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Research Article

Evaluation of the effects of *Momordica charantia* on tibial defect injury in rats

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Abstract

We aimed to examine the histological and immunohistochemical effects of Momordica charantia on bone repair, which provides positive regulation such as blood sugar, blood pressure, antilipidemic, anticarcinogenic, antibacterial, antioxidant, anti-inflammatory, wound healing, and tissue regeneration in rats with tibial bone defect. Momordica charantia (MC) (commonly called bitter melon; Goya; bitter apple; bitter gourd; bitter squash; balsam-pear; with many more names listed below) is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit. Its many varieties differ substantially in the shape and bitterness of the fruit. In this study, 32 male Sprague Dawley rats, 12 weeks old and weighing 250-300gr, were used. 8 rats in each group randomly, 1st group control, 2nd group defect (Sham), 3rd group defect + MC group (14 days), 4th group defect + MC group (28 days) separated into the group. In this experimental study, 600 mg/kg/day MC (bitter melon) extract was mixed into the drinking water of groups 3 and 4 and administered to rats by oral tube. One 6 mm diameter cylindrical defects were created in the body of the right tibia bone. No action was taken in Group 1. In Group 2, only tibial defect was made. For 14 days in group 3 and 28 days in group 4, 600 mg/kg/day MC extract was mixed with drinking water and given by oral gavage. Elevated Malondialdehyde (MDA) levels and Myeloperoxidase (MPO) decreased. In groups 3 and 4, osteoblastic activity and osteocyte development increased, while osteopontin and osteonectin expression were found to be positive, while osteoclastic activity decreased compared to the sham group. It is a good antioxidant in the groups where MC is applied. It has been observed that it can have a positive effect on bone repair.

Keywords: Momordica charantia; Tibial defect; Fracture healing; Bone repair; Antioxidant

INTRODUCTİON

Bones form the passive elements of our movement system and support the muscles and organs. And it serves as a storehouse for calcium, which is an important requirement of our body. The deterioration of the integrity of the bone as a result of internal or external stress is called a fracture [1]. Trauma is the most common cause of fracture, which is one of the main causes of workforce loss in the world. Fracture healing in the human body has become an important health problem for humans today. In fracture healing, hematoma occurs first at the fracture site. Macrophages, which are the beginning of the inflammation phase, come to this region [2]. Platelets are transferred to this region together with macrophages. Cytokines (PDGF, TGF-β, IL-I, IL-6 and PGE2) that have a critical effect on the healing of the fracture are released. Fibroblast growth factor 8 initiates new vessel formation and mesenchymal cell growth. Cells of fibroblast and mesenchyme origin replace the hematoma in the fracture area. BMP is secreted from and from these cells and the surrounding periosteum, which ensures cell growth/differentiation. Mesenchymal cells transform into chondrocytes and then callus bone tissue to form cartilage callus. While the repair process continues after the callus tissue, the remodeling process begins [3]. In addition, osteonectin (ONC) and osteopontin (OPN), which are an important indicator of fracture healing and synthesized by osteoblastic cells in the rat femoral marrow ablation model, are known as glycoproteins that are overexpressed in bone tissue that shows active remodeling [4].

Many scientific studies have been carried out on bone regeneration from past to present. Different strategies are being developed to reduce and improve the normal repair time of bone. Studies have shown that multiple factors are effective on bone regeneration. Applications for the use of plants in the treatment of diseases date back to the beginning of medicine thousands of years ago. The increasing demand for plants and products prepared from plants in recent years has led to an increase in the number of herbal products as a result of developments in both pharmacological and analytical methods, and the number of preclinical and clinical studies to obtain therapeutic efficacy and safety data for them [5]. Momordica charantia L. (M. charantia), a member of the Cucurbitaceae family, is widely distributed in tropical and subtropical regions of the world. It has biologically active chemicals such as glycosides, saponins, alkaloids and fixed

oils, triterpenes, proteins, and steroids in its structure [6]. It is a popular herb used in the treatment of many diseases such as diabetes mellitus, inflammation, constipation, ulcers, malaria, and cancers [7].

In the light of this information, we think that MC will make a protective and curative contribution to the defect in the bone in our experimental model in this study. By using MC, which is common, inexpensive, and easy to access, we will determine that it has positive effects on bone repair. In this study, it was aimed to examine histopathologically and immunohistochemically the healing or protective effects of MC on tibial defects in the tibial bone defect model created in rats.

MATERIALS AND METHODS

Animals

Every single surgical methodology and the consequent care and healing of the animals utilized as a part of this investigation were in strict understanding with the National Institutes of Health (NIH Publications No. 8023, revised 1978) rules for animal care. All techniques performed in this examination were approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine at Dicle University, Turkey (***Protocol number: 2020-37***).

The age range of the rats was determined to be 12 weeks and weighing 250-300gr. All procedures were performed in the DÜSAM experimental animals' unit. 4.2. Invasive Intervention Applied to Experimental Animals (Tibial Bone Defect Model).

Procedures on experimental animals

The invasive procedure to be performed in the experiment was performed under anesthesia. The follow-up of the depth of anesthesia was followed by skin or finger pinch responses every 2-3 minutes. Before starting the experimental procedure, 90 mg/kg intramuscular ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 8 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey) general anesthesia were administered to all experimental animals. The hairs on the body of the right proximal tibial bone, which was determined as the operation area, were shaved. The surgical site was cleaned from the center to the periphery with an antiseptic solution of povidoneiodine (Biokadin® , Biokan, Istanbul, Turkey). After the horizontal incision, the mucoperiosteal full-thickness flap was gently elevated. One cylindrical defect with a depth of 5 mm and a diameter of 6 mm were created in the right tibia of each animal, approaching from the lateral side in the trunk region of the tibial bone. All equidimensional defects were opened with a single size trefan bur designed for this purpose.

Before starting the experimental procedure, 90 mg/kg intramuscular ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 8 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey) general anesthesia were administered to all experimental animals. Rats were randomly selected, 8 rats in each group, and divided into 4 groups. The following procedures were applied to the groups. All procedures were applied to the right proximal tibial bone. The animals were fed adlibitum (unlimited access to feed and water).

Table 1: Experimental group numbers.

Experimental and Control Groups		*Number of Animals*
Group 1	*Control group*	*8*
Group 2	*Defect (Sham) group*	*8*
Group 3	*Defect + MC (14 days)*	*8*
Group 4	*Defect + MC (28 days)*	*8*

Group 1: No procedure will be applied to the tibial bone and this group will be considered as the control group. Animals were given unlimited access to water and food.

Group 2: This group will be considered as the defect (Sham) group. A cylindrical defect with a diameter of 6 mm was created on the tibial bones of the animals in this group and no further procedures were performed. Animals were given unlimited access to water and food.

Group 3: Defect + MC group (14 days) will be evaluated. A cylindrical defect with a diameter of 6 mm was created in the animals in this group, and then 600 mg/kg/day of bitter melon (Momordica charantia) extract was mixed into the drinking water and given to the rats by oral gavage for 14 days.

Group 4: Defect + MC group (28 days) will be evaluated. A cylindrical defect with a diameter of 6 mm was created in the animals in this group, and then 600 mg/kg/day of MC (bitter melon) extract was mixed into drinking water and administered by oral gavage for 28 days.

At the end of the 28th day, the animals were sacrificed, and the right tibiae were removed. Tibial tissue samples were taken into 10% neutral buffered formalin solution and routine paraffin tissue follow-up was performed. After fixation (24 hours), the tissues were kept in 10% formic acid solution for decalcification until the bone tissues were softened. Then, after washing (1 night), increasing series of alcohols (50%, 70%, 80%, 90%, 96% and absolute ethyl alcohol) and clarification (3x30 minutes in xylene), paraffin infiltration was performed at 58°C. Then, the tissues were embedded in paraffin blocks and 4-6 µm thick sections were taken from the blocks with the help of a microtome (catalog no: Leica RM2265, Wetzlar, Germany) for Hematoxylin-Eosin and immunohistochemical staining.

Tibial tissue sections taken from paraffin blocks for Hematoxylin-Eosin Staining were taken in a double boiler set at 37°C. Sections were kept in an oven at 58-62°C for 6 hours to dissolve excess paraffin on the slide. Sections were deparaffinized in xylene for 3x15 minutes. Sections were passed through decreasing alcohol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes and brought to distilled water for 5 minutes. After 8 minutes in Harris Hematoxylin stain, the sections were washed under running water for 5 minutes. After rinsing, the sections were incubated in alcoholic eosin stain for 6 minutes. Sections were rapidly immersed in increasing alcohol series (through 80%, 90%, 96% ethyl alcohol series) and kept in absolute alcohol for 2 minutes. Finally, the sections were covered with a coverslip by keeping them in xylene for 3x15 minutes and dripping Entellan onto the tissue [8].

Sections taken from paraffin blocks for immunohistochemical staining were allowed to be opened in a bain-marie set at 37°C and then transferred to polylysine slides. Sections were kept in an oven at 58-62°C for 6 hours to dissolve excess paraffin on the slide. Sections were deparaffinized in xylene for 3x15 minutes. The sections were passed through the decreasing alcohol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes and brought to distilled water for 5 minutes. Sections were washed in phosphate buffer solution (PBS) for 3x5 minutes. Sections were placed in EDTA buffer solution (pH: 8.0, catalog no: ab93680, Abcam, Cambridge, USA) and heat-induced epitope retrieval was performed. Sections left at room temperature for 20 minutes were taken back into PBS. The sections were arranged on the immunohistochemistry bar and the humidity and temperature of the bar were controlled. 3% hydrogen peroxide solution (catalog no: TA-015-HP, Thermo Fischer, Fremont, CA, USA) was dripped onto the sections and incubated for 20 minutes. Then, they were

washed with PBS for 3X5 minutes and incubated in Ultra V Block solution (Block (catalog no: TA-015-UB, Thermo Fischer, Fremont, CA, USA) for 7 minutes. Sections, osteopontin (catalog no: 14-9096-82, Thermo Fischer) , Fremont, CA, USA) and osteonectin (SPARC) (catalog no: PA5-78178, Thermo Fischer, Fremont, CA, USA) antibodies overnight at +4°C The next day, the sections were left at room temperature for 30-60 Biotin-containing secondary antibody (catalog no: TP-015-BN, Thermo Fischer, Fremont, CA, USA) was dropped on the sections washed with PBS and incubated for 14 minutes, then streptavidin-peroxidase (catalog no: TS-015-HR) , Thermo Fischer, Fremont, CA, USA) was dripped and waited for 15 minutes, then washed with PBS. Diaminobenzidine (catalog no: TA-001-HCX, Thermo Fischer, Fremont, CA, USA) was dripped onto the washed sections and the reaction was observed under the microscope and PBS After counterstaining with Harris hematoxylin, the sections were entellan (catalog number: 107961, Sigma-Aldrich, St. Louis, MO, United States) and visualized on a Zeiss Imager A2 photomicroscope [9].

RESULTS

Histopathological examination of the tibial tissue sections in the groups in terms of osteoblast, osteoclast, osteocytes, and trabecular bone

In the histopathological examination of the cross-section of group 1, it was observed that osteoblastic activity (arrow) was prominent especially in the area of bone trabeculae in the periphery, while osteoclasts in which erythrocytes and leukocytes of the bone marrow were dispersed (star) and osteocyte cells settled in the lacunae were few (Figure 1a).

In group 2, trabecular bones were irregular and lost their integrity. An increase in the number of plasma and osteoclast cells (arrow) was observed. Degeneration was observed in osteoclast cells. Inflammatory cells were scattered in places as aggregates or solitary (stars) (Figure 1b).

In the cross-section of group 3, while an increase in osteoclasts (star) was observed in the areas where bone trabeculae are located, solitary dispersed leukocyte infiltration (star) and mild hyperplasia of osteocyte cells were detected. Dilatation was observed in blood vessels (yellow arrow) (Figure 1c).

In group 4, It was observed that especially newly formed bone trabeculae (star) started to mature in the defect area. However, both osteoblastic activity (black arrow) and osteocytes located in the lacunae (yellow arrow) were prominent. While a decrease in osteoclasts was observed, a decrease in inflammation was remarkable. We can say that the use of MC in the next 6-8 weeks has a positive effect on new bone formation (Figure 1d).

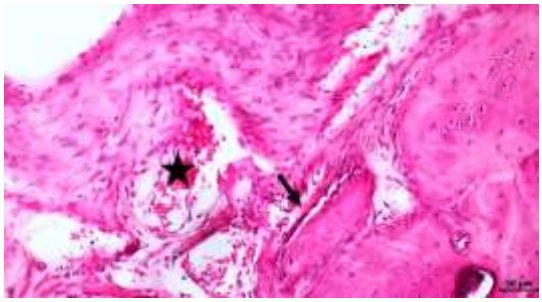

Figure 1a. Group 1 (Control) (Scale: 50 µm)

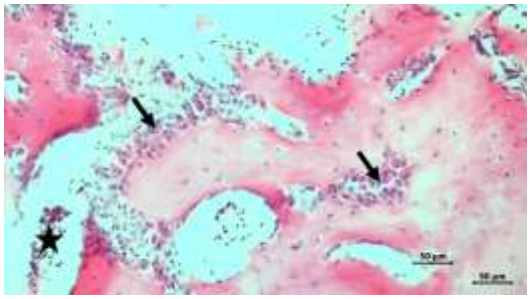

Figure 1b. Group 2 (Defect (Sham)) (Scale: 50 µm)

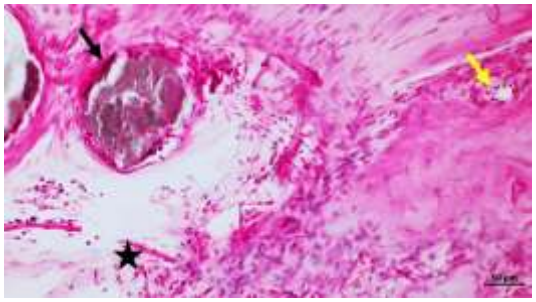

Figure 1c. Group 3 (Defect +14-day MC) (Scale: 50 µm)

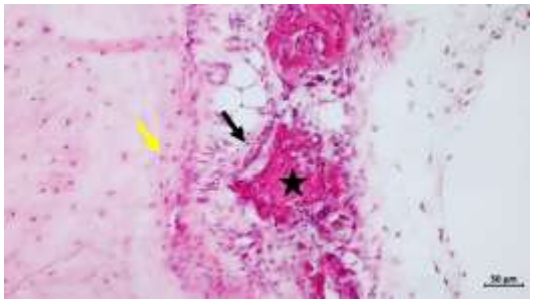

Figure 1d. Group 4 (Defect +28-day MC) (Scale: 50 µm)

Immunohistochemical examination of the tibial tissue sections in the groups for osteopontin.

In the cross-section of group 1, in the main bone trabeculae, osteopontin expression was positive in osteocyte cells (black arrow) in the lacunae, osteopontin expression was negative in some osteoclast cells (red arrow), while osteopontin expression was positive in osteoblasts (yellow arrow) in general (Figure 2a).

In the sections belonging to group 2, positive expression of osteopontin was observed in the periphery of bones and especially in osteoclasts (red arrow). Osteopontin positivity was observed in leukocytes (yellow arrow) and main bone cell (black arrow) (Figure 2b).

Intense osteoblastic activities and osteocyte activities were observed in the main bone region in the section of group 3, while osteopontin expression was positive. However, in the sections of new bone trabeculae, small bone trabeculae areas and especially osteoblast (black arrow) cells in the periphery and osteopontin expression were positive in the solitary side osteoclast (red arrow) cells in between. The expression of osteopontin in the bone matrix (yellow arrow) was not clear yet (Figure 2c).

In group 4, it was observed that osteopontin expression became evident in both osteoblasts (black arrow) and osteocytes (yellow arrow) in newly formed bone fragments outside the area of the main bone trabecula, and it accelerated matrix release in trabeculae fragments (red arrow) belonging to new bone formation (Figure 2d).

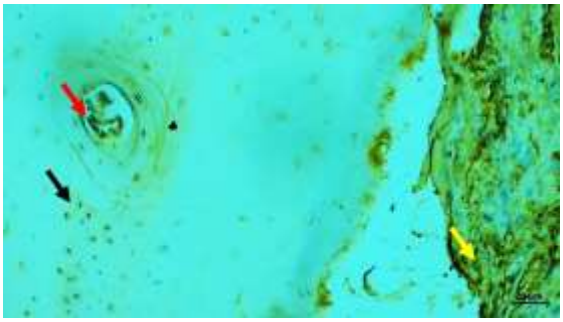

Figure 2a. Group 1 (Control) (Scale: 20 µm)

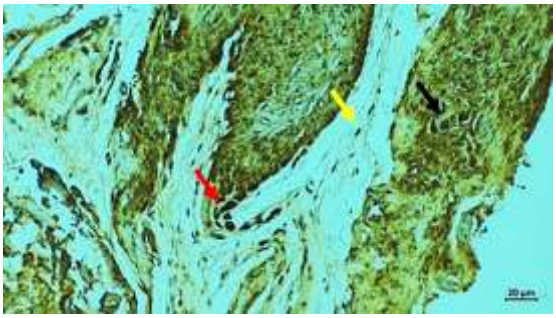

Figure 2b. Group 2 (Defect (Sham)) (Scale: 20 µm)

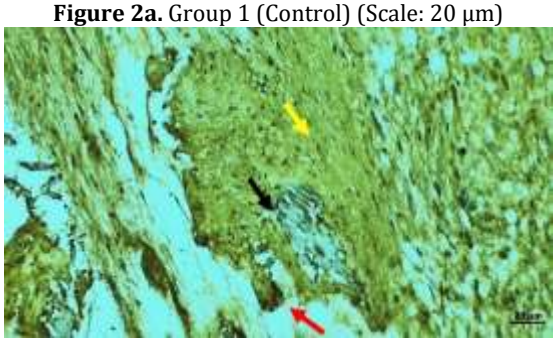

Figure 2c. Group 3 (Defect +14-day MC) (Scale: 20 µm)

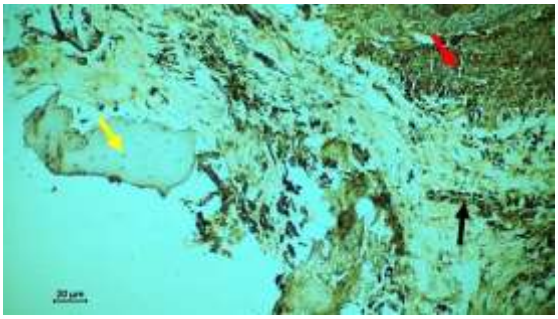

Figure 2d. Group 4 (Defect +28-day MC) (Scale: 20 µm)

Immunohistochemical examination of the tibial tissue sections in the groups for osteonectin

In the longitudinal section of the tibia region of group 1, osteoblastic activity (black arrow) was evident in general, and the nuclei of osteocytes (yellow arrow) were found to be positive with osteonectin, and osteonectin expression was positive in both fibrous structures and connective tissue cells. It was also determined that osteonectin expression of osteoma structures (red star) was also positive towards the outer region in some haversian canals (Figure 3a).

In the section of group 2, although intense inflammation and bone formations in the form of small particles are evident within the defect area, the inflammation is now quite evident and increased in the cross-section (star). It was determined that osteonectin expression was positive in osteoclast cells (red arrow) and negative interaction was observed in osteoblasts (yellow arrow) in newly formed small particles. Osteocytes have not yet formed in the region (Figure 3b).

In group 3, while a significant increase in osteonectin expression was observed in osteoblast (yellow arrow) cells in newly formed bone trabeculae in the defect area, osteonectin expression was found to be positive in osteocytes (black arrow) cells from time to time, even if the osteocytes were not prominent. However, moderate osteonectin expression was detected in the matrix area (asterisk) (Figure 3c).

In group 4, osteonectin expression was positive in osteocytes (black arrow) in osteoblast cells of enlarged trabeculae within the defect area. Positive osteonectin expression was observed in a small number of osteoclast cells (red arrow) in some areas. One of the remarkable points in the cross-section of this group is that especially the fibrous structures (stars) began to become very prominent, and their structural integrity began to develop, while osteonectin expression was also positive (Figure 3d).

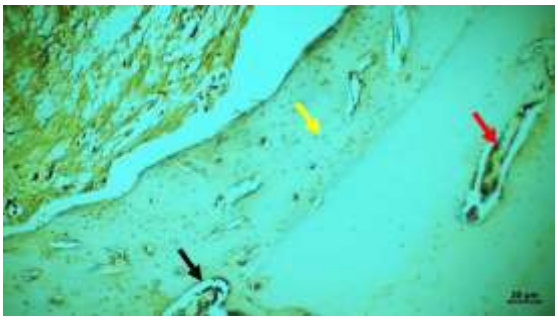

Figure 3a. Group 1 (Control) (Scale: 20 µm)

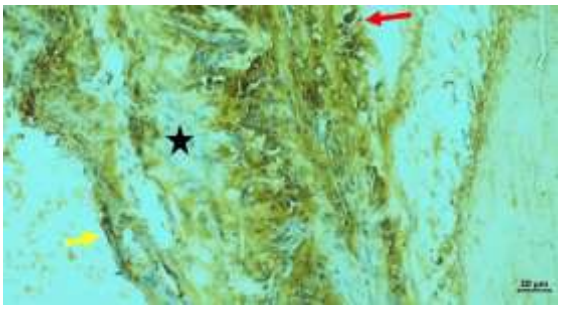

Figure 3b. Group 2 (Defect (Sham)) (Scale: 20 µm)

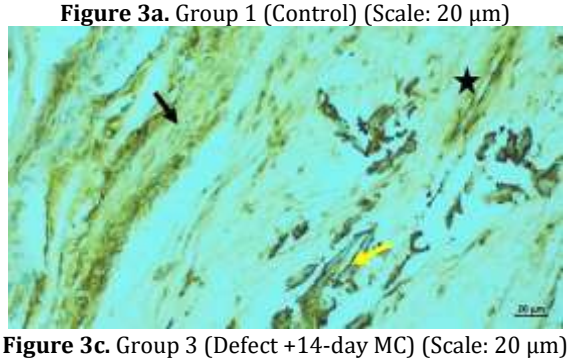

Figure 3c. Group 3 (Defect +14-day MC) (Scale: 20 µm)

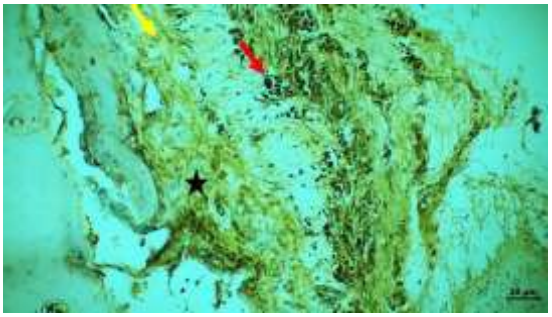

Figure 3d. Group 4 (Defect +28-day MC) (Scale: 20 µm)

DISCUSSION

Medicinal plants and their fruits have been used globally from past to present for the purpose of promoting health and curing diseases (10). Plants available or affordable in developing societies support traditional health care, which is an essential part of medicine. Today, it supports modern medicine and is also used in the production of the active substance of drugs. Approximately 25% of the drugs prescribed in the world are derived from plants. In order to protect public health, it is necessary to ensure that herbal medicines are safe and of appropriate quality and necessary precautions are taken (10,11). The amount of use of this herbal component and its content should be ensured. Consumers also need to be provided with science-based information on dosage, contraindications, and efficacy. The most important factor in determining the appropriate measures to protect public health is the data obtained as a result of academic studies. It is necessary to provide sufficient information about the use and safety of herbal medicines in studies. It is reported that if there are plant studies that provide sufficient scientific benefit, this plant should be encouraged and allowed to be used for the treatment of diseases. In our study, we preferred Momordica charantia (power pomegranate) because of its public health benefits, as a result of its advanced studies, which have been studied in many areas and positive results have been obtained. Every part of the plant is used. There are even studies on the immature parts of it. In a recent study, it was reported that the unripe fruit extract of MC had potent antilipidemic effects on rats fed a high-fat diet (12). It has many forms used in treatment in antidiabetic studies, and it has been found to be effective in regulating blood sugar regulation in the results of the study [13].

There is a significant increase in traumatic or physiological bone defects, such as traffic accidents and gunshot wounds, as a result of increasing population and developing technology globally [14]. Along with the increase in the average life expectancy with the developing technology, it has been reported that there is an increase in osteoporotic fractures that develop as a result of weakening of the bones due to reasons such as poor nutrition, sedentary life, menopause, low estrogen hormone, and gastrointestinal problems [15]. Bone defects can cause serious health problems for both patients and the treatment people. In particular, the late healing of large defects in the bone and the inadequacy of aesthetic or functional repair have led to an increase in new research on bone repair recently. In cases where bone repair is insufficient, traditional, or alternative medicine methods are used [16]. In developing countries, the majority of the population tries to solve their problem by resorting to traditional methods (plant, fruit, tea, etc.). For this reason, it has been reported that medicinal fruits and plants have been popularly used in studies in recent years.

The fact that MC is effective in many ailments increases the research areas day by day. It has been found to be effective in regulating blood sugar regulation, especially in diabetic patients. Yang et al. reported that MC exerts preventive effects against insulin resistance and diabetes through modulation of NF-κB and phospho-c-Jun N-terminal kinase pathways. They also suggested that MC may be beneficial in the prevention of insulin resistance and diabetes [17]. Irregularity in glucose and insulin metabolism causes deterioration of bone vascularization. This will cause deterioration in osteoblast, osteoclast activities and mineralization, which are necessary for bone repair [17,18]. It has been reported that vascular regeneration plays an important role in the proliferation and migration of bone cells [19]. Considering the positive results of MC on diabetes and other problems, it is thought that it may indirectly affect the bone regeneration mechanism. Thus, the lack of studies on fracture healing of this widespread, inexpensive, and easy-to-access plant contributed to the creation of our experimental model for its protective and curative effect on bone defect.

Nonunion is a common complication, accounting for 5-10% of acute fractures. Despite advances in scientific understanding and treatment methods, fracture nonunion rates still remain largely unchanged. Many studies have been conducted that positively and negatively affect the fracture healing process, and new studies continue to be conducted. However, it has been reported that insulin deficiency, hyperglycemia, and oxidative stress, calcium sulfate dihydrate, depression, and cigarette smoke have negative effects on bone development and repair. In our study, it was determined that MC significantly supported bone repair histopathologically and immunohistochemically in the groups to which it was applied [20]. It was determined that while osteoblastic activity and osteocyte development increased in the groups to which MC was applied, compared to the sham group, osteoclastic activity decreased and was regulated to the level of the normal control group. In the groups to which MC was applied, osteopontin and osteonectin expression were also found to be positive. In addition, 28 days of application of MC was found to be more effective in regulating bone repair to the level of the control group.

Osteoclast cells are active cells in fracture repair and diseases such as arthritis and tumor metastasis. It can be used as a marker in studies. In a study examining the effect of the orally available AZD0530 on bone turnover in healthy men, it was reported that it would reduce osteoclast function in humans and thus decrease bone resorption. In a study investigating the effects of Potentilla fulgens as a prophylactic agent on tibial defects in the rat, Potentilla fulgens extract provides a protective effect in new bone formation and supports osteocytes development and matrix secretion in osteoblasts, which have important roles in bone development. In addition, between the 14th and 28th day findings, it is emphasized that the expression of osteopontin and osteonectin has a positive effect on the development of osteoblasts and osteocytes [22]. In an in vivo study examining the effect of BMA and low-level laser therapy on bone development, the expression of OPN is predominantly in osteoblasts located on the surface of newly formed bone, displaying morphology consistent with high synthesis activity, and on the margins of this tissue. observed in osteocytes. It is also said that OPN expression and expression of VEGF, PCNA, Runx2, BMP-2, OPN and OCN can be improved to increase bone healing. It has been reported that it contributes to bone healing by increasing angiogenesis, cell proliferation, osteoblast differentiation and mineralization [23]. In our study, a significant increase was observed in the defect group in the trabecular structures, especially in plasma cells and osteoclast cells. Degeneration and local deterioration in osteoclastic activity, aggregate and solitary dispersed structural formations in inflammatory cells were clearly detected. And, positive osteopontin expression was observed in the periphery of bones and especially in osteoclasts. Osteopontin expression positivity in leukocytes and osteopontin positivity in main bone cell were observed. Although there are bone formations in the form of small particles within the defect area, a significant increase in inflammation was observed. It was determined that osteonectin expression was positive in osteoclast cells and negatively interacted with osteoblasts of newly formed small particles. It was observed that the newly formed bone trabeculae started to mature in the 4th group, which was applied MC. However, both osteoblastic activity and osteocytes located in the lacunae were evident. While a decrease in osteoclasts was observed, the decrease in inflammation was remarkable. It was observed that the

expression of osteopontin in osteoblasts and osteocytes became evident and the trabeculae fragments belonging to new bone formation accelerated the matrix release. In the osteoblast cells of the enlarged trabeculae within the defect area, osteonectin expression was positive in the osteocytes, and in some areas, positive osteonectin expression was observed in a small number of osteoclast cells. While it was observed that the fibrous structures began to become very prominent and their structural integrity began to develop, osteonectin expression was also positive. According to the parameters we examined affecting bone repair, it can be said that applying MC for 28 days or more instead of 14 days can increase bone repair more.

Bone healing involves interaction with the action of many cells regulated by biochemical and mechanical signals. It has been reported that hypoxia and angiogenesis are vital for successful restoration in fracture healing, which is a complex and sensitive physiological process. In a study, it was reported that the possible negative effects of ischemia on fracture healing in rats with tibial fracture associated with vascular injury or compartment syndrome can be eliminated with melatonin and caffeic acid phenethyl ester [24]. They reported that melatonin supports both osteogenesis and angiogenesis and accelerates osteoporotic bone defect repair [25]. Considering the success in blood vessel dilation and bone repair in the group in which MC was applied in our study, it can be said that it contributes to angiogenesis, which is of vital importance in bone repair.

In a study examining the effect of teicoplanin (antibiotic) on fracture healing stereologically and histopathologically, it was observed that teicoplanin increased vascularization and connective tissue. It has also been reported that Teicoplanin, which is known to have an antibacterial effect, can be used safely in the treatment of bone defects accompanying infection [26]. Non-steroidal anti-inflammatory (NSAID) drugs play a role in the development of delayed union and nonunion after fractures in animal models. Tenoxicam, which is a class of NSAID drugs, has been reported to adversely affect fracture healing. In another experimental study, it was reported that systemic administration of levofloxacin and cephalexin in rats should not be prescribed for more than 1 week after surgical treatment of bone fractures due to the possible adverse effects on fracture healing [27]. Aspirin is a nonsteroidal anti-inflammatory drug used as an anticoagulant. It is said that aspirin used in many studies can promote bone regeneration by activating cytokines and some mediators in osteoclasts, osteoblasts, and their progenitor cells in the defect area. It has been reported that it stimulates angiogenesis, which is important in bone fracture repair, by increasing the migration of endothelial cells [28]. In our study, no drugs that could positively or negatively affect the inflammatory response in fracture repair and healing in bone repair were used in order to better examine the effectiveness of MC in defect repair.

The nervous system has an important role in bone growth and remodeling, and innervation plays a regulatory role in fracture healing. Nerve injury causes mechanically unstable and weak callus formation in fracture, but callus sizes are smaller. In a study, it was reported that the negative effects of hyperbaric oxygen therapy treatment on fracture healing, including the mechanical properties of the bone, can be limited as a result of nerve injury [29] In a study investigating the effect of sciatic nerve resection on tibial fracture healing in vivo, it was reported that sciatic nerve section is not a reliable model for a total denervation of tibial fracture. Short-term muscle atrophy caused by local injection of botulinum toxin-A has been reported to adversely affect fracture healing in the rat femur. As a result of the use of muscles, it is said to be beneficial for bone repair due to its contribution to high vascularity and regional blood circulation. It has also been reported that muscle progenitors may directly contribute to bone healing.

In our study, denervation was excluded by paying attention to the existing nerves, which are effective in stimulating the muscles in the relevant region during the formation of tibial defect.

CONCLUSIONS

The biological process of fracture healing is complex and is directly or indirectly affected by many factors. In addition to many studies on bone repair to support the literature, it has been found that Momordica charantia can have a positive effect on bone repair. However, further and comprehensive studies are required to support or further investigate these results.

REFERENCES

1. Baloğlu M, Özkorkmaz EG. Biochemical and immunohistochemical investigations on bone formation and remodelling in ovariectomised rats with tamoxifen citrate administration. Folia Morphol (Warsz)., 2019; 78:789-797, https://doi.org/10.5603/FM.a2019.0035

2. Kelm RJ Jr, Swords NA, Orfeo T, Mann KG. Osteonectin in matrix remodeling. A plasminogen-osteonectin-collagen complex. J Biol Chem., 1994; 269:30147-53. https://doi.org/10.1016/S0021-9258(18)43789-1

3. Walters G, Pountos I, Giannoudis PV. The cytokines and micro-environment of fracture haematoma: Current evidence. J Tissue Eng Regen Med., 2018; 12:e1662-e1677. https://doi.org/10.1002/term.2593

4. Kuroda S, Virdi AS, Dai Y, Shott S, Sumner DR. Patterns and localization of gene expression during intramembranous bone regeneration in the rat femoral marrow ablation model. Calcif Tissue Int., 2005; 77:212-25. https://doi.org/10.1007/s00223-004-0267-x

5. Grover JK, Yadav SP. Pharmacological actions and potential uses of Momordica charantia: a review. J Ethnopharmacol., 2004; 93:123-32, https://doi.org/10.1016/j.jep.2004.03.035

6. Jia S, Shen M, Zhang F, Xie J. Recent Advances in Momordica charantia: Functional Components and Biological Activities. Int J Mol Sci., 2017; 18: 2555. https://doi.org/10.3390/ijms18122555

7. Basch E, Gabardi S, Ulbricht C. Bitter melon (Momordica charantia): a review of efficacy and safety. Am J Health Syst Pharm., 2003; 60:356-9, https://doi.org/10.1093/ajhp/60.4.356

8. Taş F, Erdemci F, Aşır F, Maraşlı M, Deveci E. Histopathological examination of the placenta after delivery in pregnant women with COVID-19. J Health Sci Med, 2022; 5(3):868-874. https://doi.org/10.32322/jhsm.1100731

9. Peker N, Deger U, Asır F. Investigation of the effects of carvacrol on experimental ischemia/reperfusion model of rat ovaries by immunohistochemistry. Anal Quant Cytopathol Histopathol, 2021; 42 (6):197-204

10. Kumar M, Rawat S, Nagar B, Kumar A, Pala NA, Bhat JA, Bussmann RW, Cabral-Pinto M, Kunwar R. Implementation of the Use of Ethnomedicinal Plants for Curing Diseases in the Indian Himalayas and Its Role in Sustainability of Livelihoods and Socioeconomic Development. Int J Environ Res Public Health., 2021; 18:1509. https://doi.org/10.3390/ijerph18041509

11. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol., 2014; 4:177. https://doi.org/10.3389/fphar.2013.00177

12. Doosti-Moghaddam M, Miri HR, Ghahghaei A, Hajinezhad MR, Saboori H. Effect of unripe fruit extract of Momordica charantia on total cholesterol, total triglyceride and blood lipoproteins in the blood of rats with hyperlipidemia. Cell. Mol. Biomed. Rep., 2022, 2:74-86. https://doi.org/10.55705/cmbr.2022.338806.1038

13. Yang YS, Wu NY, Kornelius E, Huang CN, Yang NC. A randomized, double-blind, placebo-controlled trial to evaluate the hypoglycemic efficacy of the mcIRBP-19-containing Momordica charantia L. fruit extracts in the type 2 diabetic subjects. Food Nutr Res., 2022; 66. https://doi.org/10.29219/fnr.v66.3685

20. Kayal RA, Tsatsas D, Bauer MA, Allen B, Al-Sebaei MO, Kakar S, Leone CW, Morgan EF, Gerstenfeld LC, Einhorn TA, Graves DT. Diminished bone formation during diabetic fracture healing is related to the premature resorption of cartilage associated with increased osteoclast activity. J Bone Miner Res., 2007; 22: 560-8. https://doi.org/10.1359/jbmr.070115

21. Zhang T, Han W, Zhao K, Yang W, Lu X, Jia Y, Qin A, Qian Y. Psoralen accelerates bone fracture healing by activating both osteoclasts and osteoblasts. FASEB J., 2019; 33:5399-5410. https://doi.org/10.1096/fj.201801797R

22. Ko EA, Park YJ, Yoon DS, Lee KM, Kim J, Jung S, Lee JW, Park KH. Drug repositioning of polaprezinc for bone fracture healing. Commun Biol., 2022; 5:462. https://doi.org/10.1038/s42003-022-03424-7

23. Zheng S, Zhou C, Yang H, Li J, Feng Z, Liao L, Li Y. Melatonin Accelerates Osteoporotic Bone Defect Repair by Promoting Osteogenesis-Angiogenesis Coupling. Front Endocrinol (Lausanne)., 2022; 13:826660. https://doi.org/10.3389/fendo.2022.826660

24. Haznedar B, Deveci E, Gök, E. Effect of Mancozeb Inhalation on the Olfactory Mucosa in Rats. International Journal of Otolaryngology and Head & Neck Surgery, 2022; 11(5):227-233. https://doi.org/10.4236/ijohns.2022.115024

25. Haznedar B, Ertugrul G, Deveci, E, Haznerdar B, Gök E, Deveci E. Effect of Cadmium Chloride Toxicity on Olfactory Mucosa. Int. J. Morphol, 2022; 1(2):3. https://doi.org/10.4067/S0717-95022022000601574

26. Golestani S, Golestaneh A, Gohari AA. Comparative effects of systemic administration of levofloxacin and cephalexin on fracture healing in rats. J Korean Assoc Oral Maxillofac Surg., 2022; 48:94-100. https://doi.org/10.5125/jkaoms.2022.48.2.94

27. Fattahi R, Mohebichamkhorami F, Khani MM, Soleimani M, Hosseinzadeh S. Aspirin effect on bone remodeling and skeletal regeneration. Tissue Cell., 2022; 76:101753. https://doi.org/10.1016/j.tice.2022.101753

28. Nordsletten L, Madsen JE, Almaas R, Rootwelt T, Halse J, Konttinen YT, Hukkanen M, Santavirta S. The neuronal regulation of fracture healing: effects of sciatic nerve resection in rat tibia. Acta Orthop Scand., 1994; 65:299-304. https://doi.org/10.3109/17453679408995457

29. Hao Y, Ma Y, Wang X, Jin F, Ge S. Short-term muscle atrophy caused by botulinum toxin-A local injection impairs fracture healing in the rat femur. J Orthop Res., 2012; 30:574-80. https://doi.org/10.1002/jor.21553