Original Article

Histological Effect of Flavonoid Extract of Hibiscus Sabdriffa and Mineral Trioxide Aggregate Separated and Combined on Bone Formation

Sura Saad Majeed, Ban A. Ghani
Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Baghdad, Iraq

Abstract

Background: Bone is a highly vascularized tissue that relies on blood vessels and bone cells interacting spatially and temporally. For thousands of years, people have turned to nature as a provenance of medicinal therapy and plant-based systems continue to play an important role in the primary health care of 80% of the world's developing nations. **Objectives:** This study was conducted to assess the effect of the local implementation of Hibiscus Sabdriffa Flavonoid Extract (HSFE)/mineral trioxide aggregate (MTA) separated and combined on bone healing. **Materials and Methods:** A total of 24 male adult New-Zealand rabbits were used in this research and were divided into control and experimental groups (where flavonoid, mineral trioxide aggregate, and a combination of both materials were locally used). Four induced intrabony holes of about 3 mm in depth and 4 mm in width were created in both tibias of each rabbit. Scarification was performed on the animals at 2 and 4 weeks of healing periods to prepare samples for histological, and\ histomorphometric analysis. **Results:** The/histological finding showed that bone defect treated with HSFE and HSFE/MTA showed bone trabeculae lined by osteoblast and multiple large osteocytes in 2 weeks duration which was statistically significant. At the 4-week duration of the healing period, the histomorphometric study shows highly significant differences among all studied groups for osteoblast, bone trabeculae area, and bone marrow area. **Conclusion:** Bone defects treated with HSFE, and both HSFE and MTA enhanced the process of bone healing.

Keywords: Bone healing, *Hibiscus sabdriffa* flavonoid extract, mineral trioxide aggregate

Introduction

Blood arteries control hematopoiesis and regeneration of bone production in the skeletal system by providing circulatory niches for hematopoietic stem cells.[1,2] Biomaterials used as bone substitutes are always regarded as foreign substances that could elicit an immunological response from the host. By creating inert biomaterials, traditional design approaches have long sought to minimize immunological reactions.[3] Three crucial elements are required for efficient bone regeneration: The presence of osteogenic cells for bone production, a scaffold for the development of bones, and growth factors for the stimulation of bone regeneration.[4,5]

Hibiscus sabdariffa L. from (Malvaceous) family is an annual herb shrub popularly known as Roselle or Sorrel. *Hibiscus sabdariffa* is a species of hibiscus, native to the old-world tropics, its widely cultivated/ for its strong fibers and it is well known for its edibility and its properties in medicine.[6] Sudan, India, Malaysia, and Taiwan are among the countries that cultivate the roselle plant, which has a lovely blossom and is thought to be native to Africa. It can reach a height of 2 to 2.5 m and is a woody-based subshrub, herb, or annual. The deeply 3- to 5-lobed, 8- to 15-cm long leaves are alternately placed on the stems.[7] Flavonoids, which are included in many plant foods,

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_756_23

Address for correspondence: Dr. Sura Saad Majeed, Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Baghdad, Iraq.
E-mail: sura.hussain1905@codental.uobaghdad.edu.iq

Submission: 14-Jun-2023 **Accepted:** 08-Oct-2023 **Published:** 23-Jan-2026

How to cite this article: Majeed SS, Ghani BA. Histological effect of flavonoid extract of hibiscus sabdriffa and mineral trioxide aggregate separated and combined on bone formation. Med J Babylon 2025;22:1225-30.

enhance bone health by preventing bone loss later in life and acting as a supplemental therapy in cases of chronic inflammation or excessive oxidative stress.[8] By enhancing signaling pathways that support osteoblast activity, flavonoids may prevent bone loss by minimizing reactions to oxidative stress or persistent low-grade inflammation.[9] Red clover oil contains isoflavonoid mixtures, which are the main active components of "phytoestrogens" and are regarded as osteoinductive herbal compounds that enhance and speed up the bone healing process by promoting early bone formation and maturation.[10]

Mineral/trioxide/aggregate (MTA) is a powder aggregate made up of mineral oxides with strong biological activity that may aid in bone production. It also appears to have effects on bone cells that are osteoconductive and biocompatible.[11,12] The favorable physical characteristics of MTA, as well as its capacity to promote tissue regeneration, are noteworthy. by causing a reduction of an inflammatory response, dystrophic mineralization, and complete bone formation.[13]

Material and Methods

In the present research, 24 male adult New-Zealand rabbits weighed an average of (1.5–2 kg). The intramuscular injection of xylazine (20 mg; Arendonk, Belgium; 0.2 mL/kg-B. W.) for general anesthesia. in addition to 50 mg of ketamine HCL (Holland) (20 mg/kg B.W.).[14] The right limb's proximal tibia metaphysis was the area of surgery. The tibia was exposed, and the initial intermitted drilling was carried out using a small round bur (number 010) and a fissure bur (number 10), both at a speed of 1500 rpm, and vigorous irrigation with normal saline. The flavonoid extract was then applied by using a pipette and MTA with ash (number 49). Four intra-bony holes were created in both tibias of each animal divided as follows:

1 Group A: Intra-bony defect will be left to heal spontaneously as control.
2 Group B: Intra-bony defect will be filled with flavonoid extract 2 µmL.
3 Group C: Intra-bony defect will be filled with MTA 1 mg.
4 Group D: Intra-bony defect will be filled with a combination of MTA and flavonoid extract in a ratio of 1:1

Animals' scarification was done for 2- and 4-week intervals with overdose anesthesia and After being immediately fixed in 10% freshly made formalin (Tedia, USA) for fixing, the specimens were processed into paraffin blocks with the defective bone facing the microtome knife's exterior of the block. Hematoxylin and Eosin staining was conducted on tissue sections that were 5 m thick., and histological, histomorphometric assessment using a light microscope (opticaB-350, Italy). Image processing software (ImageJ.exe) was used to do histomorphometric evaluations of bone cells (osteo blasts, osteo cytes, and osteo clasts), trabecular area (mm^2), trabecular number, and bone marrow area (mm2). A camera was attached to the microscope and took two microphotographs at X10 power, one in the upper portion and the other in the lower portion to cover nearly all defective areas.[15]

Ethical approval

The study protocol was reviewed and approved by a local ethics committee according to document number 298 on Apr 1, 2022.

Results

Histological-findings

Two weeks duration

After two weeks, the control group's microphotograph view reveals newly formed, immature bone trabeculae loaded with numerous, erratically distributed osteocytes (OC) and osteoblasts (OB), also seen at the trabeculae peripheries [Figure 1A]. The HSFE group shows newly formed bone trabeculae (BT) surrounding bone marrow (BM), with many unevenly distributed OC [Figure 1B]. The bone section at the defect site filled with MTA shows new BT rimmed by OB, and large irregularly distributed OC are embedded in bone tissue [Figure 1C]. However, the HSFE/MTA group shows bone trabeculae rimmed by osteoblasts, osteocytes embedded in bone, and osteoclast (OCL) [Figure 1D].

Four-week duration

After 4 weeks of histological section of the control group showed mature bone with BT filled by OC, and BM surrounded at the periphery by OB, Moreover, reversal lines (arrows) separating the old from the new bone are shown [Figure 2A]. The HSFE group shows mature, dense bone, OC, and the OB-lined Haversian system (HS) [Figure 2B]. In the MTA group, we can see mature bone with multiple large OC and BM which is lined by OB [Figure 2C]. The defect filled with combination group (HSFE/MTA) showed thick dense new bone that is separated from basal bone by reversal line(arrows), osteon lamellae were almost visible due to the alignment of OC around the HS with OB at the bone periphery [Figure 2D].

Bone architecture parameters histomorphometric ally analysis' of studied groups

In all of the investigated groups, the statistical analysis for the trabecular-number, trabecular-area, bone-marrow area, and bone cell counts (OB, OC, and OCL) were estimated over a period of two weeks [Table 1], whereas 4-week duration shown in Table 2.

Bone cells

At 2 weeks in comparison between experimental groups and the control group, the mean count difference of osteoblast cells shows a slight increase between the control

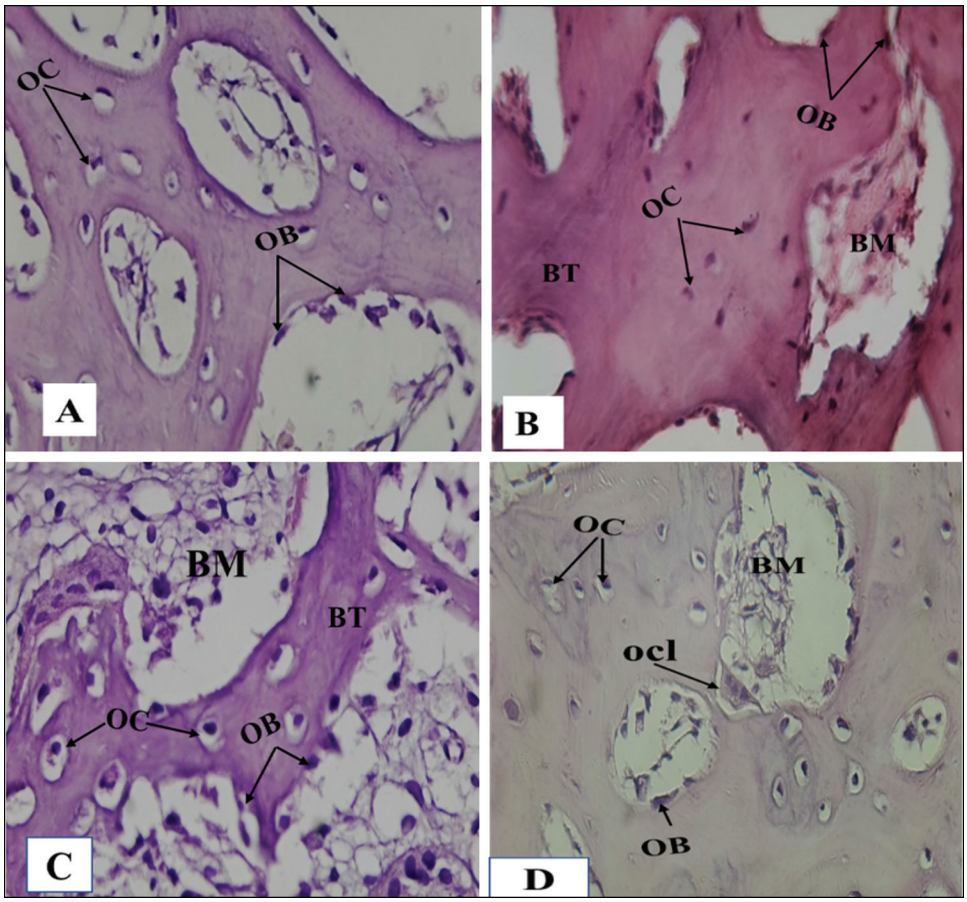

Figure 1: Histological section with v H&E x40: (A) control group, (B) HSFE Group, (C) MTA group, and (D) filled with HSFE/MTA group

groups and HSFE group, whereas slight decrees in both MTA and HSFE/MTA groups with statically significant change. Although OC shows a slight decrease in the MTA group and an obvious increase in both flavonoid and mixed groups with statistically significant changes. Regarding osteoclast, the mean count difference shows a nonsignificant decrease statistically.

After 4 weeks of comparison between the experimental groups, and the control group, the mean count difference of osteoblast shows an obvious increase in all experimental groups with highly significant change statically, whereas osteocyte shows a slight increase in both HSFE and HSFE/MTA groups and a slight decrease in MTA group which statistically significant change.in regard to osteoclast, there was a slight decrease in the HSFE group and a slight increase in both MTA and mixed groups with statistically nonsignificant change.

Trabecular area, trabecular number, and bone marrow area at two weeks, there was an increase in the mean count difference of the trabeculae number (TN), and the BM area revealed a little decline in mean values, and no statistically significant data were found in any of the experimental groups, whereas the TB area reviled an increase in the mean count difference and statistically highly significant data.

After 4 weeks of comparison between the control and all experimental groups TB area and BM area revealed an increase in mean count difference with highly significant statistical analysis, whereas the TN showed a slight increase in mean values of all experimental groups which were the statically nonsignificant change.

Discussion

The objectives of this experimental study were to estimate the effect of topical treatment of bone defect in rabbits' tibiae with HSFE and MTA separated and in combination.

Adult rabbits were chosen for the study because they have many appealing characteristics and satisfy the criteria. The rabbit tibia model has been effectively used as an operative site in numerous earlier research. New-Zealand-bred white rabbit strains are commonly used in studies.[16]

Beyond calcium and vitamin D, flavonoids, which are found in a wide variety of plant foods such as fruits and vegetables, herbs and spices, essential oils, and beverages, have the most potential of any dietary component for bone health promotion.[17,18]

The defect area in HSFE and HSFE/MTA groups had more and more substantial bone trabeculae than the other groups in agreement with Hejazi *et al*.[19] which stated that

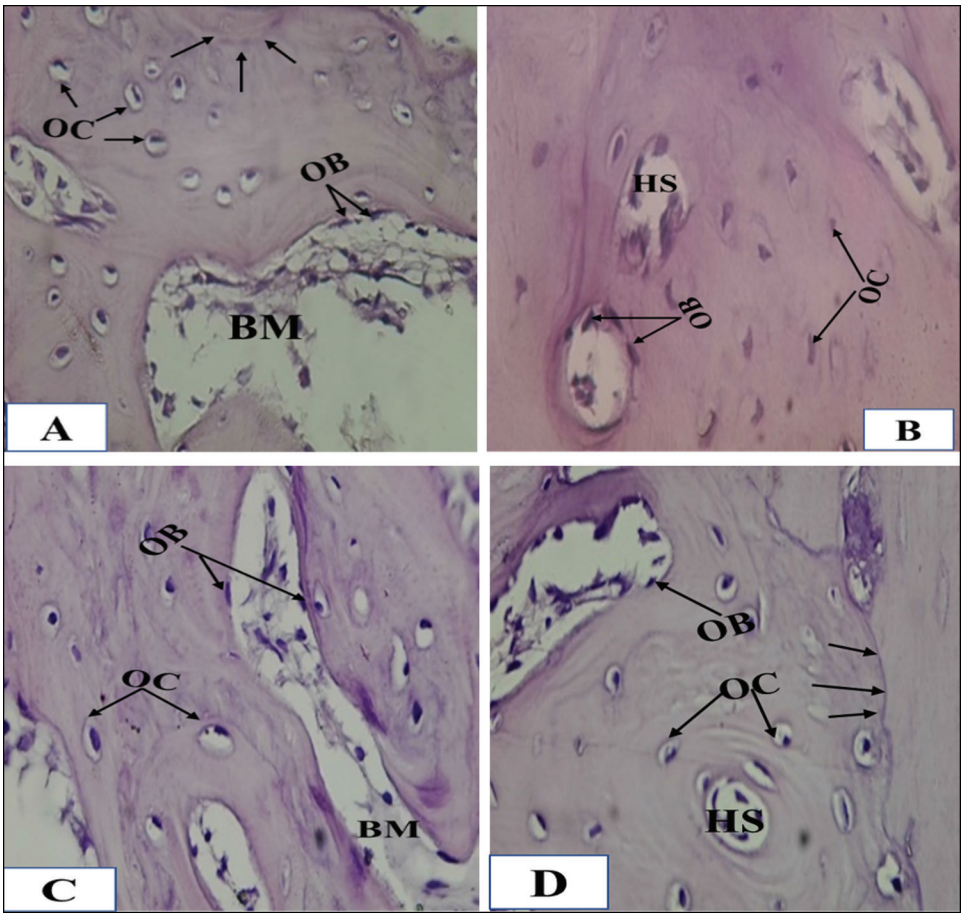

Figure 2: Histological view of H&E stain x40: (A) control group, (B) HSFE group, (C) MTA group, and (D) HSFE/MTA group

Table 1: Descriptive-statistical analysis of the 2-week duration of different groups

Parameters	Controls (*N* = 12)	HSFE (*N* = 12)	MTA (*N* = 12)	HSFE/MTA (*N* = 12)	*P* Value
Osteoblast, No					
Mean ± SD	16.7 ± 2.0	17.4 ± 1.6	13.82 ± 3.5	12.1 ± 1.28	<0.001
Range	14.8-21	14.6-19.8	8.0-18.8	10.4-14.6	
Osteocyte, No					
Mean ± SD	15.47 ± 2.33	27.38 ± 3.541	15.19 ± 3.27	23.86 ± 3.23	<0.001
Range	12.4-19.2	22.0-33.0	10.8-23.0	18.7-27.8	
Osteoclast, No					
Mean ± SD	0.2 ± 0.21	0.1 ± 0.16	0.13 ± 0.2	0.18 ± 0.18	0.545
Range	0.0-0.6	0.0-0.40	0.0-0.60	0.0-0.4	
Trabeculae, No					
Mean ± SD	8.08 ± 2.11	8.33 ± 1.67	8.25 ± 2.14	10.42 ± 1.97	0.018
Range	6.0-12.0	7.0-11.0	5.0-13	8.0-14.0	
Trabeculae area, mm					
Mean ± SD	3.02 ± 1.04	4.17 ± 0.68	2.19 ± 0.98	3.25 ± 0.92	<0.001
Range	1.16-4.75	3.09-5.09	0.74-3.4	2.34-4.8	
BM area, mm					
Mean ± SD	2.87 ± 0.7	2.18 ± 0.64	2.75 ± 1.95	2.87 ± 0.97	0.435
Range	1.79-4.0	1.19-3.64	0.61-6.29	098-4.18	

Isoflavone compounds have shown the ability to enhance the osteogenic impact by promoting the proliferation of osteoblast cells and activating the matrix.

After two weeks, we found that the MTA group had lower levels of OB and OC activity as compared to the groups treated with HSFE/MTA and the C and MTA

Table 2: Descriptive-statistical analysis of 4-week duration for different groups

Parameters	Controls ($N = 12$)	HSFE ($N = 12$)	MTA ($N = 12$)	HSFE/MTA ($N = 12$)	*P* Value
Osteoblast, No					
Mean ± SD	4.02 ± 176	6.73 ± 2.05	7.85 ± 2.69	11.43 ± 1.91	<0.001
Range	2.0-7.6	3.6-10.0	3.40-13.0	9.0-15	
Osteocyte, No					
Mean ± SD	20.68 ± 2.76	21.32 ± 3.0	18.0 ± 2.91	21.7 ± 2.46	0.011
Range	17.2-25.6	17.8-26.0	11.0-23.1	18.6 ± 26.0	
Osteoclast, No					
Mean ± SD	0.12 ± 0.16	0.07 ± 0.1	0.13 ± 0.2	0.15 ± 0.17	0.618
Range	0.0-0.4	0.0-0.20	0.0-0.6	0.0-0.40	
Trabeculae, No					
Mean ± SD	6.58 ± 2.91	6.25 ± 3.63	7.67 ± 1.77	8.0 ± 2.0	0.303
Range	2.0-12.0	4.0-15.0	5.0-11.0	5.0-11.0	
Trabeculae area, mm					
Mean ± SD	4.06 ± 2.41	5.84 ± 1.51	4.76 ± 0.55	5.08 ± 0.87	<0.001
Range	0.87-6.87	0.86-6.47	3.0-4.96	2.88-6.7	
BM area, mm					
Mean ± SD	1.14 ± 0.59	1.32 ± 0.57	2.4 ± 0.74	2.06 ± 0.87	<0.001
Range	0.55-2.37	0.54-2.36	1.57-3.91	1.0-3.14	

groups. Comparing this group to other groups revealed a statistically significant difference. this is in line with the findings of Xi *et al*.[20] Which suggested that taking the herb Epimedium's total flavonoid extract would be able to help adolescents reach their peak bone mass.

Near 4 weeks, histologic analysis of bone sections from the C, HSFE, and HSFE/MTA groups revealed mature bone tissue with many embedded OC and OB near the periphery. this is consistent with the findings of Elbahnasawy *et al*.[21,22] who found that at 4 weeks thick bony trabeculae were detected in regions of bone growth. According to Castelo-Branco, and Cancelo-Hidalgo,[23] Iso, flavone chemicals are effective physiological inducers of OB differentiation and angiogenesis and activate the conversion of mesenchymal cells into OB. They also stimulate bone formation during remodeling and repair processes which is compatible with our study findings.

Histomorphometric analysis has shown that the experimental groups had higher values for mean count difference of all parameters examined for micro-architecture-records than in the control group. The findings also revealed that there was a significant difference in bone architecture parameters at different intervals of time. Trabecular number and trabecular width cortical width were increased at two and four weeks which would be attributed to the time consumed for the bone maturation and its apposition in agreement with the result of Li *et al*.[24] which suggested that flavonoids could improve the trabecular bone number and bone mineral density (BMD), enhance bone tissue architecture, encourage the creation of new bone, and boost biomechanical strength in bone defects.

Examination of serial sections from two weeks following the procedure in the intervention site under a microscope revealed that the new bone trabecular development in all experimental groups highly increased the number of OB and OC, compared to the control group. This result was in line with those of a study carried out by Jassim, and AlGhaban[25] who examined bone sections of the control group after 2 weeks.

According to the present study, dense bone trabecular filled the bone defect site in the control group after 4 weeks from the procedure. However, in the experimental group treated by HSFE, mature bone was seen by the presence of Haversian lamellae which concentrated around the Haversian canal by the arrangement of OC. This finding is in agreement with those of a study by Yang *et al*.[26] which showed that after 4 weeks, Flavonoids and other Traditional Chinese medicines derived active components have been shown to have an effect on promoting osteoblast proliferation and differentiation.[27]

Conclusion

According to the results obtained in this study, it can be concluded that the combined local application of HSFE, and HSFE/MTA accelerates the process of bone healing through increasing activity of bone-forming cells.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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