ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
June2025 • Vol. 38

Castor oil effects on bone healing defect in rats: An experimental study

Nawar Bahjet Kamil [1]

Aim: evaluation the effect of castor oil on bone healing in rats.
Materials and methods: study done in 24 rats with two-bone defect in each rats so 48 bone defect studied in this research. General anesthesia given intramuscularly to all rats. Make an incision in the right and left femur, muscles reflection and expose the bone. Using the round bur for the hand piece of microingen, make a hole about 3 mm deep and 2 mm wide, with continues washing. Holes in the right side left without treatment while holes in the left side treated with 0.5 ml castor oil. After that, we sew the muscle and skin and randomly dividing the rats into two healing periods: the second and sixth weeks. At each healing period twelve rats (24 holes) used. After giving general anesthesia, exposed the defect bone and cutting, then putting in formalin after that doing the processing procedure to get a tissue with Hematoxylin and Eosin stain. The slide examined under light microscope and evaluated histologically.
Results: Castor oil group recorded higher mean value in all parameter except bone marrow area than the control group at second week. At 6th week, the experimental group recorded higher mean value in all parameter except osteoclasts account and bone marrow area than control group.
Conclusion: castor oil accelerated bone healing in rats.

Key word: Castor oil, histology, bone healing.

1. Department of Oral Diagnostic Sciences, College of Dentistry, University of Baghdad, Iraq.
Corresponding author: Nawar Bahjet Kamil, email: nawar.bahjat@codental.uobaghdad.edu.iq

Introduction

Bones form the skeleton of the body, consists from water, cells, organic and inorganic compound. Bone is forming from osteoblast cells that then become osteocytes. Bone tissue formed and remodeling throughout life 1. The majority of the crystalline calcium phosphate ions that make up the inorganic part of bone matrix found as hydroxylapatite 2, 3. Bone formation either by formation of cartilage called endochondral bone formation or bone formation without cartilage formation called intramembranous form or bones are forming from these two form 4. There are three phases of bone healing inflammatory phase, proliferation phase and remodeling phase 5. The hematoma that forms in the broken bone is the first stage that happens right after a fracture 6. The arrangement of the fracture hematoma has used to define the proliferative or fibroplasia process. Necrotic bone resorption is initiating during the fibroplasia phase by osteoclasts produced from circulating monocytes in the bloodstream and by monoblastic precursor cells arising from the local bone marrow. The fibroplasia phase, which starts with collagen fibers present, osteoid secretion, and ongoing vascular ingrowth, is marking by the creation of callus 7.

Castor oil taken from the seed, the solitary point of unsaturation, carboxylic group, hydroxyl group, and structure of ricinoleic acid form the basis of castor oil's chemistry. The oil structure strengthened further by these characteristics. Among the fatty acid profiles found in castor oil are linoleic, linolenic, stearic, palmitic, and ricinoleic 8, 9. Ricinoleic acid is a monounsaturated fatty acid, the most prevalent, making up between 75 and 90 percent of the oil's overall makeup, used in herbal remedies 10. Castor oil absorbs readily into the skin, after absorption, it can activate the lymphoid tissues and/or the thymus gland, which increases the quantity of lymphocytes 11, 12.

The aim of this study to evaluate the effects of castor seed oil on bone healing defect histologically.

Materials And Methods

Ethical approval

This study conducted after obtaining the approval of the Ethics Committee of the College of Dentistry, University of Baghdad, on 12-5-2024, No: 917.

Sample size collection

Twenty-four adult healthy male rats, aged between 8 and 12 months and weighing 2.7 -3.2 kg, were using in this study. Two holes, one in the right femur and one in the left femur, were doing in each rat by surgical operation so total number of holes (48) dividing randomly in to two period (2& 6 week) each period with 12 rat 13.

The SPSS 25 program was using to examine the data.

Each parameter's mean, median, standard deviations, and p values ascertained. P values equal or less 0.01 high significant wile equal or less than 0.05 statistically mean significant.

Surgical process and slide preparation

General anesthesia given intramuscularly to all rabbits. Make an incision in the right and left femur, muscles reflection and expose the bone. Using the round bur for the hand piece of microingen, make a hole about 3 mm deep and 2 mm wide, with continues washing 14. Holes in the right side left without treatment while holes in the left side treated with 0.5 ml castor oil. After that, we sew the muscle and skin and randomly dividing the rats into two healing periods: the second and sixth weeks. At each

healing period twelve rats (24 holes) used. After giving general anesthesia, exposed the defect bone and cutting, then putting in formalin after that doing the processing procedure to get a tissue with Hematoxylin and Eosin (H&E) stain. Histological preparation included, first: Fixation, the samples treated in blocks of standard paraffin and instantly fixed in 10% freshly made neutral formalin. Second: dehydration, the specimens underwent a succession of increasing alcohol concentrations (50%, 70%, 80%, 95%, and 100% alcohol) before being placing in two xylene jars and left for a half-hour in each jar. Third: Embedding, the samples arranged in dishes with melted embedding paraffin, and the dishes then placed in an oven set to a constant temperature of around 60ºC. The specimens moved to two separate paraffin dishes during the course of the several-hour operation so that the paraffin could completely replace the xylene in the tissue (each dish for one hour). After that, the specimens were positioning in the middle of the paraffin blocks. Fourth: Sectioning, a microtome employed to obtain sections of five micrometers in thickness, which subsequently mounted on clean glass slides for H&E staining 15. The slide examine under light microscope and evaluated histologically. Trabecular bone area measured in µm by using image J program in 1.40 × 1.46 tissue area. Osteoclast, osteoblast and osteocyte account measured by taking a mean in 1.43 × 1.08 mm dimension under power 40 16.

Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk used in current study to test normality of values, which showed the data statistically homogenous. Anova test used and descriptive analysis done. P-value equal or less than 0.05 mean statistically significant and when P-value eq ual or less than 0.01 mean statically high significant.

Results

Histological finding

After two week: microscopic feature illustrated bone trabecular enclosed bone marrow and a reversal line separated between the old and new bone. The experimental group showed large area of trabecular bone with osteocytes embedded in the bone and osteoblasts on the surface fig (1: B) while the control group showed few osteocytes in the bone as shown in fig (1: A).

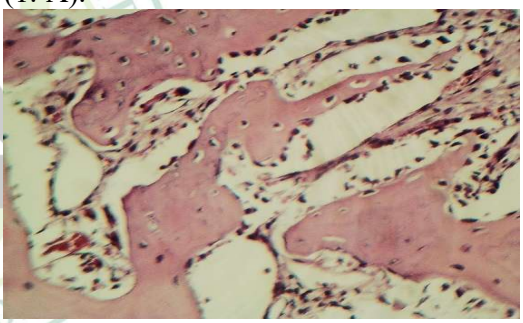

Fig 1 (a)

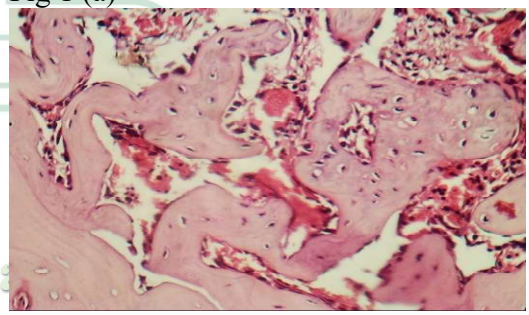

Fig 1 (b)

Figure 1: Histological feature at second week, A: Control group. B: Experimental group.

After 6 week: microscopic feature showed mature bone in the experimental group with osteon formation and regular osteocytes surrounded it fig (2: B) while the control one still with woven bone with mature bone and osteocyte irregular arrangement around haversion canal (2: A). Osteoblast arrangement on the surface of the bone in both groups.

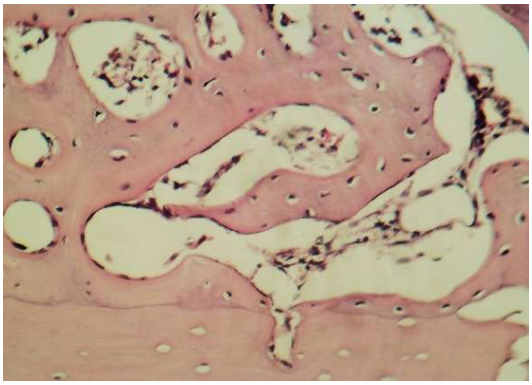

Fig 2(a)

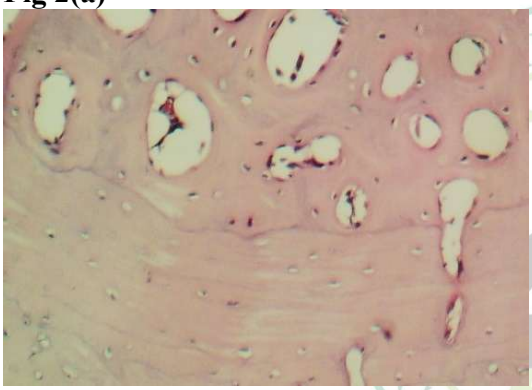

Fig 2(b)

Figure 2: Histological features at 6th week, A: Control group. B: Experimental group.

Statistical results

At the second week, castor oil group recorded the higher mean value in the osteoblasts, osteocytes, osteoclasts and trabecular bone area than the control group. The control group recorded higher mean value in the bone marrow area. There was a high significant difference (P value ≤ 0.01) between the control group and castor oil group in the all parameter except osteoclasts account table (1).

At the sixth week, the control group recorded a higher mean value in osteoclasts account and bone marrow area than the castor oil while castor oil group recorded a higher mean value in the osteoblasts, osteocytes and trabecular bone area than the control one. There were a significant difference (P value ≤ 0.05) between the control and castor oil groups in the osteoblasts, osteocytes account, bone marrow area and trabecular bone area and there was a non-significant difference in osteoclasts account table (1).

Table 1: Statistical result at second and 6th week.

duration	parameter	group	number	mean	SD	SE	P value
2 week	osteoblast	Control	12	3.35	1.23	0.43	0.001
		Castor	12	7.24	1.56	0.51	
	osteocyte	Control	12	4.47	0.85	0.29	0.001
		Castor	12	8.14	1.56	0.58	
	osteoclast	Control	12	0.41	0.25	0.15	0.08
		Castor	12	1.01	0.44	0.13	
	Trabecular area	Control	12	0.42	0.02	0.01	0.00
		Castor	12	0.86	0.07	0.06	
	Bone marrow area	Control	12	2.11	0.29	1.56	0.001
		Castor	12	1.25	0.13	1.11	
6 week	osteoblast	Control	12	4.89	0.73	0.27	0.02
		Castor	12	6.25	0.72	0.28	
	osteocyte	Control	12	12.40	2.67	1.02	0.02
		Castor	12	15.40	3.38	1.42	
	osteoclast	Control	12	0.51	0.44	0.12	0.67
		Castor	12	0.33	0.43	0.14	
	Trabecular area	Control	12	1.56	0.22	0.11	0.03
		Castor	12	3.38	0.15	0.08	
	Bone marrow area	Control	12	0.83	0.27	0.12	0.04
		Castor	12	0.55	0.06	0.02	

High significant P-value ≤ 0.01 Significant P-value ≤ 0.05

Discussion

Castor oil has an anti- oxidant and anti- inflammatory effects [10] therefore chosen in the current study. Trabecular bone formation increased in thickness, but decreases in number throughout the healing process, and the deposition of bone matrix are indicators of the bone healing process. One of the most important factors in determining the degree of bone regeneration in osseous defects is the amount of newly formed bone formation [17]. Histological study in the current study showed, as the newly created trabecular bone matured and mineralized, the area of the bone marrow decreased with time. New bone almost filled up the defect areas, suggesting that the extract may stimulate the production of osteoblasts cell. Current study illustrated the trabecular bone formed in both groups with different degree in deposition. Trabecular bone contains lacunae with osteocytes cell and osteoblasts on the

border which surrounding bone marrow. Castor oil contains fatty acid as an isolated hydroxyl acid as ricinoleic acid, which is a major component in castor oil [18] also contains linolenic acid, oleic acid and stearic acid, which have, benefit effects on human health [19]. These compound have an effect on bone metabolism, which regulate osteoblastogenesis, osteoclast activity and decrease inflammatory cytockine [20] also the surgical procedure have an effective role in stimulation the bone formation [21]. This may explained the significant increasing in bone trabeculae thickness in the castor oil group than the control group at both period. Castor oil contains phenolic compound [22] which able to mediate osteoblasts differentiation through activation of Wnt pathway and up regulated B-catenin expression. Wnt pathway through the activation of B-catenin expression leading to differentiation of bone marrow stromal cells, which formed osteoblasts cell and decrease the differentiation of osteoclasts cell [23]. This is may explain why castor oil recorded a higher mean value in the osteoblasts account at both period and lower mean value in osteoclasts account at sixth week than the control one. Carotenoid in castor oil working as scavenger free radicals and function as antioxidants, perhaps enhancing the effectiveness of fat-soluble vitamins like vitamin A. The antioxidant action of oils' tocopherols and β-carotene combination may work in concert [24]. This is in return promote osteogenic activity of bone marrow stromal cells, angiogenesis process and facilitated bone healing [25]. For this reason, castor oil group recorded higher mean value in osteocytes account than the control group at both period, and the control group still with immature bone but the experimental group converted in to mature lamellate bone at sixth week. This finding agree with Mohamed [26] who illustrated, the bone healing accelerated in Punica granatum seed oil than the control group. Also came in line with Razouki [27] who studied the effect of beta-Tricalcium Phosphate on bone healing, which showed an acceleration in bone healing with the experimental group and these researches showed increasing in osteocytes account in the experimental group with time. Current study showed bone marrow area decreased with time in both groups with the lowest mean value recorded in the experimental group. This was due to acceleration of bone maturation in the castor oil and microscopic feature showed osteon surrounded by lamella with osteocytes related to anti-oxidant and anti-inflammatory effect of castor oil [10]. This agree with Jassim [28] who studied the effect of eucommia ulmoides on bone healing which showed the bone marrow area decreased with time and the lower mean value recorded in the experimental group than the control one. Current study cleared the castor oil group accelerated the bone healing process by early cells differentiation and coordination of working between osteogenesis and angiogenesis; this was consistent with Fadhil [29]. Who showed the combination of BMP9 and Ang1 have an effective role in accelerating bone healing than the control group by coordination the osteogenesis and angiogenesis working.

Conclusion

Castor oil has an effective role in acceleratinon bone healing by early differentiation of cells from bone marrow stromal cells and coordination the working of these cells. Castor oil suggested using in osseointegration and oral mucosa healing.

Funding: This study is self-funded.
Data Availability: Full data is available for anyone.
Ethics Approval and Consent to participate the research plan reviewed and accepted by the research ethical committee, College of dentistry University of Baghdad No: 917 in 12-5-2024.
Competing Interests: The author has no conflicts of interest to declare.

References

1- Cowin SC. Tissue growth and remodeling. Annu Rev Biomed Eng. 2004; 6:77–107.
2- Bliziotes M, Gunness M, Eshleman A, Wiren K. The role of dop-amine and serotonin in regulating bone mass and strength: studieson dopamine and serotonin transporter null mice. J MusculoskeletNeuronal Interact. 2002; 2 (3):291–5.
3- Ulstrup AK. Biomechanical concepts of fracture healing in weight-bearing long bones. Acta Orthopaedica Belgica. 2008; 74, 291.
4- Shapiro F. Bone development and its relation to fracture repair. The role of mesenchymal osteoblasts and surface osteoblasts. Eur Cell Mater. 2008; 15, 53-76.
5- Thompson DD. Introduction-Mechanisms of fracture healing and pharmacologic control. J Musculoskel Neuron Interact. 2003; 3, 295-6.
6- Mountziaris PM, Mikos AG. Modulation of the inflammatory response for enhanced bone tissue regeneration. Tissue Engineering Part B: Reviews, 2008; 14, 179-86.
7- Pilitsis JG, Lucas DR, Rengachary SR. Bone healing and spinal fusion. Neurosurgical focus.2002; 13, 1-6.
8- Anjani, K. A re-evaluation of castor (Ricinus communis L.) as acrop plant. Perspectives in Agriculture, Veterinary Science, Nutritionand Natural Resources.2014; 9(1), 1-21.
9- Naik, S. N., Saxena, D. K., Dole, B. R., & Khare, S. K. Potentialand perspective of castor biorefinery waste biorefinery.USA: Elsevier. 2018; 623-656
10-Mubofu, E. B. Castor oil as a potential renewable resource for the production of functional materials. Sustainable ChemicalProcesses.2016; 4(1).
11-Akpan UG, Jimoh A, and Mohammed AD. Extraction, Characterization and Modification of Castor Seed Oil. Leonardo Journal of Sciences. 2006; (8): 43-52.
12-Budai L, Antal I, Klebovich I and Budai M. Natural oils and waxes: studies on stick bases. J Cosmet Sci. 2012; 63(2):93–101.
13-Kamil NB, AL-Ghaban NMH, Aamery A. Osseointegration effects of whey protein (histological and histomorphological observations): An experimental study on rabbits. J Bagh Coll Dent. 2023; 35(3):28-36.
14-AL-Ghaban Nada M.H. , Jasem G H. Histomorphometric evaluation of the effects of local application of red cloveroil (trifolium pratense) on bone healing in rats. J Baghdad College Dentistry. 2020; 32(2):26-31.
15-Elsherif, N., Abdelhamid, M., Taha, A. 'Histopathological Evaluation of Cancer Tendency in the Buccal Mucosa of Aged Albino Rats (Histological and Immunohistochemical Study)', Ain Shams Dental Journal. (2023); 32(4). pp. 37-48.
16-Kamil NB, Al-Ghaban NMH. Evaluation of the Effects of Whey Protein and Moringa Oleifera Leaves Extract Mixture on Osseointegration in Rabbits. Iran Biomed J. 2024; 1;28 (2&3):82-9.
17-E. Pepelassi, D. Perrea, I. Dontas, C. Ulm, I. Vrotsos, and S. Tangl, "Porous titanium granules in comparison with autogenous bone graft in femoral osseous defects: a histomorphometric study of bone regeneration and osseointegration in rabbits," BioMed Research International. 2019, vol. 2019: 12
18-Omohu, O. J., & Omale, A. C. Physicochemical properties and fatty acid composition of castor bean Ricinus communis L. seed oil. European Journal of Biophysics.2017; 5(4), 62-65.
19-Ganesan, K., Sukalingam, K., & Xu, B. Impact of consumption and cooking manners of vegetable oils on cardiovascular diseases-A critical review. Trends in Food Science & Technology.2018; 71, 132-154.
20-Maggio M., Artoni A., Fulvio F. Lauretani et al., "*e effect of polyunsaturated fatty acids on bone health," Reviews in Clinical Gerontology. 2011; 21.
21-Ebrahem, A., Gaber, R., Mekhemer, S., El-Abedeen, M. (2024). 'Radiologic and Histological Evaluation of Allogenic Bone graft Blocks in Bounded Anterior Maxillary Alveolar Ridge Defects', Ain Shams Dental Journal, 35(3), pp. 182-188.
22-Chew, S. C., & Nyam, K. L. Kenaf (Hibiscus cannabinus L.) seed oil. In M. F. Ramadan (Ed.), Fruit oils: chemistry and functionality .Switzerl and Springer.2019; 451-494.
23-Kim TH, Jung JW, Ha BG, et al. The effects of luteolin on osteoclast differentiation, function in vitro and ovariectomy-induced bone loss. J Nutr Biochem. 2011; 22(1):8-15.
24-Atta, N. M., & Mohamed, E. S. A. Determination of fat–soluble vitamins and natural

antioxidants in seventeen vegetable oils. Journal of Food and Dairy Sciences. (2017); 8(8), 323-330.
25-Huang, L.; Lu, S.; Bian, M.; Wang, J.; Yu, J.; Ge, J.; Zhang, J.; Xu, Q. Punicalagin attenuates TNF-alpha-induced oxidative damage and promotes osteogenic differentiation of bone mesenchymal stem cells by activating the Nrf2/HO-1 pathway. Exp. Cell Res. 2023; 430, 113717.
26-Mohamed I F, Ghani B A., and Fathalla A A. Histological Evaluation of the Effect of LocalApplication ofPunica granatum Seed Oil on Bone Healing. International Journal of Biomaterials. 2022; 8.
27-Razouki N A, Ghani B A. Histological Evaluation of Effect of beta-Tricalcium Phosphate on Bone healing in Alloxan-Induced diabetes. Journal of Baghdad College of Dentistry. 2016; 28(3):75-81.
28-Jassim O H and Al-Ghaban, N. M. H. Effect of Eucommia Ulmoides on Healing of Bon Defect Using Histological and Histomorphometric Analysis in Rat: in vivo Study. Razi Vaccine & Serum Research Institute. Archives of Razi Institute. 2023; 78(2):691-697.
29-Fadhil E, Dosh R H., Wally Z J., and Haider J. Histological evaluation of the effects of bone morphogenetic protein 9 and angiopoietin 1 on bone healing. Journal of Taibah University Medical Sciences. 2023; 18(5), 954e963.