anlamlı bir fark bulunmuştur ($p<0,05$). Gruplar arasında yapılan ikili karşılaştırmalarda Grup 3 ile Grup 5 ve Grup 1'in ikili karşılaştırmaları arasında istatistiksel olarak anlamlı fark bulunmuştur ($p<0,05$). Kontrol grubuyla karşılaştırıldığında, lokal olarak EPO verilen grup en az kemik yapımı gözlenen grup olmuştur. Beyaz kan hücreleri ve trombositlerin ortalama değerleri hematolojik açıdan çalışma grupları arasında değerlendirildiğinde istatistiksel olarak

anlamlı bir fark bulunmamıştır ($p>0.05$). Hemoglobin ve hematokrit ortalama değerleri değerlendirildiğinde gruplara göre istatistiksel olarak anlamlı bir fark bulunmuştur ($p<0.05$). Grup 2 ile Grup 4'ün ikili karşılaştırmaları arasında istatistiksel olarak anlamlı fark bulunmuştur ($p<0.05$). Deneysel olarak oluşturulan bu modelde, diş çekimi sonrasında yüksek doz sistemik olarak verilen eritropoietinin kemik iyileşmesi üzerinde pozitif anlamlı bir etkisi olup, hematokrit değerini artırmadığı sonucuna varılmıştır. Sonuç olarak eritropoietin'in kemik iyileşmesi üzerinde pozitif bir etkisi olduğu söylenebilir.

Anahtar Kelimeler: Eritropoietin, Diş çekimi, Sıçan

1. INTRODUCTION AND PURPOSE

One of the main purposes of dentoalveolar surgery is the reconstruction of anatomic integrity and reinstatement of the absent tissue with function. Tooth extraction is the most common type of surgical procedure in daily practice. Thus its healing is very important for the rehabilitation of the patient.

After tooth extraction, the initial phase of wound healing occurs. Inflammation, coagulation, and, granulation tissue generation are the events that take place in the initial phase. Healing starts from the apical portion of the extraction socket with the migration of endothelial cells and pre-osteoblastic cells. The new bone matrix starts to form there 4 days after tooth extraction. As a result, trabecular bone is formed sequentially and fills the extraction socket by the end of the 14^{th} day. The healing solely depends on the proliferation, specification, and maturation of osteoblasts. Furthermore, cytokines and several intrinsic factors regulate the whole process. Consequently, local or systemic drug application has an effect on the activity of such signaling molecules and on the healing of bone tissue after tooth extraction (1).

Prior to tooth extraction; if the bone tissue has underlying issues such as trauma due to periodontal or endodontic disease, the remaining bone tissue after extraction might be even more impaired. Currently, aesthetics have become a very significant part of treatment planning. Since the loss of bone tissue directly affects the aesthetic appearance of the patient, its healing and preservation have become more critical. Therefore, to grant the patient a functional and aesthetic appearance, an adequate amount of alveolar bone must be present (2).

The human erythropoietin (EPO) is a glycoprotein hormone that is 30.4 kDa. It is made up of a single 165 amino acid chain that is attached to four glycans. The primary source of EPO is the kidneys and its production is regulated by hypoxia-inducible transcription factors (HIFs) (3). One of the fundamental roles of EPO is the production of erythrocytes. As a result, the treatment of patients with chronic kidney disease who suffer from EPO deficient anemia is EPO. In adult humans, most EPO production occurs in the peritubular renal cortex. When the tissue O_2 pressure decreases, a response is generated by the hypoxia-induced transcription factor in the kidney, and EPO is produced (4,5). EPO also has non-hematopoietic effects (6). It was found that systemic EPO

administration causes the increase of the expression of vascular endothelial growth factor (VEGF) and thus VEGF mediated angiogenesis in mice with femoral segmental defects (7). It was also discovered that EPO causes the increase of chondrogenic and angiogenic responses in the course of bone repair and might be used as a therapeutic agent for the promotion of skeletal regeneration (8). Moreover, it was reported that the new bone formation was increased significantly when EPO was applied with xenogenic grafts in critical-size defects (9).

The main purpose of this study is to examine the histologic, histomorphometric, and hematologic effects of local and systemic EPO administration on alveolar bone healing after tooth extraction in rats.

2. LITERATURE REVIEW

2.1 Bone Tissue

Bone is a specialized connective tissue characterized by its rigidity and hardness and is the main component of the skeletal system. It consists of cells embedded in an intercellular tissue called extracellular matrix (ECM) which consists of collagen fibers, non-collagenous proteins, and minerals. Bone tissue differs from other connective tissues by the feature of this ECM in its structure to become saturated with inorganic salts (10).

Bone tissue has a structure that can renew itself and change its shape, volume, and content in line with mechanical stimuli from outside. In addition to mechanical functions such as building the skeleton and providing support to skeletal muscles, it also participates in many metabolic events such as being a mineral reservoir for calcium homeostasis, providing a blood-forming environment for bone marrow, and playing a role in acid-base balance (10,11).

2.1.1 Bone Matrix

Bone is composed of about %60 inorganic components, %30 organic components, and %10 water by weight (12,13).

2.1.1.1 Inorganic Component

The inorganic component of bone matrix is impure hydroxyapatite with the chemical formula Ca_{10} $(PO4)_6(OH)_2$ which contains carbonate in place of phosphate groups, chloride, and fluoride in place of hydroxyl groups, and sodium, potassium, and magnesium as a replacement of calcium ions (14,15).

2.1.1.2 Organic Component

The organic component of bone mainly consists of %90 collagen. Collagen is a fibrous protein synthesized by osteoblasts to provide mechanical stiffness and enables tissues to maintain their shape by resisting deformation. Type I collagen (%97) is the most abundant protein existing in bone ECM among other types which are also found in small amounts (16).

The remaining %10 of the organic component of bone is composed of various non-collagenous proteins such as osteopontin, bone sialoprotein, osteocalcin, and osteonectin (15).

2.1.1.3 Water Component

About %10 of water is contained by the bone and its volume decreases with age and can decline to %5. This drop can cause a negative effect on the mechanical properties of bone (13).

2.1.2. Bone Types

Bone classification can be made in many different ways in regard to the shape, location, size, or consistency of bones but histologically there are two different types of bones; primary/woven bone and secondary/lamellar bone (17).

2.1.2.1 Primary Bone Tissue (Woven Bone)

Primary bone is produced by osteoprogenitor cells near blood vessels during prenatal development, growth, and bone healing. It forms the embryonic skeleton with the newly formed indiscriminately oriented collagen fibrils in its structure. It does not contain lamellae, but contains more osteocytes than secondary bone tissue. Therefore primary bone forms quicker than secondary bone (17).

2.1.2.2 Secondary Bone Tissue (Lamellar Bone)

Secondary bone is formed as a result of the calcification of primary bone during growth and later stage of bone healing. Unlike primary bone tissue, it has a highly organized structure represented by multiple layers of firmly packed collagen fibrils. Due to its lamellar organization, secondary bone is stronger and less flexible than primary bone (17,18).

2.1.3 Bone Structure

Bone tissue is morphologically composed of compact (cortical bone) and spongy (trabecular bone) tissue.

Cortical bone, which covers the outer surfaces of the diaphysis and metaphysis of the long bones and forms the inner and outer surfaces of flat bones, has a dense and solid structure that shows resistance to external forces. The adult human skeleton consists of %80 cortical bones by weight overall. Inside the cortical bone, there is a cylindrical structure with canals of different widths extending longitudinally from the periosteum to the bone marrow. These canals contain veins and connective tissue comes from periosteum and bone marrow. The system consisting of 8-15 lamellae around a canal is called an osteon (12,18,19).

The cortical bone has an outer layer called periosteum, which provides the connection between the cortical bone and the surrounding soft tissue or muscles, and an inner layer called the endosteum, which is more vascularized and contains blood vessels, osteoblasts, and osteoclasts (12,18,19).

Trabecular bone, which is softer and weaker than cortical bone, constitutes %20 of body bones by weight. Trabecular bone does not contain osteon but it consists of honeycomb-like trabecular layers intermixed with bone marrow that is called a packet, which can be observed macroscopically. Trabecular bone contains blood vessels and bone marrow (12,18,19).

2.1.4 Bone Cells

The basic cellular elements of bone are osteoprogenitor cells, osteoblasts, osteocytes, osteoclasts, and bone lining cells that line through the inner surface of the bone and bone marrow cells. Osteoblasts, osteocytes, and bone lining cells are derived from mesenchymal stem cells (MSCs) in the osteoblast lineage representing different stages of maturation of a single cell type known as osteoprogenitor cells. Osteoclasts originate from hematopoietic stem cells of the monocyte-macrophage lineage (12,20).

2.1.4.1 Osteoprogenitor Cells

Osteoprogenitor cells have the capacity to proliferate and differentiate. They are found on the endosteal and periosteal surfaces of the bone (20). These cells, which are active during the normal growth of the bone, become inactive when bone growth is completed. However, in cases where bone formation is stimulated such as the regulation of the internal bone structure, fracture healing, or different types of injuries, they can be distinguished by activating both in number and transformation into bone-forming cells such as osteoblasts (21).

2.1.4.2 Osteoblasts

Osteoblasts are mature, cuboidal, and metabolically active bone-forming cells that differentiate from mesenchymal cells in embryonic life. Osteoblasts can also be derived from fibroblasts in the later stage of life via reprogramming. They are arranged close to the surface of bone tissue and found in ossification areas. The primary function of osteoblasts is to synthesize the organic portion of bone matrix called osteoid which consists of mainly Type I collagen, and the proteins and growth factors required for matrix synthesis. They also take a role in matrix mineralization and blood-calcium homeostasis (22).In addition, they play an active role in the regulation of bone resorption together with osteoclasts and provide new bone formation on bone surfaces that were previously resorbed by osteoclasts (23).

The lifespan of osteoblasts is up to 3 months. When new active bone formation ceases, osteoblasts can undergo apoptosis or with the function of growth factors, some osteoblasts remain as bone lining cells while others turn into osteocytes (24).

2.1.4.3 Bone Lining Cells

Bone lining cells, which arise from inactive osteoblasts, cover the endosteal region. They have a significant role in bone remodeling and hemopoiesis (25,26). In addition, bone lining cells aid in bone formation when the osteoblasts are absent (27).

2.1.4.4 Osteocytes

Osteocytes are mature osteoblast cells that lose most of their organelles during the formation of bone tissue and are entrapped within lacunae surrounded by a mineralized bone matrix. They make up %90-95 of total bone cells and therefore they constitute the main cell group in mature bone. When compared with osteoblasts' cuboidal pattern, osteocytes are flat and almond-shaped. Originating from osteoblasts, osteocytes take an active role in the reconstruction of the bone. Osteocytes stimulate bone remodeling and strengthen the bone through their extensive lacuna-canalicular system in response to physical stresses and other local stimuli on the bone (24,28).

2.1.4.5 Osteoclasts

Osteoclasts are giant multi-nucleated cells that originate from monocyte or monocyte-like cells in the bone marrow under the influence of several factors (29,30). Osteoclasts, which have acidophilic cytoplasms, are found in clusters along the surface of the bone and inside the canals. Their main function is bone resorption. In addition, homeostasis of bone formation and resorption that occur when the bone is being remodeled or damaged is maintained by osteoclasts. They contain proteolytic enzymes such as cathepsin K and matrix metalloproteinase and absorb the ECM of the bone in areas where bone resorption begins (31).

2.1.5 Bone Formation and Types of Ossification

During embryogenesis, the formation of bone tissue can be seen in two ways: endochondral and intramembranous ossifications (32).

2.1.5.1 Endochondral Ossification

Endochondral ossification occurs when the cartilage plate is present and cartilage cells play a significant role in this process. Long bones are formed by this type of ossification (33). First, mesenchyme cells transform into chondroblasts and form a membrane, which is perichondrium, around the cartilage template. Then these chondroblasts differentiate into chondrocytes that are observed in their lacunae and secrete an enzyme and growth factors to promote mineral deposition and blood vessel invasion. Periosteum, whose inner osteogenic layer hosts osteoprogenitor cells, is formed

by this process. Chondrocytes undergo death by apoptosis and osteoprogenitor cells and blood vessels fill in the space leftover by chondrocytes. Osteoprogenitor cells differentiate into osteoblasts and form the bone matrix. Finally, this bone matrix is calcified and forms the bone tissue (32,34,35).

2.1.5.2 Intramembranous Ossification

Intramembranous ossification is direct ossification that occurs without relying on the cartilage plate such as endochondral ossification. Flat bones such as the skull bones, clavicle, and parts of the mandible are formed by intramembranous ossification (36). MSCs proliferate and condense to promote the first immature form of bone tissue. MSCs go through differentiation to first become osteoprogenitor cells and then osteoblasts. As the osteoid tissue, which is formed by osteoblasts, increases in amount to form rudimentary bone tissue known as bone spicule, the osteoblasts turn into osteocytes, and new ones that differ from MSCs replace them. Thereby, bone trabeculae are formed by these bone spicules that grow and are fused with each other. Trabeculae then fuse to form primary bone which is remodeled to become secondary bone (37,38).

2.1.6 Growth and Development of Bone Tissue

Throughout life, processes of formation and resorption occur continuously for the growth and transformation of bone tissue. Two basic processes are required for the formation of new bone tissue and skeletal growth; modeling and remodeling. Although these two processes appear to be two similar mechanisms, they represent two different mechanisms due to the fact that the activities of the bone cells involved in the process are interconnected or separate. On the one hand, new bone tissue formation occurs, on the other hand, bone resorption occurs (39).

2.1.6.1 Bone Modeling

Modeling is a process that occurs mainly during the period of skeletal growth, in which bones change shape and size to allow the bone to grow harmonic and adapt to mechanical forces. While both modeling and remodeling occur together in early childhood, the dominant process is modeling (40). Bone formation at the periosteal surface of bone without prior bone resorption increases the bone size and changes bone

shape (41). Cartilage proliferates in the epiphyseal and metaphyseal areas of long bones before undergoing mineralization to form new bone. Bone is formed by the deposition of mineralized tissues into areas encoded for development after the cartilage framework settles (40).

2.1.6.2 Bone Remodeling

Remodeling is the condition of a balanced continuation of the destruction and construction of bone tissue in order to preserve the normal bone structure and remove micro-damaged bone tissues due to mechanical forces and replace it with a new one, which prevails throughout the entire adulthood (42). Most areas of remodeling are random, even if remodeling takes place in areas of bone that require repair to prevent the accumulation of microdamage.

Bone resorption and bone formation are closely interconnected in the remodeling process. An imbalance between these two processes results in bone mineral diseases such as osteoporosis with excessive bone loss or osteopetrosis with excessive bone formation (40).

In the activation phase of the remodeling process, the interaction of osteoclast and osteoblast precursor cells is observed depending on the effect of local or systemic factors on osteoblast mesenchymal stem cells. As a result of this interaction, osteoclasts differentiate and migrate towards the mineralized bone surface and fusion occurs there. These cells initiate resorption by secreting hydrogen ions at low pH and lysosomal enzymes such as Cathepsin K (43).

As a result of resorption, irregular cavities are formed on the trabecular bone surface. Mononuclear cells such as macrophages on the bone surface complete the resorption process in the recycling phase of the remodeling process and create signals that will initiate bone formation. With the onset of the bone formation phase, osteoblasts that differentiate from mesenchymal precursor cells and fill the cavities provide bone formation (44,45).

2.1.7 Regulatory Factors of Bone Formation Mechanism

Various local and systemic factors are required in order to harmoniously maintain the cellular functions that occur in the modeling and remodeling processes involved in the bone formation process.

2.1.7.1 Local Regulatory Factors

2.1.7.1.1 Growth Factors

Growth factors are polypeptides that are produced by the bone cells themselves or in non-osseous tissue and affect bone metabolism mainly by acting as modulators of cellular functions such as growth, differentiation, and proliferation. During bone healing, these structures that provide vascularization, stiffness, and mechanical function of the bone are insulin-like growth factors (IGF-I and II), transforming growth factors (TGF), bone morphogenetic proteins (BMP), platelet-derived growth factors (PDGF), fibroblastic growth factor (FGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) (46,47).

2.1.7.1.2 Matrix Proteins

Matrix proteins are proteins that act as modulators of growth factors and play a greater role in the regulation of many different cell functions such as cell adhesion, proliferation, and differentiation, as they have a concentration up to a thousand times greater than growth factors (48).

2.1.7.1.3 Cytokines

Cytokines are polypeptides that play an important role in many cellular functions such as immunological response, inflammation, and hematopoiesis and have both autocrine and paracrine effects. Interleukin 1 (IL-1), Interleukin 6 (IL-6), Interleukin 11 (IL-11), tumor necrosis factor (TNF), granulocyte/macrophage-colony stimulating factor (GM-CSF), and macrophage-colony stimulating factor (M-CSF) are osteoclastogenic cytokines that directly or indirectly increase bone resorption by stimulating the bone resorption process and play an important role in the bone remodeling process (49).

2.1.7.1.4 Prostaglandins

Prostaglandins are enzymatically derived metabolites from arachidonic acid that are involved in bone remodeling by mediating the regulation of osteoblast and osteoclast functions. Among the prostaglandins produced by almost all cells of the body, Prostaglandin E_2 (PGE2), in particular, is involved in the adaptation of bone to stress responses, while it is widely involved in various processes of inflammation and in the regulation of bone metabolism (50).

2.1.7.2 Systemic Regulatory Factors

2.1.7.2.1 Parathyroid Hormone

Parathyroid hormone (PTH) is synthesized and secreted by the parathyroid glands. The main function of PTH is to provide blood calcium hemostasis and in addition to this, it regulates bone mass endocrine. PTH can have both anabolic and catabolic effects on bone. PTH has no direct effect on osteoclasts. A persistently high level of PTH in the body indirectly induces bone resorption due to its effects on osteoblasts. The anabolic effects of PTH are thought to be through its effects to increase proliferation and differentiation of osteoblasts, reduce osteoblast apoptosis, and activate bone-lining cells. Thus, the osteoclast stimulating factor is secreted and the bone matrix is resorbed (51).

2.1.7.2.2 1,25 $(OH)_2$ Vitamin D_3

Vitamin D is essential for normal skeletal development. Vitamin D regulates intestinal calcium and phosphate absorption, which provides substrates for bone mineralization. Active vitamin D plays a central role in calcium and bone homeostasis by binding to the vitamin D receptor (VDR). While the intestinal VDR has a primary role in regulating calcium supply for skeletal mineralization, vitamin D also has direct effects on skeletal cells. Thus, bone mineralization is maintained and calcium and phosphate are found at normal levels in the serum (52).

2.1.7.2.3 Calcitonin

Calcitonin is a polypeptide hormone synthesized in the C cells of the thyroid gland. Calcitonin has a significant inhibitory effect on osteoclasts. Bone resorption is inhibited by the main mechanism of the rapid decrease in serum calcium level induced by calcitonin. Thus, an inhibitory effect on osteoclastic activity is observed in mature osteoclasts (53).

2.1.7.2.4 Sex Hormones

Sex hormones decrease the rate of bone remodeling and increase bone mineral density (BMD). Both androgens and estrogens suppress osteoblast apoptosis and increase osteoclast apoptosis. Androgens inhibit resorption and stimulate bone formation. Estrogens have a dual effect on bone metabolism. On the one hand, they inhibit bone resorption and limit the remodeling process by reducing osteoclast activity and cytokines involved in bone resorption. On the other hand, they increase the number and function of osteoblasts (54,55).

2.1.7.2.5 Thyroid Hormones

The hypothalamic-pituitary-thyroid axis (HPT) has an important role in the development of the skeletal system, access to peak bone mass, and regulation of bone turnover (56). Thyroid hormones have direct effects on osteoblasts. They directly stimulate osteoblast differentiation and mineralization. Hypothyroidism causes a decrease in osteoblast formation and osteoclast resorption, leading to a prolongation of the remodeling process. Thyrotoxicosis, which means an excess of thyroid hormone in the body, is associated with osteoblastic bone formation and osteoclastic bone resorption. Hyperthyroidism, the most common cause of thyrotoxicosis, increases bone turnover with an impaired bone formation cycle and shortens the duration of the remodeling process (57,58).

2.1.7.2.6 Glucocorticoids

Glucocorticoids exert anabolic and catabolic effects on bone metabolism in a dose-dependent manner. Glucocorticoids at physiological doses have anabolic effects on

bone turnover (59). Glucocorticoids above the physiological dose cause osteoporosis. High doses of glucocorticoids inhibit the differentiation and function of osteoblasts and increase osteoblast apoptosis (60,61). Thus, bone resorption accelerates and bone formation decreases, affecting the remodeling process (62). However, increased bone resorption due to high-dose glucocorticoid levels is temporary, and long-term glucocorticoid therapy causes a decrease in osteoclast numbers and resorption (63).

2.1.7.2.7 Growth Hormone

Growth hormone (GH) induces the release of insulin-like growth factor 1 (IGF-1). It directly and/or indirectly increases the production of IGF-1 and increases bone turnover by stimulating both production and resorption (64). The main function of IGF-1 is to maintain bone mass and homeostasis during the remodeling process (65). In addition, IGF-1 facilitates osteoclast differentiation. With aging, there is a decrease in GH release and IGF-1 production. In GH deficiency, bone resorption increases, and as a result, osteoporosis develops (66,67).

2.1.8 Alveolar Bone

Alveolar bone is the part of the jawbone that contains tooth sockets and supports teeth. Alveolar bone is the most dynamic tissue in both the periodontal system and the skeletal system. Its structure varies from person to person and usually becomes more intense with age. The main functions of the alveolar bone are to protect the tooth roots and support the chewing function. In addition, alveolar bone serves as a source for hematopoietic and mesenchymal stem cells and a reserve for calcium, phosphate, and magnesium (68,69).

Alveolar bone consists of bundle bone that supports trabecular and cortical bone. Bundle bone is the layer of bone that covers the tooth socket and supports the attachment of Sharpey's fibers. The inner surface of the bundle bone is surrounded by the periodontal ligament, and the outer surface is surrounded by supporting trabecular and cortical bone (70).

In order to adapt to various mechanical forces on it, alveolar bone is reshaped by resorption in the pressure areas where the force comes from and by the physiological new

bone formation in the areas of tension. The most important factor affecting the structure of the alveolar bone is the presence of teeth and their active function. Tooth extraction affects the vertical and horizontal dimensions of the alveolar bone and may cause dimensional reduction (71).

2.1.9 Healing of Extraction Wound

Bleeding occurs following tooth extraction and erythrocytes cluster and settles into the extraction socket. The vessels in the periodontal membrane contract as fibrin meshes form. Moderate inflammatory response develops with tissue damage (72).

Day 1: After 24 hours, leukocyte migration from the periphery to the clot occurs following vasodilation. Fibrin filling the extraction socket is the basis of healing.

Day 2: Fibroblasts emerging from the periodontal membrane begin to spread inward from the periphery of the wound. The surface epithelium begins to proliferate. Bone resorption begins with osteoclastic activity in the alveolar bone surrounding the tooth socket.

Day 3: Fibroblast migration from the surrounding connective tissue and the development of capillaries continue until the clot is replaced by granulation tissue.

Day 7: The fibroblasts within the fibrin network began to proliferate and organize. The number of new capillaries has increased. The surface epithelium is advanced, epithelialization may be completed in small wounds. Small alveolar particles damaged during extraction are either resorbed or excreted as sequestra.

Day 14: The granulation tissue begins to take the place of the clot. Young trabeculae that are not yet mineralized are noticed in the nymphs of the wound. Young bone trabeculae result from the transformation of potential cells from periodontal membrane remnants into osteoblasts. The cortical bone of the outer alveoli is reconstructed. But it is not as high as before. Superficial epithelialization is complete. The original shape of the alveoli begins to form on the 14th day.

Day 21: As a result of the deposition, the socket is filled with incompletely calcified bone. In approximately four weeks, the primary bone filling the extraction cavity

is first resorbed and then filled with a trabecular bone until it regains its normal shape. Partially mineralized trabecular structures may also be seen.

2.2 Erythropoietin

Human erythropoietin (EPO) is a glycoprotein hormone with an atomic mass of 30.4 kDa. It consists of a single chain of 165 amino acid residues with four glycans attached. The main production site of EPO is the renal peritubular cells in the kidneys but in adult life, 10% of EPO is produced by the liver. EPO synthesis is controlled by hypoxia-inducible transcription factors (HIFs). EPO keeps the blood hemoglobin (Hb) concentration constant by regulating red blood cell (RBC) production. EPO deficiency results in anemia (3,73).

2.2.1 Pharmacology of Erythropoietin

Erythropoietin is mainly produced in the renal cortex by the peritubular fibroblasts. The mRNA of EPO can be found in other organs such as the spleen, liver, bone marrow, brain, and lungs since it can also be translated into these organs as well. The main synthesis site of EPO is the liver in the fetal stages of life. The circulating EPO concentration is controlled by the pO_2 and the affinity of Hb-O_2. The synthesis of EPO occurs by the rate of transcription of the EPO gene which is EPO in chromosome 7. This process is affected by various transcription factors. GATA-2 and nuclear factor-kB (NF-kB) inhibit the EPO promoter. In inflammatory diseases, this inhibition causes compromised EPO expression. Heterodimeric (/, 100– 120 kDa each), hypoxia-inducible transcription factors (HIFs) activate the EPO enhancer which has a hypoxia-response element (HRE) (3,74).

2.2.2 Administration of Erythropoietin

The international non-proprietary drug name (INN) of eucaryotic cell-derived rhEpo is Epoetin. Its amino acid sequence is exactly the same as endogenous human EPO. A random prefix is used to identify the differences in the amino acid residues chain. A Greek letter such as alpha, or beta is used to name the glycosylation patterns. Approximately 25 years ago, two types of Chinese hamster ovary cell-derived rhEpo, which are called epoetin alfa and epoetin beta, were produced as anti-anemic agents. Both

epoetin alpha and beta have been used for the same indications which are anemia occurring as a result of chronic kidney disease (CKD) and cancer treated with myelosuppressive chemotherapy.

Clinically, an appropriate single dose of 50 IU/kg body weight (b.w.) of RhEpo is administered intravenously (IV) and it is disposed of at a first-order kinetic rate after the rapid distribution phase (volume of distribution 0.03–0.09 l/kg b.w.). Accordingly, after IV administration; peak plasma rhEpo concentrations (IU/l) can be approximately predicted by multiplying the dose (IU/kg) with the factor 20 (3,75). Peak plasma concentrations of rhEpo following subcutaneous (SC) administration are established after 12–18 h with bioavailability adding up to roughly 30%. When applied subcutaneously, peak plasma concentrations are roughly one-twentieth of the primary values measured following IV administration. On the other hand, the slow absorption enables about 30% lower drug requirements with SC when compared with IV administration (3,76).

2.2.3 Action Mechanism of Erythropoietin

CD34+ hematopoietic stem cells, colony-forming units generating granulocytes, erythrocytes, monocytes, and megakaryocytes (CFU-GEMMs) give rise to red blood cells. The erythrocytic compartment's earliest offspring are the burst-forming units-erythroid (BFU-Es). Their descendants undergo 12 divisions and produce several hundred erythroblasts in 10 to 20 days. Colony-forming units- erythroid (CFU-E)'s are next to BFU-Es. Their function is to express abundant EPO receptor molecules (EpoR) and go through apoptosis when EPO is absent. When EPO is present, CFU-Es and their offspring undergo 3–5 divisions and generate 8 to 64 erythroblasts in 7–8 days. The cells lose their nuclei and turn into reticulocytes when the level of orthochromatic erythroblasts) is achieved (3,77). Mature erythrocytes and reticulocytes do not contain EpoR. When EpoR dephosphorylation by the tyrosine phosphatase, Src homology phosphatase-1 (SHP-1), occurs and the Epo/EpoR-complex is internalized, the action of EPO is finished. Circulating EPO's main mechanism of degradation is mediated by EpoR since it affects EPO uptake by its target cells (3,78).

2.2.4 Side Effects and Reliability of Erythropoietin

rhEpo formulations or its analogs do not have reports of acute toxic effects. Patients who are hypersensitive to non-human cell-derived products are contraindicated for EPO application. Moreover, rhEpo administration should be done very carefully because EPO's effect on the fetus have not been assessed in thorough studies. In patients who have CKD, EPO administration has the side effect of increased arterial blood pressure and likely hypertension. As a result, EPO is contraindicated for patients with uncontrolled hypertension. This is likely due to increase of hemoglobin concentration causing raised blood viscosity and the reversal of hypoxia-induced vasodilatation. Patients who have non-renal anemia do not exhibit hypertension as a result of EPO treatment (3,79,80).

Administration of EPO can also elevate the occurrence of thromboembolism and the risk of cardiovascular episodes which include death. Most likely, the increase of hemoglobin and hematocrit concentration levels cause the incidence of cardiovascular events. A different critical issue with EPO administration is its effect on tumor growth. EPO and its analog's effect on the promotion of tumor growth by angiogenesis stimulation is the focus of several meta-analysis studies. Studies by Bennett et al. and Bohlius et al. address the negative impact risk on mortality by the application of EPO, but not on how EPO affects the progression of cancer (79,80). Other meta-analysis studies have demonstrated that EPO administration do not usually impact the progression of cancer (3,81,82).

2.3 Clinical Applications of Erythropoietin

EPO is mainly indicated for use in chronic forms of anemia. However, it is not an alternative to transfusion of red blood cells in cases of severe trauma-induced hemorrhage, severe or life-threatening anemia, and considerable blood loss during surgery such as in cardiothoracic or liver surgery. EPO has been approved for use in patients who have anemia as a result of CKD before and during dialysis, cancer patients who receive chemotherapy, and patients with HIV who have anemia due to zidovudine treatment. EPO has also been approved for elective surgery, to support autologous blood collection in preterm infants who have anemia (3).

3. MATERIALS AND METHODS

This study was carried out on 30 Sprague Dawley adult male rats, 10-12 weeks old and weighing 272 grams on average, obtained from Yeditepe University Experimental Animals Study Center (YUDETAM). The content of the study was approved by the decision of the Yeditepe University Experimental Animals Ethics Committee (YUDHEK) dated 24.06.2019 and numbered 766 (Appendix-1). All rats were housed in metal cages in triplicate at 21˚C, at 40% to 60% humidity, in continuous warm and fresh air, in a cycle of 12 hours of light and 12 hours of dark. The rats were fed with continuous and unrestricted water and without any food restrictions.

The rats used in the study were randomly divided into five groups, with six animals in each group.

- Group 1 (n=6) Tooth extraction + Saline solution (SS)
- Group 2 (n=6) Tooth extraction + Systemic Erythropoietin (450 IU/kg) (EPO 450)
- Group 3 (n=6) Tooth extraction + Systemic Erythropoietin (1350 IU/kg) (EPO 1350)
- Group 4 (n=6) Tooth extraction + Geletamp (GEL)
- Group 5 (n=6) Tooth extraction + Geletamp + Local Erythropoietin (GEL/EPO LOCAL)

The day on which the experimental animals were received was considered the first day of the study (day 1). On day 1, rats were administered intramuscular (IM) injections of 80-100 mg/kg Ketamine hydrochloride (Ketalar®, Pfizer, Istanbul, Turkey) and 10 mg/kg 2% Xylazine hydrochloride (Rompun®, Bayer) as both analgesic and anesthetic. General anesthesia was provided before tooth extraction by these injections. To prevent ophthalmic complications during general anesthesia, bacitracin and neomycin sulfate ointment (Thiocilline®, Abdi İbrahim, Turkey) was applied to the eyes of rats.

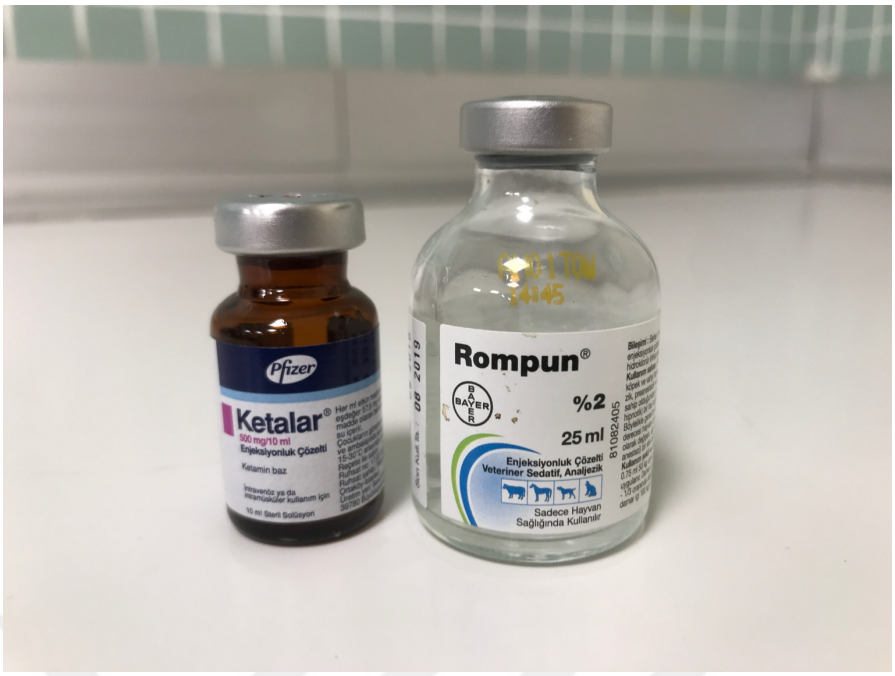

Figure 3.1. Ketamine hydrochloride and Xylazine hydrochloride

Figure 3.2. IM injection of ketamine hydrochloride and xylazine hydrochloride into rats

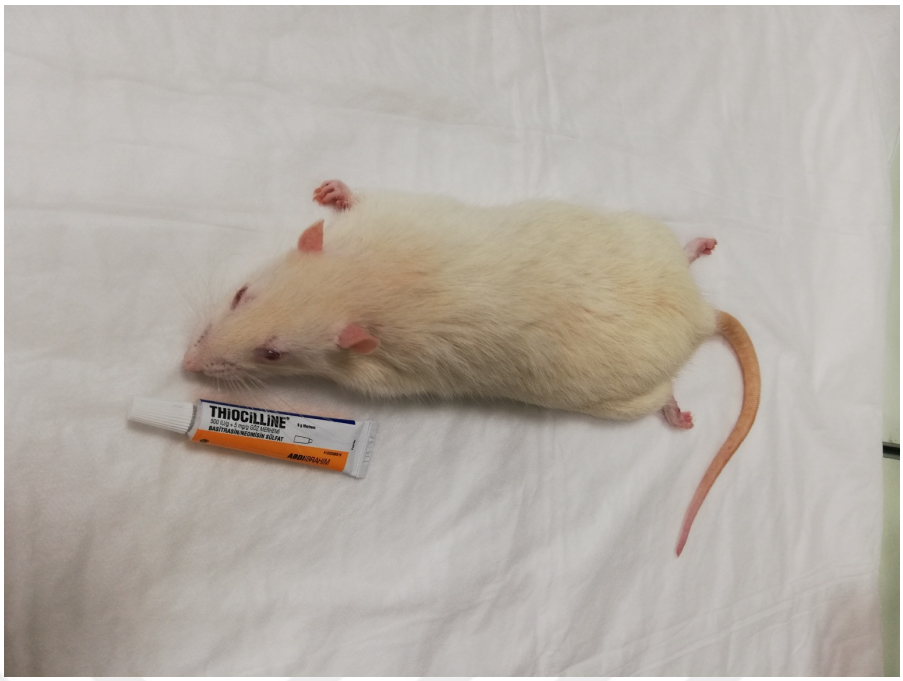

Figure 3.3. The ointment was applied to the eyes of rats to prevent ophthalmic complications

Under general anesthesia, the tongue and cheeks of the rats, which were prepared for surgery by complying with asepsis and antisepsis conditions, were retracted with the help of sterile surgical instruments and rubber bands, and the left upper 1st molar was luxated and extracted with a probe and hemostat.

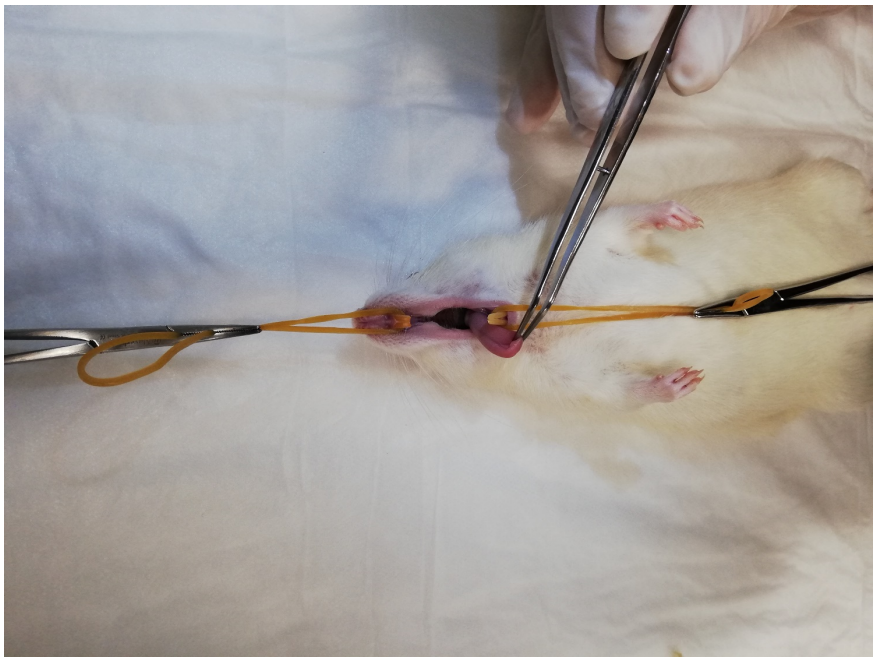

Figure 3.4. The field of view was ensured by opening the jaws with a rubber band

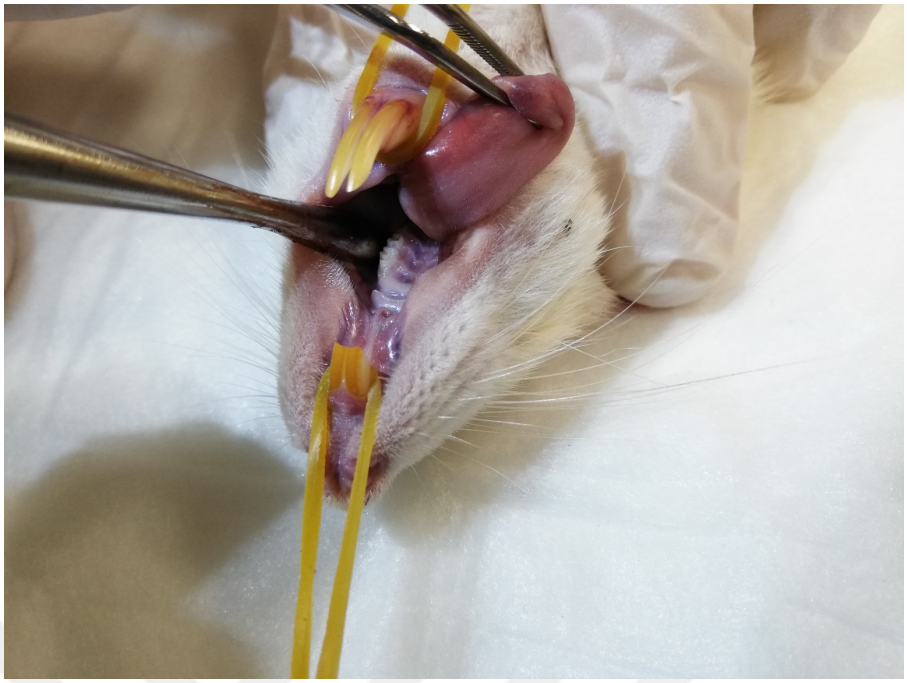

Figure 3.5. Intraoral view before tooth extraction

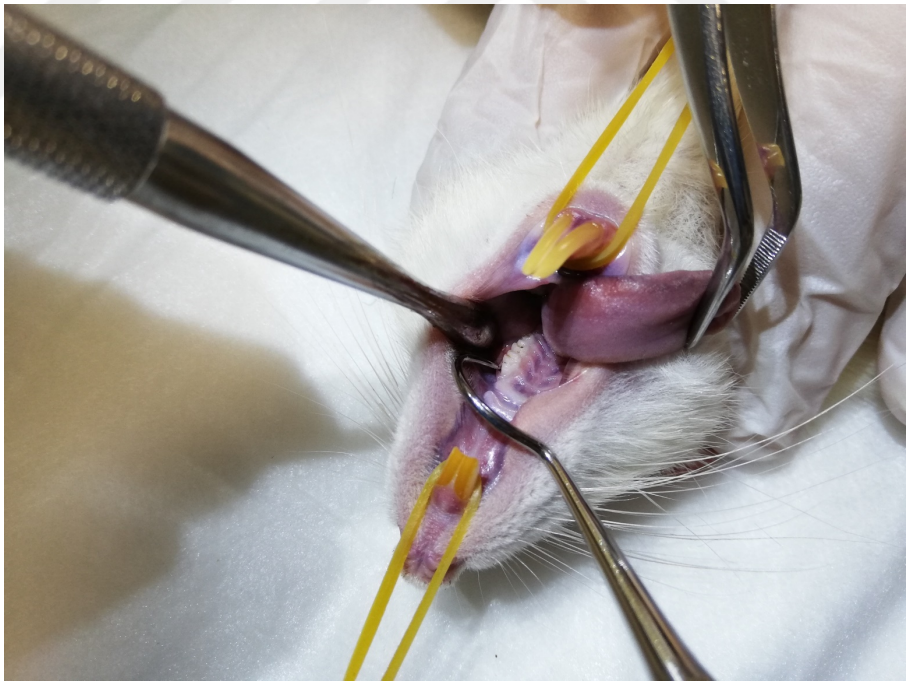

Figure 3.6. Luxation of the left upper first molar with a probe

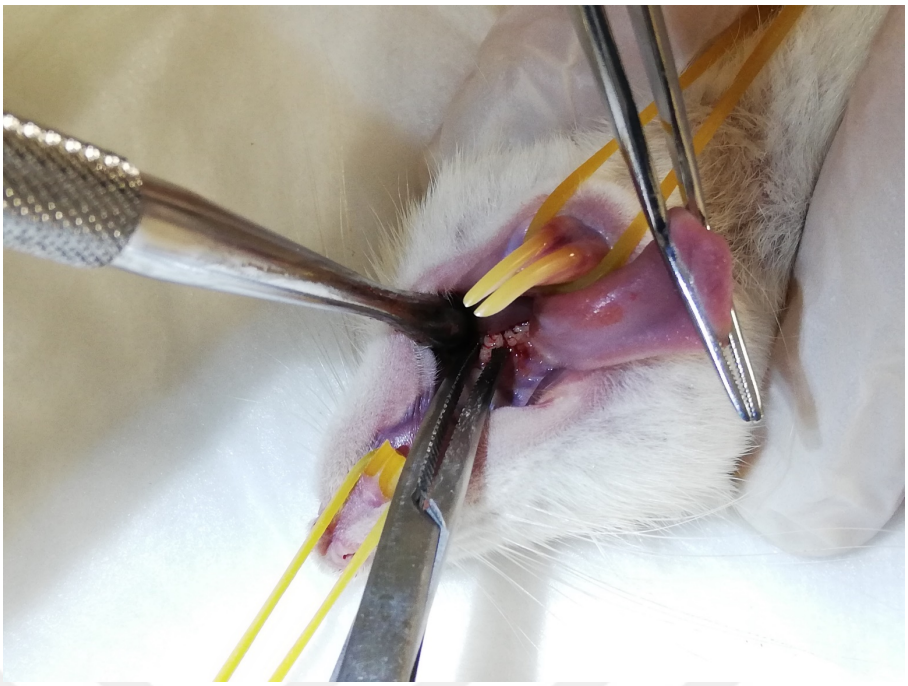

Figure 3.7. Luxation of the left upper first molar with a clamp

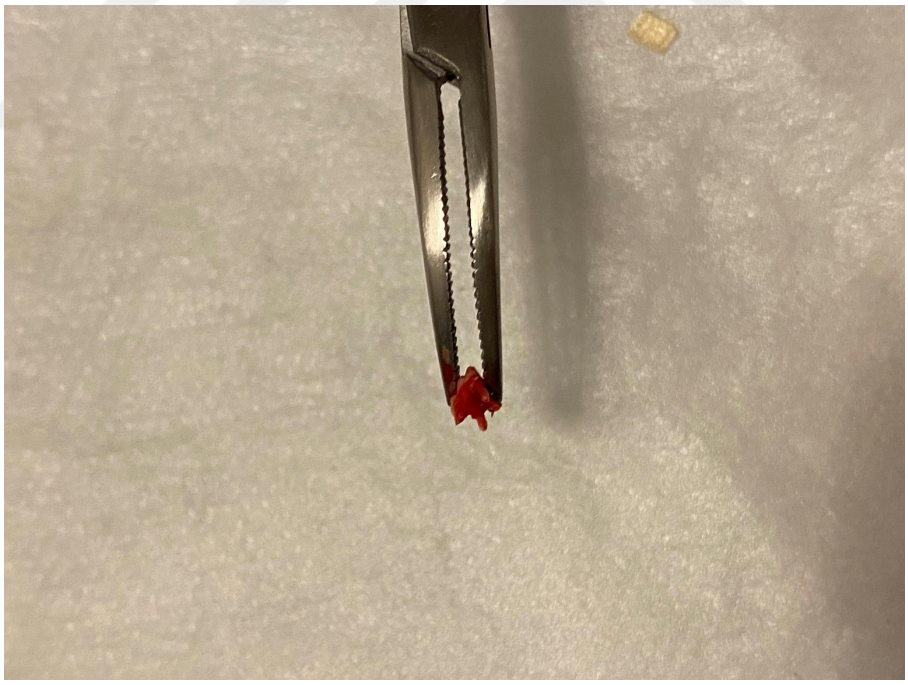

Figure 3.8. Extracted left upper first molar

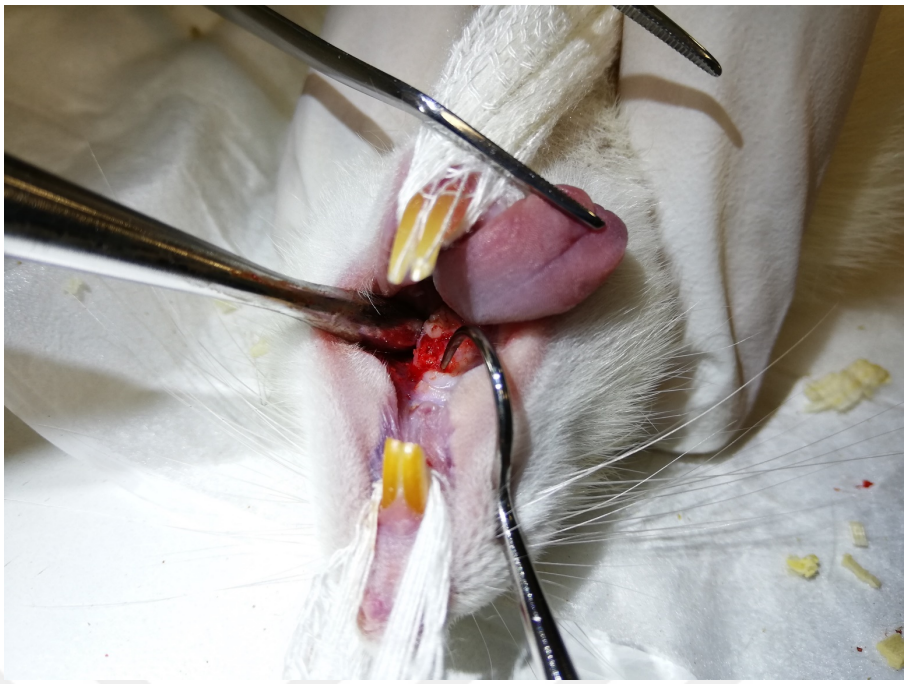

Figure 3.9. View of the extraction socket

After tooth extraction, the extraction site was compressed with a sterile sponge. Intraperitoneal (IP) saline solution (SS) (0.1 mg/ml) injection was applied to the rats in Group 1. Rats in Group 2 and Group 3 were injected subcutaneously (SC) with a single dose of 450 IU/kg and 1350 IU/kg of erythropoietin (EPO) (BINOCRIT 4000 IU/0.4 ml, Sandoz, Austria), respectively. While Geletamp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) was placed alone in the extraction sockets of the rats in Group 4, Geletamp® was locally placed in the extraction sockets of the rats in Group 5 together with EPO. Locally administered EPO's effect was examined following Rölfing et al.'s study. In Rölfing's study, a collagen scaffold, which was soaked with EPO, was used and resorbed amount of EPO was measured (83). The same principle was applied in the present study and Geletamp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) was used as the collagen scaffold. An empty dappen glass was filled with EPO and then a piece of Gelatemp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) (length 7mm, height 3.5mm, width 3.5mm) was put inside the dappen glass. Afterward, Gelatemp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) which was soaked with EPO was removed and the amount of EPO left on the dappen glass was measured; which was equal to 20 units of EPO. After tooth extraction, powder feed was placed in the cages in order to help the rats to be fed.

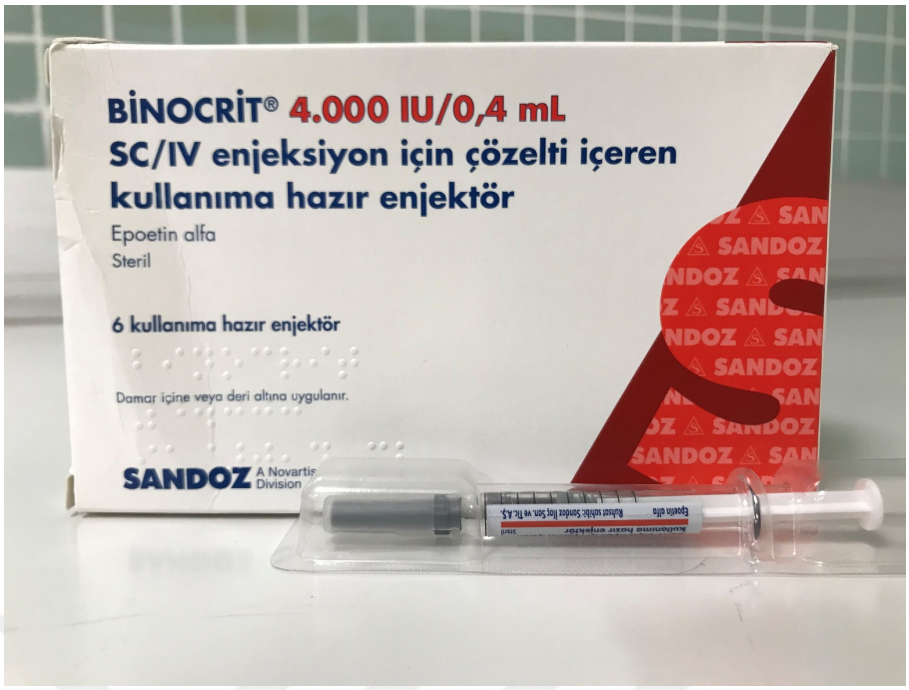

Figure 3.10. Erythropoietin

Figure 3.11. SC administration of EPO

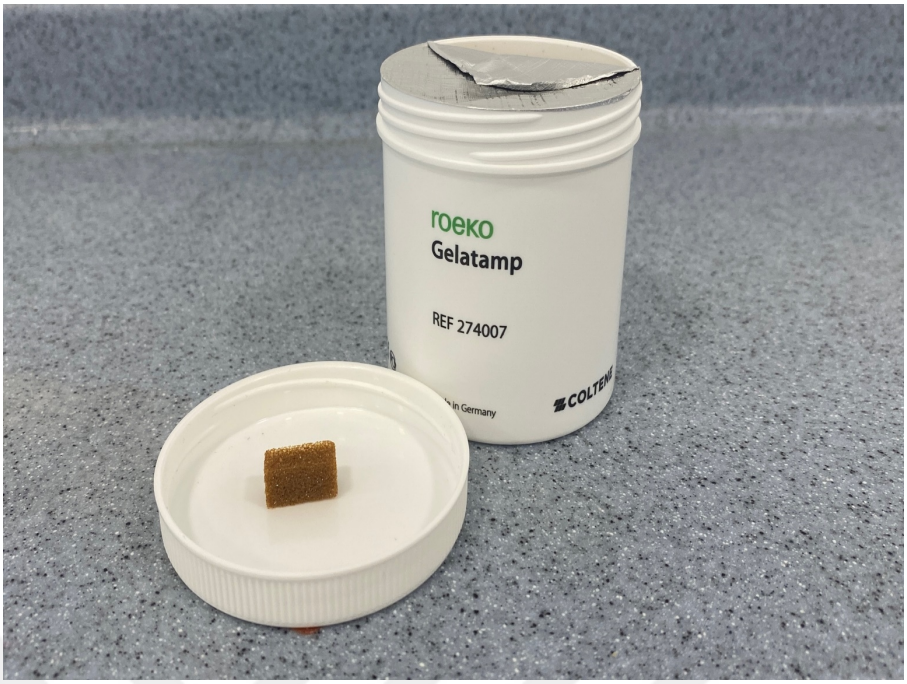

Figure 3.12. Gelatemp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany)

Figure 3.13. Local administration of EPO with Gelatemp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany)

The study animals tolerated the study procedures. No study animals died during or after the operation. At the end of the study period, which was on the 8th day, 2.5 ml of blood sample was taken from the jugular vein of all experimental animals before sacrification and put into purple capped tubes.

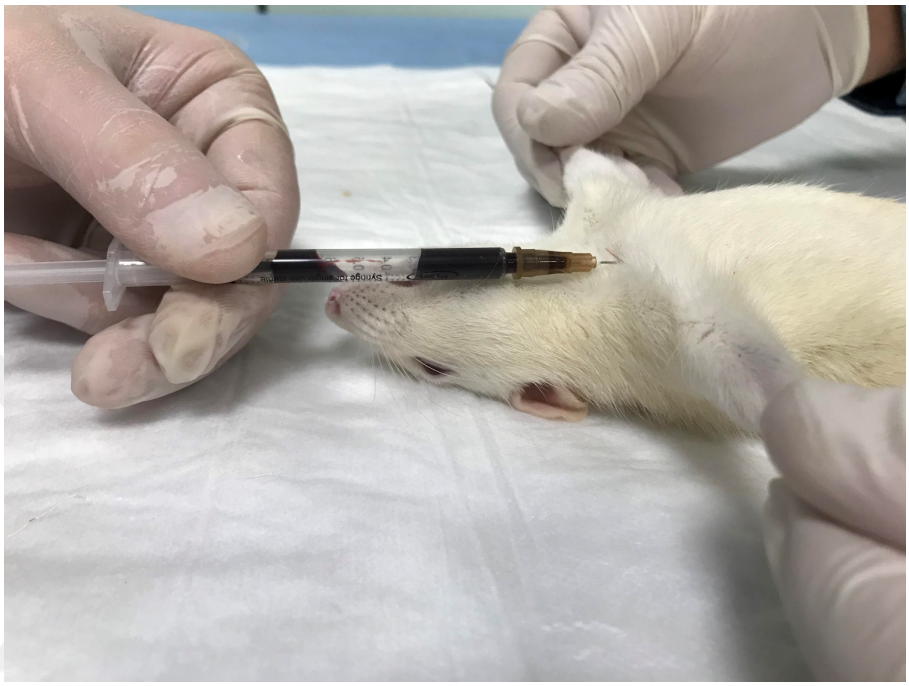

Figure 3.14. Collection of the blood sample

A hemogram test was performed at YÜDETAM for each blood sample taken. All experimental animals were sacrificed on the same day by the decapitation method. The maxilla of each rat was then dissected and removed. For histological and histomorphometric evaluations, the tissues were placed to 10% neutral buffered formalin (NBF) (pH = 7.0 ± 0.3) and kept in this solution for 1 week for fixation. Histological and histomorphometric evaluations were carried out in the Department of Tumor Pathology, Institute of Oncology, Istanbul University.

3.1 Clinical Evaluation

The relevant region of the maxilla of the rats was examined with the naked eye to assess the presence of abscess, fistula orifice, and tissue healing.

3.2 Histological and Histomorphometric Evaluation

Tissue samples to be examined under the light microscope were taken into tissue containers containing 10% neutral buffered formalin at +4˚C. After the fixation phase, the tissues were decalcified in 50% formic acid and 20% sodium citrate solution for 4 weeks. Decalcified tissues were dehydrated by passing through alcohol series. After they were cleared in xylene, the buccal surfaces were blocked by embedding in paraffin at 58ºC-65ºC to form the cross-sectional surface.

Paraffin blocks prepared from routinely processed decalcified samples were cut into 4 µm slices and dewaxed by placing them in xylene. Sections were rehydrated by passing through an alcohol series. Sections immersed in water were stained with hematoxylin for 10 minutes and washed in tap water for 10 minutes. After bluing in phosphate-buffered saline (PBS), it was stained with eosin containing 90% alcohol for 1 minute. Sections were dehydrated by passing through alcohol series and cleared with xylene. The sections were covered with a coverslip after a drop of xylene-based sealing solution was applied to them.

Sections were evaluated with an Olympus BX60 light microscope connected to a color video camera and computer. Extraction sockets were captured using the camera and displayed on a computer monitor. All measurements were made with the Olympus Image Analysis System 5. Sections were evaluated at x40, x100 and x200 magnification for new bone formation. Inflammation and fibrosis were evaluated as mild (1+), moderate (2+), and severe (3+). In addition, it was evaluated as "-" if there was no necrosis and foreign body reaction, and "+" if there was (Table 3.1).

Table 3.1 Histological Scoring Criteria

Score	Result
1 mild; 2 moderate; 3 severe	Inflammation
1 mild; 2 moderate; 3 severe	Fibrosis
- absent; + present	Foreign body reaction

3.3 Statistical Analysis

Data were analyzed using the SPSS 25.0 (Statistical Packages of Social Sciences) program. Explanatory statistics are shown as mean ± standard deviation, median, minimum and maximum values for continuous variables. The conformity of the data to the normal distribution was evaluated with the Shapiro Wilks test. Homogeneity of variances was evaluated with Levene's test. ANOVA test was used to compare the measurements that fit the normal distribution between the groups and provide the assumption of homogeneity of variances. Tukey test was used for pairwise comparison of groups. Welch ANOVA test was used to compare the measurements that fit the normal distribution between the groups and did not provide the homogeneity of variances assumption. Dunnett T3 test was used for pairwise comparison of the groups. The Kruskal Wallis test was used to compare the measurements and those that did not fit the normal distribution between the groups. Mann-Whitney U test was performed for pairwise comparisons of the groups, and Bonferroni correction was applied to p values. $P<0.05$ was considered statistically significant.

4. RESULTS

4.1 Clinical Results

Procedures and injections throughout the study were well tolerated by rats. There was no loss of any rats in any of the groups.

In the clinical examination of the rats before the sacrification procedure, no mucosal ulceration at the extraction site, fistula formation in the gingiva, or exposed necrotic bone was observed in any of the study groups.

Clinically, the absence of any signs of infection due to the complete coverage of the mucosa of the extraction area and the absence of exposed bone gave the impression that the extraction cavities were successfully healed.

4.2 Histological Results

4.2.1 Inflammation

When the study animals in all groups were evaluated histologically in terms of inflammation, no statistically significant difference was found between the median values of inflammation according to the groups ($p>0.05$).

Table 4.1. Comparison of the mean inflammation scores by groups

Inflammation							
Group	N	Mean	SD	Median	Minimum	Maximum	P value
1.00	6	1.667	.8165	1.500	1.0	3.0	0.137*
2.00	6	1.500	.5477	1.500	1.0	2.0	
3.00	6	1.000	.8944	1.000	.0	2.0	
4.00	6	2.000	.6325	2.000	1.0	3.0	
5.00	6	2.167	.7528	2.000	1.0	3.0	
Total	30	1.667	.8023	2.000	.0	3.0	

*Kruskal-Wallis Test ($p>0,05$); Standard Deviation: SD

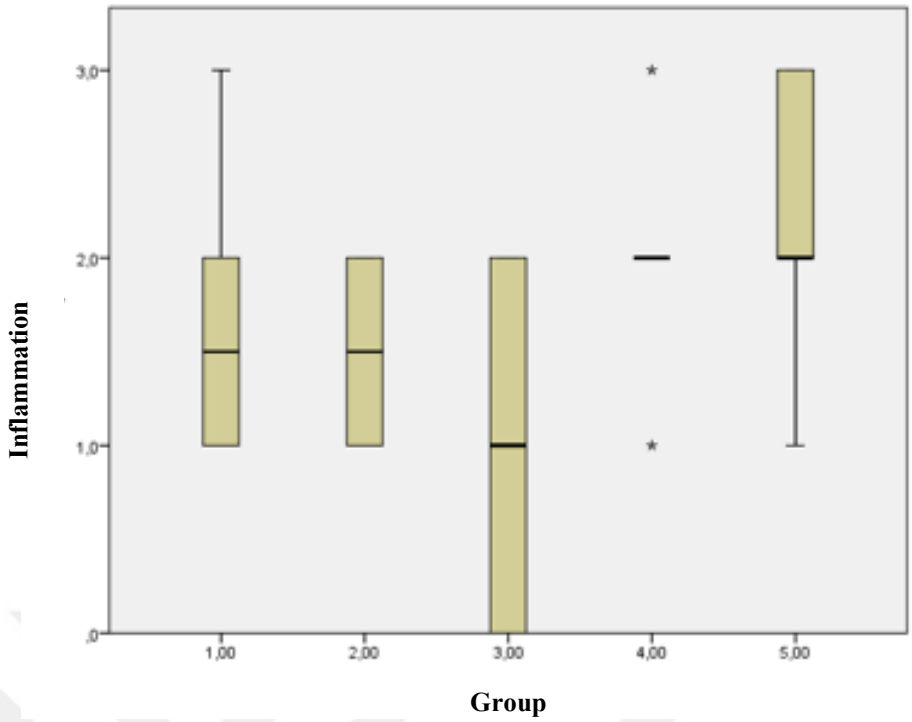

Figure 4.1. Comparison of the inflammation severity of the groups

4.2.2 Fibrosis

When the animals in all groups were evaluated histologically in terms of fibrosis, no statistically significant difference was found between the fibrosis median values of the groups ($p>0.05$).

Table 4.2. Comparison of the mean fibrosis scores by groups

Fibrosis							
Group	N	Mean	SD	Median	Minimum	Maximum	P value
1.00	6	1.667	.8165	1.500	1.0	3.0	0,055*
2.00	6	1.500	.5477	1.500	1.0	2.0	
3.00	6	1.333	.5164	1.000	1.0	2.0	
4.00	6	2.333	.5164	2.000	2.0	3.0	
5.00	6	2.167	.7528	2.000	1.0	3.0	
Total	30	1.800	.7144	2.000	1.0	3.0	

*Kruskal-Wallis Test (p>0,05); Standard Deviation: SD

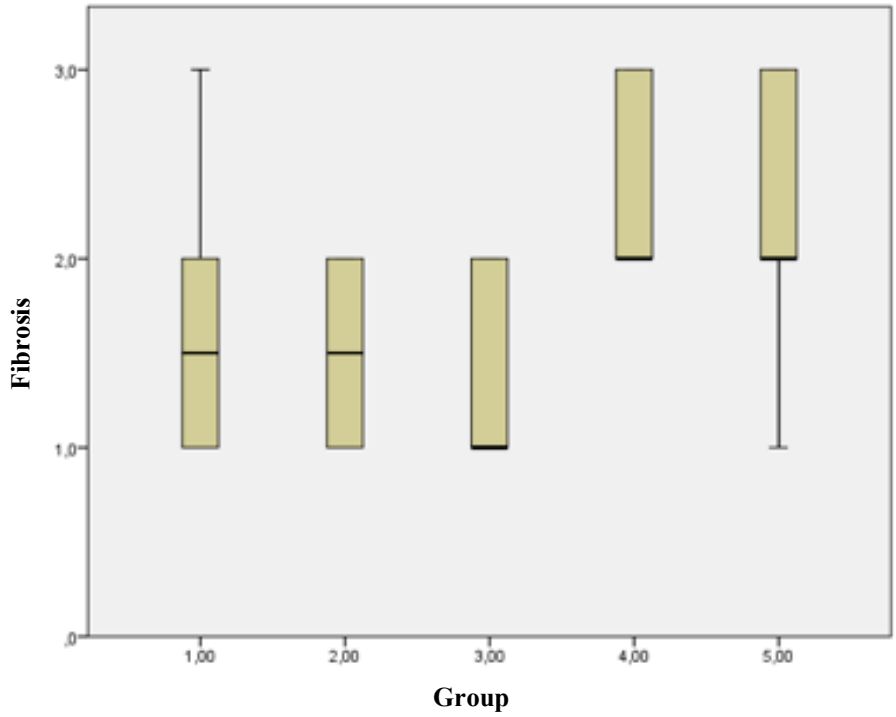

Figure 4.2. Comparison of the fibrosis values of the groups

4.2.3 Foreign Body Reaction

Since foreign body reaction was observed in only one study animal in the second study group, statistical evaluation was not required.

4.3 Histomorphometric Results

4.3.1 New Bone Formation

The amount of new bone formation measured in tooth extraction cavities in the study groups is shown in Table 4.3. When the extraction sockets of the animals in all groups were examined histomorphometrically, a statistically significant difference was found between the mean values of new bone formation according to the groups ($p<0.05$). The group, which was administered with a high dose of systemic EPO, had the highest amount of new bone formation when compared with the other groups. The group, which was administered locally with EPO, had the least amount of new bone formation when compared with the other groups.

Table 4.3. Comparison of the mean new bone formation amount by groups

New Bone Formation (mm)									
	N	Mean	SD	SE	95% Confidence Intervals for Mean		Minimum	Maximum	P value
					Lower Limit	Upper Limit			
1.00	6	9.0883	8.01257	3.27112	.6797	17.4970	.00	22.42	0.007*
2.00	6	11.2250	4.74261	1.93616	6.2479	16.2021	8.15	20.34	
3.00	6	23.3517	4.28876	1.75088	18.8509	27.,8524	18.14	30.27	
4.00	6	10.1400	4.46299	1.82201	5.4564	14.8236	4.37	18.12	
5.00	6	6.9850	2.18302	.89122	4.6941	9.2759	4.65	10.35	
Total	30	11.8247	7.41114	1.35308	9.0573	14.5920	.00	30.27	

*Kruskal Wallis test (*p<0,05); SD: Standard Deviation; SE: Standard Error

In order to determine between which groups the difference in new bone formation occurred, the Mann-Whitney U test, which is a pairwise comparison test, was performed and Bonferroni correction was applied to the *p* values.

Table 4.4. Pairwise comparisons of groups in terms of new bone formation

Pairwise comparisons of groups	
Groups	*P* value
5.00-1.00	1.000
5.00-4.00	1.000
5.00-2.00	1.000
5.00-3.00	.005*
1.00-4.00	1.000
1.00-2.00	1.000
1.00-3.00	.032*
4.00-2.00	1.000
4.00-3.00	.139
2.00-3.00	.237

*p<0.05

There is a statistically significant difference between Group 3 and Group 1; and Group 3 and Group 5 pairwise comparisons (p<0.05). The mean value of Group 3 is higher than the mean values of Group 1 and Group 5.

Even though the mean value of new bone formation is higher in Group 3 than in Group 2, there was no statistically significant difference between the groups.

Group 5 had a lower amount of new bone formation when compared with Group 4, but there was no statistically significant difference between the groups.

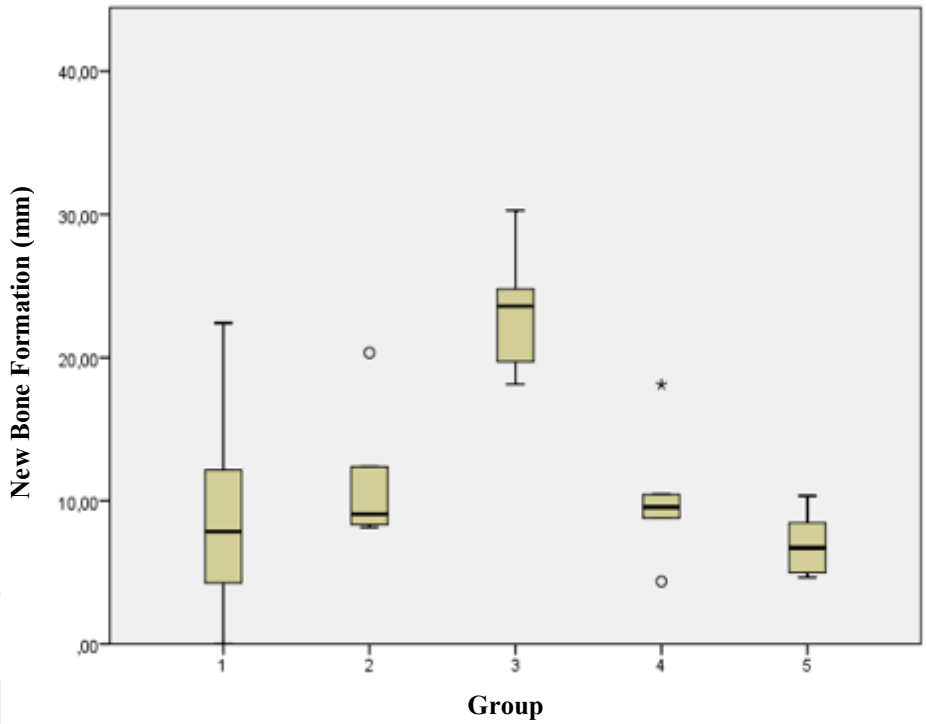

Figure 4.3. Comparison of the mean new bone formation values of the groups

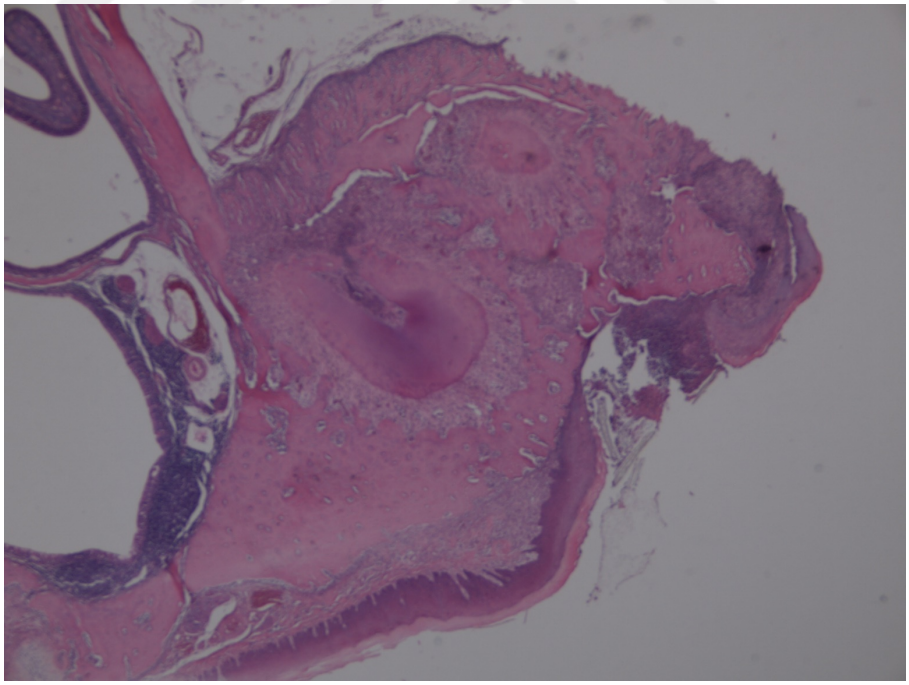

Figure 4.4. Representative histologic H&E stained image (40X magnification) from Group 1. Under the partly ulcerated surface epithelium, severe inflammation, a few new bone formations, and, severe fibrosis were observed on the defect side (H&E X40).

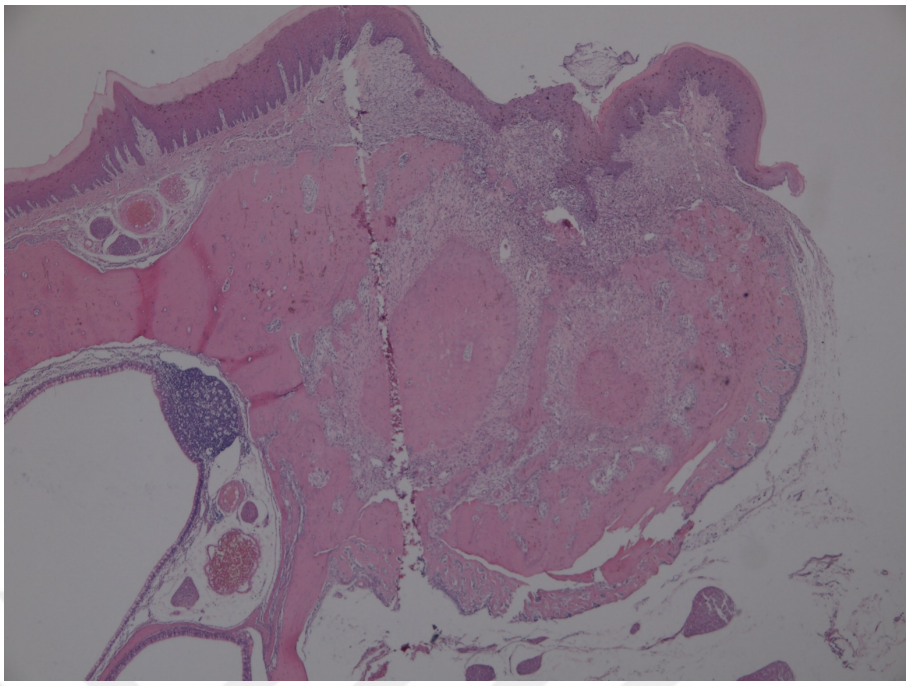

Figure 4.5. Representative histologic H&E stained image (40X magnification) from Group 2 mild inflammation, moderate fibrosis, and new bone formation was identified under the surface epithelium (H&E X40).

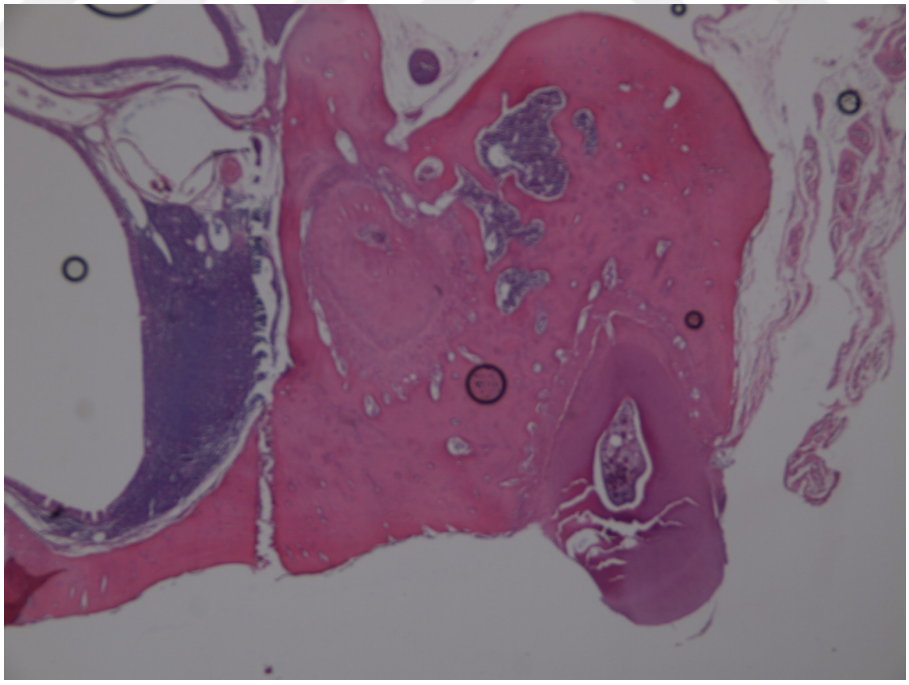

Figure 4.6. Representative histologic H&E stained image (40X magnification) from Group 3. On the defect side, moderate inflammation, moderate fibrosis, and new bone formation were observed. (H&E X40)

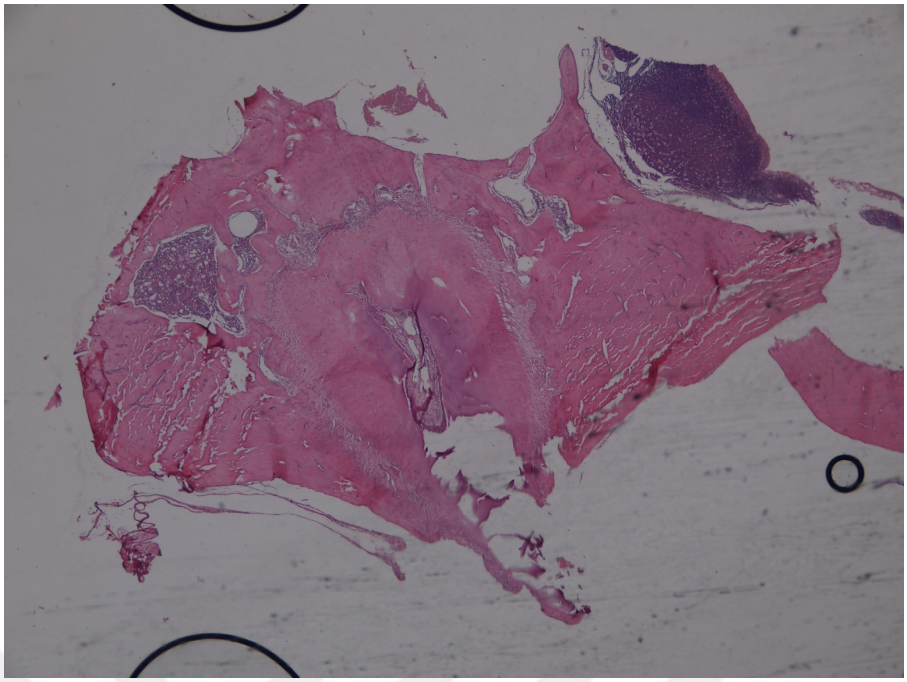

Figure 4.7. Representative histologic H&E stained image (40x magnification) from Group 4. Under the ulcerated epithelium, moderate inflammation, severe fibrosis, and a new bone formation were detected in the defect area. (H&E x40)

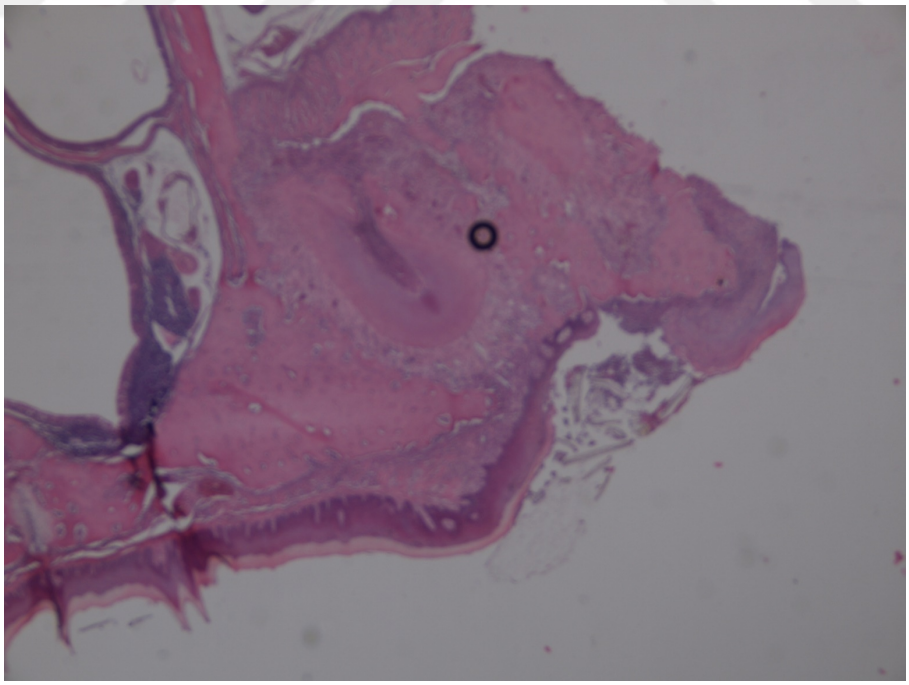

Figure 4.8. Representative histologic H&E stained image (40x magnification) from Group 5. (H&E x40). Severe inflammation and fibrosis were observed; also new bone formation was seen to some degree. (H&E x40)

4.4 Hematological Results

4.4.1 White Blood Cells (WBC)

When the animals in all groups were evaluated hematologically in terms of the amount of white blood cells, no statistically significant difference was found between the mean values of white blood cells according to the groups ($p>0.05$).

Table 4.5. Comparison of the mean white blood cell amount by groups

WBC (10^3/uL)									
	N	Mean	SD	SE	95% Confidence Intervals for Mean		Minimum	Maximum	P value
					Lower Limit	Upper Limit			
1.00	6	17.617	2.7448	1.1205	14.736	20.497	15.1	22.5	0.112*
2.00	6	15.400	1.9287	.7874	13.376	17.424	13.7	18.5	
3.00	6	23.550	6.1591	2.5145	17.086	30.014	15.2	30.4	
4.00	6	15.933	3.1494	1.2857	12.628	19.238	10.6	19.2	
5.00	6	16.650	6.2708	2.5600	10.069	23.231	11.0	28.1	
Total	30	17.830	5.0994	.9310	15.926	19.734	10.6	30.4	

*Welch ANOVA Test ($p>0.05$); SD: Standard Deviation; SE: Standard Error

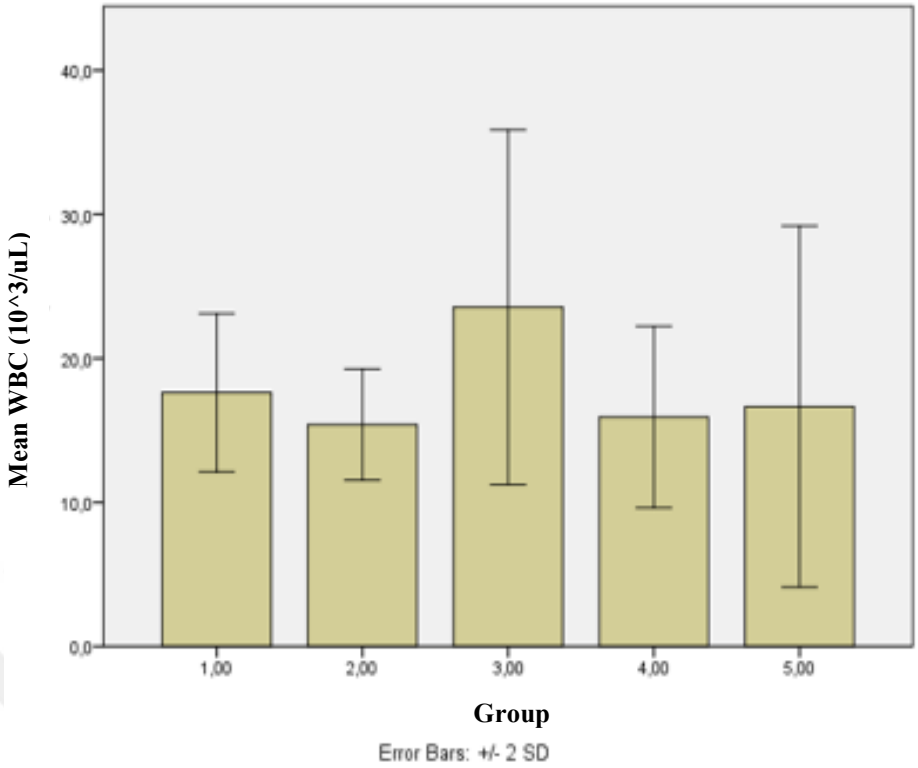

Figure 4.9. Comparison of the WBC mean values of the groups

4.4.2 Platelets (PLT)

When the study animals in all groups were evaluated hematologically in terms of platelet amount, no statistically significant difference was found between the mean platelet values of the groups ($p>0.05$).

Table 4.6. Comparison of the mean platelet amount by groups

PLT (10^3/uL)									
	N	Mean	SD	SE	95% Confidence Intervals for Mean		Minimum	Maximum	P value
					Lower Limit	Upper Limit			
1.00	6	492.33	94.137	38.431	393.54	591.12	397	629	0.056*
2.00	6	613.83	80.420	32.831	529.44	698.23	510	694	
3.00	6	516.67	233.726	95.418	271.39	761.95	204	836	
4.00	6	710.33	114.500	46.744	590.17	830.49	604	897	
5.00	6	617.67	127.312	51.975	484.06	751.27	423	772	
Total	30	590.17	153.214	27.973	532.96	647.38	204	897	

*Welch ANOVA test (p>0.05); Standard Deviation: SD; Standard Error: SE

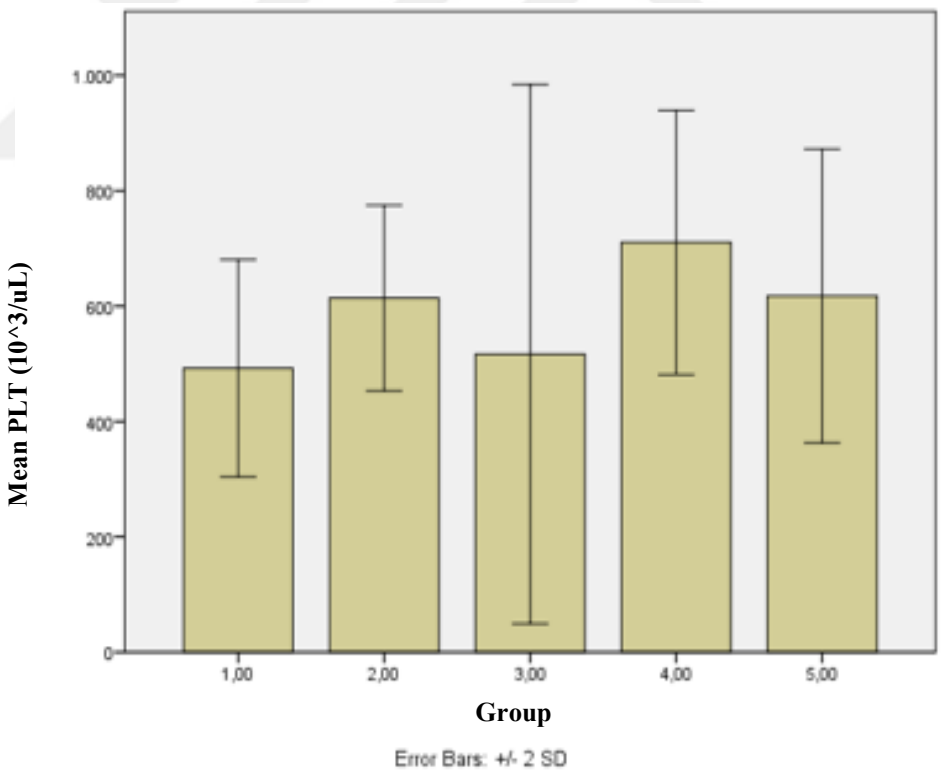

Figure 4.10. Comparison of the PLT mean values of the groups

4.4.3 Hemoglobin (Hb)

When the animals in all groups were evaluated in terms of hematological hemoglobin values, a statistically significant difference was found between the mean hemoglobin values of the groups ($p<0.05$).

Table 4.7. Comparison of mean hemoglobin values by groups

Hb (g/dL)									
	N	Mean	SD	SE	95% Confidence Intervals for Mean		Minimum	Maximum	P-value
					Lower Limit	Upper Limit			
1.00	6	18.717	1.4905	.6085	17.152	20.281	16.3	20.4	0.004*
2.00	6	20.433	1.2323	.5031	19.140	21.727	18.9	21.7	
3.00	6	19.250	1.3867	.5661	17.795	20.705	16.9	20.8	
4.00	6	17.400	.8099	.3307	16.550	18.250	16.5	18.7	
5.00	6	18.700	.8922	.3642	17.764	19.636	18.0	20.0	
Total	30	18.900	1.4897	.2720	18.344	19.456	16.3	21.7	

Welch ANOVA Test (*$p<0.05$); SD: Standard Deviation; SE: Standard Error

Tukey test, which is a pairwise comparison test, was used to determine between which groups the difference occurred in Hb measurements.

Table 4.8. Pairwise comparisons of groups in terms of hemoglobin values

Pairwise Comparisons						
Hb (g/dL)						
(I) Group	(J) Group	Average Difference (I-J)	SE	P-value	95% Confidence Intervals	
					Lower Limit	Upper Limit
1.00	2.00	-1.7167	.6887	.124	-3.739	.306
	3.00	-.5333	.6887	.936	-2.556	1.489
	4.00	1.3167	.6887	.337	-.706	3.339
	5.00	.0167	.6887	1.000	-2.006	2.039
2,00	1.00	1.7167	.6887	.124	-.306	3.739
	3.00	1.1833	.6887	.442	-.839	3.206
	4.00	3.0333	.6887	,.02*	1.011	5.056
	5.00	1.7333	.6887	.119	-.289	3.756
3,00	1.00	.5333	.6887	.936	-1.489	2.556
	2.00	-1.1833	.6887	.442	-3.206	.839
	4.00	1.8500	.6887	.085	-.173	3.873
	.,00	.5500	.6887	.929	-1.473	2.573
4,00	1.00	-1.3167	.6887	.337	-3.339	.706
	2.00	-3.0333·	.6887	,.02*	-5.056	-1.011
	3.00	-1.8500	.6887	.085	-3.873	.173
	5.00	-1.3000	.6887	.350	-3.323	.723
5,00	1.00	-.0167	.6887	1.000	-2.039	2.006
	2.00	-1.7333	.6887	.119	-3.756	.289
	3.00	-.5500	.6887	.929	-2.573	1.473
	4.00	1.3000	.6887	.350	-.723	3.323

Tukey test (*p<0,05); SE: Standard Error

A statistically significant difference was found only between the pairwise comparisons of Group 2 and Group 4 ($p<0.05$). The mean value of Group 2 is higher than the mean value of Group 4.

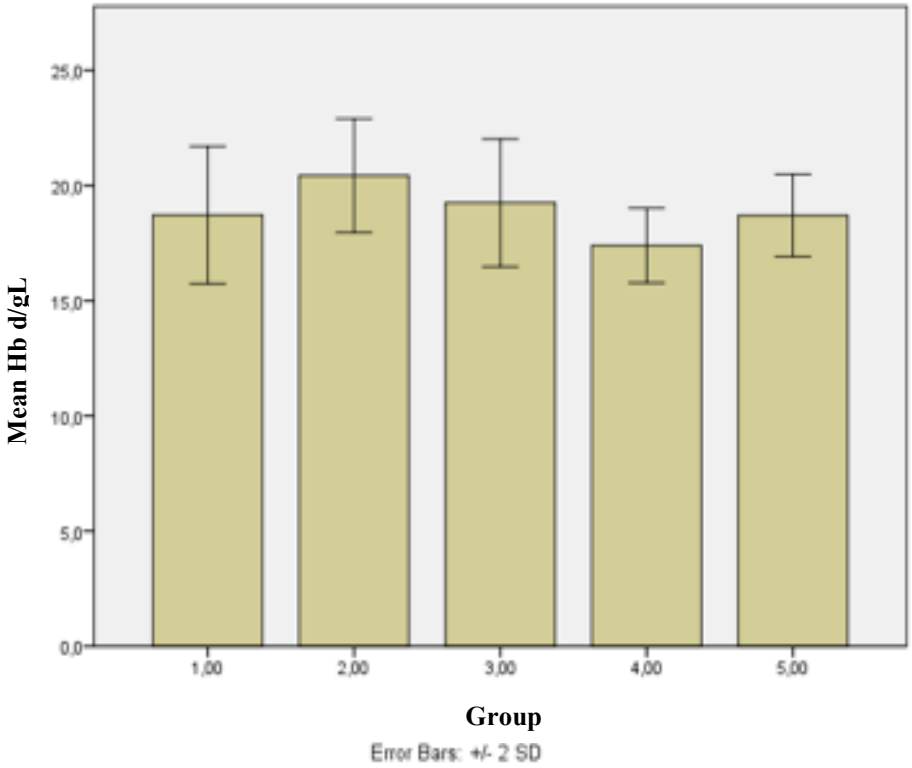

Figure 4.11. Comparison of the Hb mean values of the groups

4.4.4 Hematocrit (HCT)

When the animals in all groups were evaluated hematologically in terms of hematocrit value, a statistically significant difference was found between the mean hematocrit values according to the groups ($p<0.05$).

Table 4.9. Comparison of the mean hematocrit levels by groups

HCT (%)									
	N	Mean	SD	SE	95% Confidence Intervals for Mean		Minimum	Maximum	P-Value
					Lower Limit	Upper Limit			
1.00	6	44.950	3.9738	1.6223	40.780	49.120	38.6	50.2	0.015*
2.00	6	48.050	2.4040	.9814	45.527	50.573	44.7	50.4	
3.00	6	45.367	2.6942	1.0999	42.539	48.194	40.7	48.2	
4.00	6	41.933	2.3526	.9604	39.464	44.402	39.6	45.4	
5.00	6	44.300	1.9667	.8029	42.236	46.364	42.1	47.3	
Total	30	44.920	3.2535	.5940	43.705	46.135	38.6	50.4	

Welch ANOVA Test (*p<0.05); SD: Standard Deviation; SE: Standard Error

Tukey test, which is a pairwise comparison test, was used to determine between which groups the difference in hematocrit measurements occurred.

Table 4.10. Pairwise comparisons of groups in terms of hematocrit levels

Pairwise Comparisons						
HCT (%)						
(I) Group	(J) Group	Average Difference (I-J)	SE	P value	95% Confidence Intervals	
					Lower Limit	Upper Limit
1.00	.00	-3.1000	1.5965	.323	-7.789	1.589
	3.00	-.4167	1.5965	.999	-5.105	4.272
	4.00	3.0167	1.5965	.349	-1.672	7.705
	5.00	.6500	1.5965	.994	-4.039	5.339
2.00	1.00	3.1000	1.5965	.323	-1.589	7.789
	3.00	2.6833	1.5965	.463	-.2.005	7.372
	4.00	6.1167	1.5965	.006*	1.428	10.805
	5.00	3.7500	1.5965	.163	-.939	8.439
3.00	1.00	.4167	1.5965	.999	-4.272	5.105
	2.00	-2.6833	1.5965	.463	-7.372	2.005
	4.00	3.4333	1.5965	.231	-1.255	8.122
	5.00	1.0667	1.5965	.961	-3.622	5.755
4.00	1.00	-3.0167	1.5965	.349	-7.705	1.672
	2.00	-6.1167	1.5965	.006*	-10.805	-1.428
	3.00	-3.4333	1.5965	.231	-8.122	1.255
	5.00	-2.3667	1.5965	.583	-7.055	2.322
5.00	1.00	-.6500	1.5965	.994	-5.339	4.039
	2.00	-3.7500	1.5965	.163	-8.439	.939
	3.00	-1.0667	1.5965	.961	-5.755	3.622
	4.00	2.3667	1.5965	.583	-2.322	7.055

Tukey Test (*p<0.05); SE: Standard Error

A statistically significant difference was found only between the pairwise comparison of Group 2 and Group 4 ($p<0.05$). The mean value of Group 2 is higher than the mean value of Group 4.

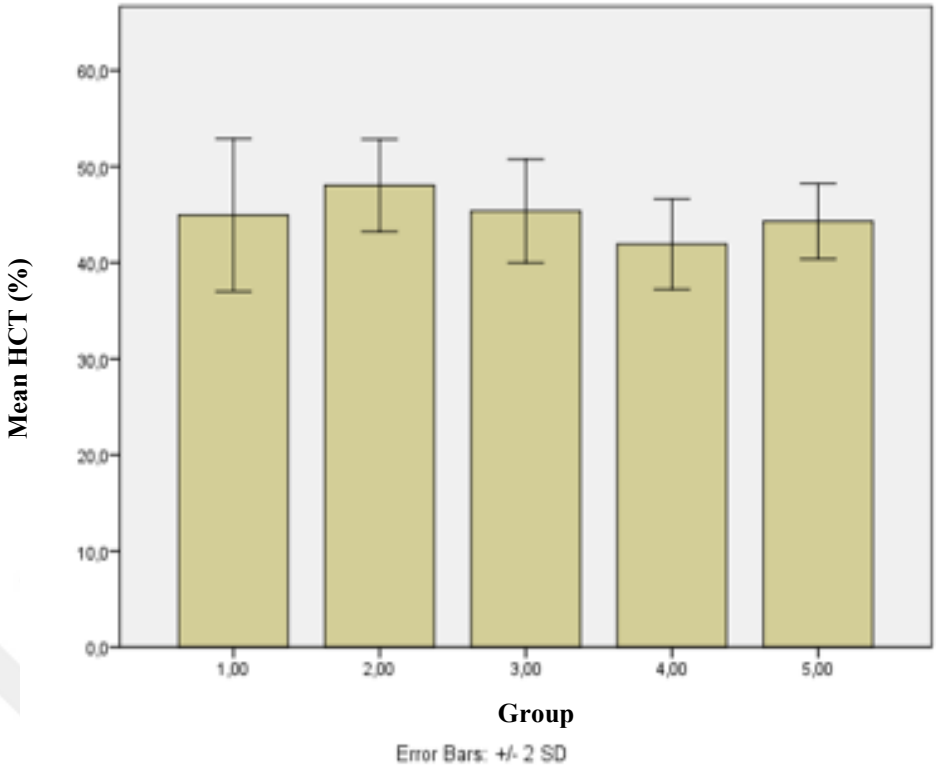

Figure 4.12. Comparison of the HCT mean values of the groups

5. DISCUSSION

Tooth-extraction sockets are among the most frequently seen defects in maxillofacial bone structures. Inflammation, coagulation, and the generation of the granulation tissue are the events that take place as the initial phase of the wound healing process begins. Cell apoptosis and osteoclastic activities aid in the production of the blood cloth that consists of proliferative fibrous tissue (1,84).

4 days after tooth extraction, a new bone matrix starts to form from the apical portion of the socket as the endothelial cells and pre-osteoblastic cells migrate there[1]. Thus, new trabecular bone is produced progressively and it fills the extraction socket in 14 days. This whole process depends on the proliferation, specification, and maturation of osteoblasts. Moreover, it is regulated by cytokines and several intrinsic growth factors. As a result, the application of drugs either locally or systemically affects the activity of these signaling molecules; thus the repair of bone after the extraction of a tooth is affected (1,85).

After tooth extraction, atrophy will occur in the alveolar bone. The atrophy occurs due to the fact that the bundle bone loses its function and ceases to exist. Resorption of the alveolar bone occurs simultaneously with the bone growth in the extraction socket. The utmost quantity of bone loss takes place in the horizontal dimension of bone and on its facial side. Moreover, vertical bone loss occurs mostly on the buccal side. Thus, a narrow and a short alveolar ridge is observed. The change in the alveolar ridge results in its shift to a more lingual or palatal location (2).

Deficient bone structure left after extraction might be even more damaged if the previous bone structure was already traumatized due to periodontal or endodontic disease. Most rapid loss of residual bone structure occurs during the first 6 months following extraction. This resorption continues throughout the lifespan of the patient at a slower rate and ensures the loss of huge amounts of alveolar bone tissue (2).

In recent years, aesthetics have become even more important in treatment planning. If the repair of bone after extraction is defective; problems such as functional inadequacy, complications in prosthetic rehabilitation, and unsatisfactory aesthetic results

might occur. To provide the patient with functional and aesthetic prosthetic rehabilitation, a satisfactory volume and structure of alveolar bone must be present (1,2).

In studies that examine bone tissues histologically, histomorphometrically, and mechanically; the use of experimental animals is the first choice. The chosen experimental animal must be suitable for the study model. Rats are among the group of animals that are usually preferred in studies (86,87,88). In the present study, to examine the explained importance of the extraction socket with the effect of EPO, rats were preferred as the study animal. Rats are convenient animals since they can be easily found, and surgical methods can be applied to them with ease. The fact that rats do not require intubation for general anesthesia is another reason why they are convenient animals to use in study models.In addition, rats were preferred as the study animal is that they can be produced conveniently and since they are small animals, less amount of drug is administered. Therefore, their usage is fairly economic (89). Moreover, rats are ideal laboratory testing animals since they have anatomic and physiologic similarities with humans. Approximately 95% of human genes are analogous to rats. Rats are also preferred in research studies since they are small animals, need limited spaces to live, and do not need much amount of food to survive (90).

All subjects in the current study were 12 weeks old. This age was preferred because bone healing is better in younger rats and to waive any changes that differences in age may cause. Furthermore, rats are also chosen since their extraction sockets heal within one to two weeks (89).

In the current study, extraction of the maxillary first molar of the rats was planned. There are several reasons for this. First of all, rats consume food by using their superior incisors thus the extraction of molars does not cause them to have nutritional problems. The mandible of rats is mostly made up of cortical bone and has high bone density and resistance. All these properties increase the likelihood of accidents and fractures of the mandible during extraction, so the maxillary molars are preferred in this study.

Molar extraction has more biological advantages such as; not having continuous eruption patterns, and having roots and the periodontal ligaments that are similar to humans. Moreover, since the molar extraction area has no cartilage tissue and heals in a very short time, it makes an efficient model to study bone repair (91).

Human EPO is mainly produced by the kidneys and it is a hematopoietic growth factor. Endogenous EPO is a cytokine for erythrocyte precursors of the bone marrow, thus it manages the production of red blood cells (92). It is secreted when hypoxia occurs and HIF-1α is activated. As a response to low oxygen levels, EPO enters the peripheral circulation and binds to the receptor, which is called the preformed homodimer transmembrane receptor. Subsequently, proliferation and differentiation of hematopoietic precursors are induced and erythrocyte production increases (93).

Another property of EPO is bone regeneration and formation and it is the main reason why EPO was the drug of choice in this study. Intraperitoneal administration of EPO's effect on femoral fracture healing was investigated on mice by Holstein. It was reported that the callus formation in the EPO administered subjects were more resistant to torsional forces (94,95,96). Holstein et al. did another study in which mice with femoral segmental defects were administered EPO. The results showed that EPO triggers bone formation, cell proliferation, and angiogenesis (7,94). The density of the bone in mice that were administered EPO was shown to be higher 2 weeks following fracture (94). EPO's effect on the alveolar extraction socket has not been examined before and previous studies found that EPO had a positive effect on new bone formation. These findings also support the reason why EPO was chosen in the present study.

In a study done by Mihmanli et al, EPO was administered subcutaneously to examine its effects on mandibular distraction osteogenesis. As a result, it was found that EPO enhanced the healing of bone, causes the bone to have better vascularity and increases bone strength (97). Turkeli et al. also did a study on EPO and investigated its effect on the ulnar and radial fractures of rats. They found that EPO has a positive effect on fracture healing and its low dose administration might protect against the delayed union or nonunion (98). These findings also support the reason why EPO was administered systemically in the current study.

In another study by Bakhshi et al, local EPO injection was performed to examine its effects on tibiofibular fracture healing. Their results were that EPO administered group's fractures healed 2 weeks faster and had a lower nonunion rate (99). Moreover, Rölfing et al's study examined the local EPO application's effect. They used a porcine calvarial defect model. They applied EPO locally by using a collagen scaffold. They

found that bone volume increased in the local administration group after 5 weeks (83). These findings support the reason why EPO was applied locally in the current study.

The FDA recommendation for the administration regimen of EPO is 50 to 100 units/kg three times weekly for adults with chronic kidney disease. The regimen for patients that use Zidovudine for the treatment of HIV is 100 units/kg 3 times weekly and for patients with cancer treated with chemotherapy, the regimen is 40,000 units weekly or 150 units/kg 3 times weekly in adults.

When studies were searched for the administration of EPO applied to study animals, most of the studies did not give reliable information on the correct dosage and how they chose the correct administration regimen. Chen et al. administered 500 U/kg of EPO to the rats (100). Diker et al. did a study on rats and applied EPO. Their dosage was a daily intraperitoneal injection of EPO by 500U/kg body weight. Both of the researchers did not disclose any information on why they chose this dosage (9,100). As mentioned before, most of the studies did not give reliable information on the correct dosage. Only one study by Omlor et al. disclosed an argument on dosage. They calculated the dosage by using the formula; metabolic bodyweight of the animal equals individual bodyweight of the animal to the power of 0.75 (101). However, they did not give any information on why they used this formula.

Since there is no consensus on the EPO administration on bone defects, the FDA dosage recommendations were thought to be the most reliable source of information available in the literature. However, they did not apply to the current study since it only gave dosage recommendations to patients with illnesses and the mice in this study were healthy. Be that as it may, there is a study by Kryzansky et al. in which they applied FDA recommendations to healthy patients (102). As a result, in the present study, EPO's systemic effect on alveolar bone healing was tested according to Kryzansky et al's study. Subcutaneous injection dosages of 450 units and 1350 units were used in the systemic EPO study groups in this study.

EPO's local effect was tested according to Rölfing et al.'s study (83). In Rölfing's study, they used a collagen scaffold in which they soaked the EPO and they measured the amount of EPO resorbed. The same principle was applied in the present study and

Geletamp® (Roeko Geletamp, Coltene/Whaledent GmbH, Germany) was used as the collagen scaffold and it was measured to soak 20 units of EPO.

In the present study, results were examined in three categories which were histopathologic, histomorphometric, and hematologic.

Histopathologically; inflammation, fibrosis, and foreign substance reaction were examined. None of the results was statistically significant in all groups. Consequently; in the current study both locally and systemically, EPO has no effect on inflammation, fibrosis, and foreign substance reaction. Moreover, in the literature, there was no study in which the researchers looked for the effect of EPO on inflammation, fibrosis, or foreign substance reaction histopathologically.

When the median values of inflammatory response were compared between the groups, the locally applied EPO group had higher scores than the other EPO applied groups but had the same score as its control group. Moreover, there was no statistically significant difference between any of the groups. Therefore, EPO's effect on inflammation could not be demonstrated in the current study.

The fibrosis median scores of the groups were quite similar to the inflammatory response median scores. The locally applied EPO group had the exact median score as its control group but had the highest score when compared with the other EPO applied groups. There was no statistically significant difference between the groups. Consequently, EPO's effect on fibrosis could not be shown in the current study.

When the foreign substance reaction was examined in the groups, only one study animal in the low dose systemic group had a foreign substance reaction. None of the other study animals demonstrated such a response. Therefore, statistical analysis was not performed since only one out of thirty study animals had a foreign substance reaction. This reaction could be caused by the powder of the dental gloves when the study was performed in the laboratory.

Moreover, in the literature, there was no study in which the researchers looked for the effect of EPO on inflammation, fibrosis, or foreign substance reaction

histopathologically. As a result, the current study's histopathological results could not be compared with any study.

When bone healing is examined in experimental studies, it is essential to evaluate the data histologically to comprehend the degree of healing on a cellular level. On the other hand, the gold standard is histomorphometric evaluation since it directly and quantitatively examines bone cells, activities, and the bone matrix (103).

In the histomorphometric analysis of the current study, new bone formation was examined. When all groups are compared according to the data obtained, there was a statistically significant difference in only the high dose systemic group. However, there is no statistically significant difference between high dose and low dose systemic groups. This finding indicates that EPO is more effective when administered systemically high doses.

In the study done by Mihmanli et al., mandibular distraction osteogenesis was performed on rabbits and four doses of 150 IU/kg EPO were administered systemically. When the experimental group was compared with the control group, the experimental group had more new bone formation ($p<0.05$) (97). Their results correlate with the current study's findings of the high-dose systemic administration group.

Turkeli et al. did a study on mice with ulnar and radial fracture models. They gave 500 IU/kg low dose intraperitoneal injections for five days to the experimental group. They looked at fracture healing in the 7^{th} and 21^{st} days. According to their analysis, the experimental group had statistically higher bone healing scores (98). Their results correlate with the findings of the current study in the high-dose systemic group.

Garcia et al. made a study on mice with 500 U/kg EPO every day intraperitoneally for 2 and 5 weeks. Their results demonstrated that EPO significantly fastens bone healing. Their findings were supported by the following data; significantly increased biomechanics stiffness, increased radiological density of the periosteal callus, and significantly more bone, less cartilage, and fibrous tissue at the periosteal callus (95). Their finding that EPO application increases new bone formation correlates with the current study's findings in the high dose systemic administration group.

Holstein et al. did a study on mice that were treated with 5000 U/kg intraperitoneal EPO injections for 6 days and had closed femoral fractures. According to their findings, the experimental group had significantly more torsional stiffness and increased callus density. They also found significantly more amount of mineralized bone and osteoid in the experimental group (96). Consequently, their results are parallel with the current study's findings since it was also found that EPO administration increases bone formation.

Holstein et al. did another study in which mice had femoral segmental defects and were given 500 IU/kg intraperitoneal injections of EPO for 10 weeks. They found that the experimental group had a significantly increased rate of bridging in the bone defects. In addition, the experimental group had significantly more bone volume within the fracture defects. The findings of Holstein et al's study correlate with the current study's findings in terms of new bone formation (7).

Diker et al. did a study on rats to explore the effect of EPO on bone regeneration. They administered EPO systemically alone or combined it with xenogenic bone graft augmentation. The rats in the EPO group were administered 500 U/kg daily intraperitoneal injections for four weeks. The control group was only injected with saline solution intraperitoneally. Only the results of the group in which EPO was administered alone were relevant to the current study since no grafts were used to administer EPO. The result of the systemic EPO alone group demonstrated that there was no statistically significant difference in bone formation between the EPO group and the control group (9). The results of Diker et al and the current study do not correlate with each other since in the current study; it was found that there was a significant difference in bone formation between the high dose systemic group and its control group.

Orth et al. made a study on aged mice with femoral fracture models. They gave 500 U/kg EPO intraperitoneal injections every day during the entire study duration to the experimental group. Their histomorphometric analysis result demonstrated that the experimental and control group had no statistically different results in terms of new bone formation between the fracture segments ($p<0.05$) (104). Orth et al's results do not correlate with the current study since they did not find any statistically significant difference between the groups. Even though in the current study a single dose was

applied, a statistically significant increase in new bone formation was observed in the high dose systemic group.

Omlor et al. made a study on rabbits with defects in the radius diaphysis. They had local and systemic administration groups and their respective control groups. The local group was implanted with a gelatin sponge that was soaked with EPO. The systemic group had subcutaneous injections of a single high dose of 4900 IU EPO. According to their results, 2.3 to 2.5 times more bone formation was seen in the experimental group of both local and systemic groups. Moreover, they found that the EPO-treated groups had 3 to 3.4 times superior bone healing in terms of bone and tissue volume. Their systemic group findings correlate with the results of the current study's high-dose systemic group in terms of new bone formation. However, they also found an increase in new bone formation in the local group whereas in the current study there was no significant difference in new bone formation in the local group (101). The reason behind this difference could be due to the fact that a different study animal, a different study model, and a different dosage regimen were preferred in the study.

Bakshi et al. did a study on human patients' tibiofibular fracture. They applied 4000 IU EPO locally into the fracture site two weeks after surgery and monitored healing. The experimental group had 2.1 weeks shorter duration of fracture union when compared with the placebo group ($p<0.01$) (105). The current study did not correlate with Bakshi et al's study. Bakshi et al's study have a different study model, a different study animal, and a different dosage regimen. Therefore, these factors might be the reason behind why different results were obtained.

Rölfing et al. did a study on porcine with a calvarial defect model. They administered EPO or placebo in combination with an autologous bone graft or with a collagen scaffold. The subjects were examined after five weeks and it was found that bone volume increased in the collagen scaffold group. These results do not correlate with the current study's findings since the local administration group was found to be the group that had the least amount of new bone formation (83). The reason behind this difference could be due to the fact that Rölfing et al. had a different study model and a different experimental animal.

In the hematologic analysis of the current study, white blood cells, platelet, hemoglobin, and hematocrit levels were examined.

The high-dose systemic administration group had the highest mean value of white blood cells. However, when compared with the other EPO groups and their control groups, there was no statistically significant difference between the groups. When the platelet mean values of groups were compared, the control group of local EPO administration had the highest value. However, the control group and the local EPO administration group had no statistically significant difference between them. The low dose systemic EPO group had higher platelet mean value than the high dose systemic EPO and control group, but there was no statistically significant difference between the groups. Since none of the other studies examined the white blood cell and platelet levels, the current study's findings could not be compared with other studies' findings.

When the hemoglobin and hematocrit mean values of the present study are compared, the low dose systemic EPO administration group had the highest value. When the mean values are compared between the groups, a statistically significant difference was found. The low dose systemic EPO had no statistically significant difference when compared with its control group and high dose systemic EPO group. However, the low-dose systemic EPO group and the control group of local EPO administration had a statistically significant difference ($p<0.05$). The mean hemoglobin and hematocrit value of the low dose systemic group had a higher mean value than the control group of local EPO administration.

The low dose systemic EPO administration group also had the highest score of hematocrit mean value when compared with the other groups. There was a statistically significant difference between the groups, which was similar to the results of the hemoglobin mean values. Low dose systemic EPO had no statistically significant difference when compared with its control group and high dose systemic EPO group. However, the low dose systemic EPO group and the control group of local EPO administration had a statistically significant difference ($p<0.05$). Interestingly, the low dose systemic group's mean hematocrit values are higher than the high dose systemic group. However, there is no statistically significant difference between them. This result

is quite different than the other studies that analyzed EPO because according to their results hematocrit levels increase when the total dosage of EPO increases (7,95,104).

Holstein et al. (2007) also analyzed the hemoglobin concentrations and found no statistically significant difference between the experimental and control groups (96). Since there was no statistically significant difference in hemoglobin levels in the current study, the two study's results correlate with each other.

Omlor et al. examined the hematocrit levels study on rabbits with defects in the radius diaphysis. They applied EPO both locally with a gelatin sponge and systemically. They found that there was no statistically significant difference in hematocrit levels between the control groups and the locally treated group. However, they found that the systemically treated group had a significant increase in hematocrit levels until day 14. For all of their groups, hematocrit levels decreased after day 21 (101). When the hematocrit levels are compared, their results only correlated with the current study's findings in terms of no statistically significant change in hematocrit levels between the local administration and control groups. However, the current study's results do not correlate when the systemic groups are compared because they found a significant increase in hematocrit levels.

Orth et al. made a study on aged mice with femoral fracture models. They gave 500 U/kg EPO intraperitoneal injections every day during the entire study duration to the experimental group. Their analysis on days 14 and 35 showed that hemoglobin and hematocrit concentrations were significantly higher in the experimental group ($p<0.05$) (104). Orth et al.'s results do not correlate with the current study since the current study's high dose systemic group had no statistically significant increase in hemoglobin and hematocrit values. The reason behind this difference between studies could be due to the fact that Orth et al. applied a much higher dosage over a longer period of time and had a different study animal.

Garcia et al. made a study on mice with 500 U/kg EPO every day for 2 and 5 weeks intraperitoneally. They also examined hemoglobin levels and found that hemoglobin concentrations were significantly greater in both experimental groups when compared with the control group (95). Garcia et al.'s result did not correlate with the current study in terms of hemoglobin concentrations because the systemic administration

groups of the current study did not have a statistically significant increase in hemoglobin. The cause of this different outcome could be due to the fact that Garcia et al. had a higher dosage regimen and performed the experiment on a different animal.

Holstein et al. (2011) did another study in which mice had femoral segmental defects and were given 500 IU/kg intraperitoneal injections of EPO for 10 weeks. They found that the hemoglobin levels in the EPO-treated group had significantly increased (7). The reason why Holstein found increased hemoglobin concentrations could be that they administered a significantly higher total amount of EPO for 10 weeks.

6. CONCLUSION

1. The current study is the first experimental study to examine the effect of erythropoietin in extraction socket healing.

2. In the current study, it was observed that EPO, which is given systemically at high doses (1350 IU) after tooth extraction, has a positive effect on new bone formation.

3. High dose systemic EPO group (1350 IU) does not display an increase in hemoglobin and hematocrit levels.

4. In the current study, bone healing was examined at only the end of the 7^{th} day in rats. If healing is evaluated in different periods, different results might be obtained. Further studies are needed to explore the effect of a different time period.

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8. APPENDIX

8.1 Ethics Committee Approval Form

YEDİTEPE ÜNİVERSİTESİ

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU

(YÜDHEK)

ETİK KURUL KARARI

Toplantı Tarihi	Karar No	İlgi	Proje Yürütücüsü
24.06.2019	766	18.06.2019	Ceyda ÖZÇAKIR TOMRUK

'Sıçanlarda diş çekimi sonrası lokal ve sistemik eritropoietin tedavisinin alveoler kemik iyileşmesi üzerine etkisinin histolojik ve histomorfometrik olarak değerlendirilmesi' adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

Etik Onay Geçerlilik Süresi: 3 Yıl	Hayvan Türü ve cinsiyeti: Sıçan ♂	Hayvan Sayısı: 30

GÖREVİ	ADI SOYADI
Başkan	Prof. Dr. Bayram YILMAZ
Başkan Yardımcısı	Prof. Dr. Erdem YEŞİLADA
Raportör	Vet. Hekim Engin SÜMER
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Üye	Prof. Dr. Rukset ATTAR
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Educational Status

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Doctoral	Oral and Maxillofacial Surgery	Yeditepe University	2022
Graduate	Faculty of Dentistry	Yeditepe University	2016
High School	Science	ISTEK Bilge Kağan Science High School	2010

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Publications
