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Histological Effect of Artichoke Leaf Extract on Bone Healing in Rats

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Abstract
Objectives: This study evaluated the effect of artichoke leaves extract on bone healing.
Methods: A total of 30 rats were used in the current study, 60 bone defects were made, divided into 30 bone defects left without treatment and an other 30 bone defects were treated with 0.5 ml of Artichocke oil. Each group was divided randomly into three period intervals (1 week, 2 and 5 weeks).
Results: Current study had showed a high significant difference between the control and the experimental group in osteoclast cells account, a significant difference in osteoblast and osteocyte cells account. Also there was a high significant difference in the formation of the blood vessels and the trabecular bone between the experimental and the control group.
Conclusion: Artichoke leaves extract was effective and faster in bone healing without any complication in comparison with the control group.
Keywords: Artichoke oil, bone healing

Introduction

The bone is a hard structure that forms the vertebrates skeletal framework. It consists of an organic matrix that is composed of about 40% collagen type 1, water and inorganic minerals which is composed of hydroxylapatite. The bone is formed by osteoblasts and when the bone is formed some of them will be embedded in the bone and become osteocytes.[1]

Bone development happens in two ways, intra membranous and endochondral ossification (Ossification is a process of bone formation). The formation of woven bone happens by the replacement of the collagenous mesenchymal tissues by bones.[2]

The woven bone is considered as a primitive form of the bone with a randomly organized collagenous fibers and by remodeling it is converted into a mature lamellar bone. The lamellar bone can possesses a regular parallel multiple rings of collagen, which is then remodeled by the osteoblasts and osteoclasts.[3]

Bone Healing

There are three stages of bone healing which includes stage of inflammation, repair stage and the remodeling stage.[4] Inflammatory phase occurs immediately following a fracture and is characterized by the formation of the hematoma.[5] Within the first few days after the injury, the levels of interleukin-1 (IL-1), (IL-6), (IL-11), (IL-18) and the tumor necrosis factor-α (TNF-α) are elevated.[6]

The proliferative phase is a second phase of healing which is characterized by the formation of the callus and it begins with a continued vascular ingrowth, the secretion of the osteoid and resorption of necrotic bone which is carried out by the osteoclasts,[7] and it is also characterized by the formation of the connective tissue, angiogenesis and the soft callus which is then replaced by the immature woven bone that is formed via the intramembranous or the endochondral bone formation.[8]

The remodeling phase is the third phase characterized by the formation and the mineralization of the callus and replaced with a mineralized bone and return the bones to its original size and shape through the remodeling.[9]

Artichoke belongs to the family of Asteraceae, which represents an important part of the Mediterranean diet. It is considered as a rich source of a bioactive phenolic compound.[10] It had been used widely in medicine because of its health benefits which are due to its high content of phenolic compound and inulin.[11] The extracts from the artichoke had been used for the hepato-protection. The artichoke is a very rich source of the dietary anti-oxidants and therefore it can be used in many phytopharmaceutical applications.[12]

Materials and Methods

The artichoke plant material was taken and mixed with water. The mixture underwent heating process with a special vaporizing system. The vapor then passed through a cooling tubes for distillation process of the product. The essential oil product is then got separated from the mixture. The whole procedure was done in the chemical labs of the college of science.

Thirty male Albino rats weighing (300–400 g) were used in the study. They were put in the animal house in a separated cage for each one under optimum heat and feeding conditions with an ethical approval from the college of dentistry university of Baghdad. A bone defect with a 3 mm depth and 2 mm width was created in the right and left side of the femurs for each rat. The total bony defects (60) was divided into three period intervals 1, 2 and 5 weeks, each period interval included (10) control bone defects left to heal normally and (10) bone defects treated with (0.5 ml) artichoke oil.

The whole procedure was done under general anesthesia that was given intramuscularly using ketamin hydrochloride (1 ml/kg of body weight) plus xylazin. The access openings were made on the left and right side of the femur by a microengine using a round bur until a hole of about

(2 mm diameter and 3 mm depth),[13] then the bone defects were washed with normal saline to remove the bone fragments. In thirty rats, the left bone defects were left to heal normally, while the right bone defects were treated with 0.5 ml of artichoke oil.

Result

Clinical findings: there was no sign of any infection and all rats were healthy at the day of scarifying and at 5 weeks the experimental group showed higher stages of healing in the defect in comparison to the control group which showed incomplete healing.

Histological Result

In the first week, the histological study showed more trabecular bone formation in the experimental group than the control group and the woven bone was seen more clearly than the control group as shown in Figure 1.

In the second week, the histological findings showed early formation of blood vessels and more osteocytes in the experimental group than the control group as shown in Figure 2.

In the fifth week, we can see clearly a reversal line in the experimental group, more blood vessels and a difference in osteocytes number between control and experimental group Figure 3.

Statistical Result

Osteoclasts

As shown in Table 1 Artichoke oil group recorded a high significant difference at second and fifth week in reducing the number of osteoclast cells.

Osteoblast

Table 2 showing that an Artichoke group recorded a high significant difference at the first week, also recorded significant difference at the fifth week than control group.

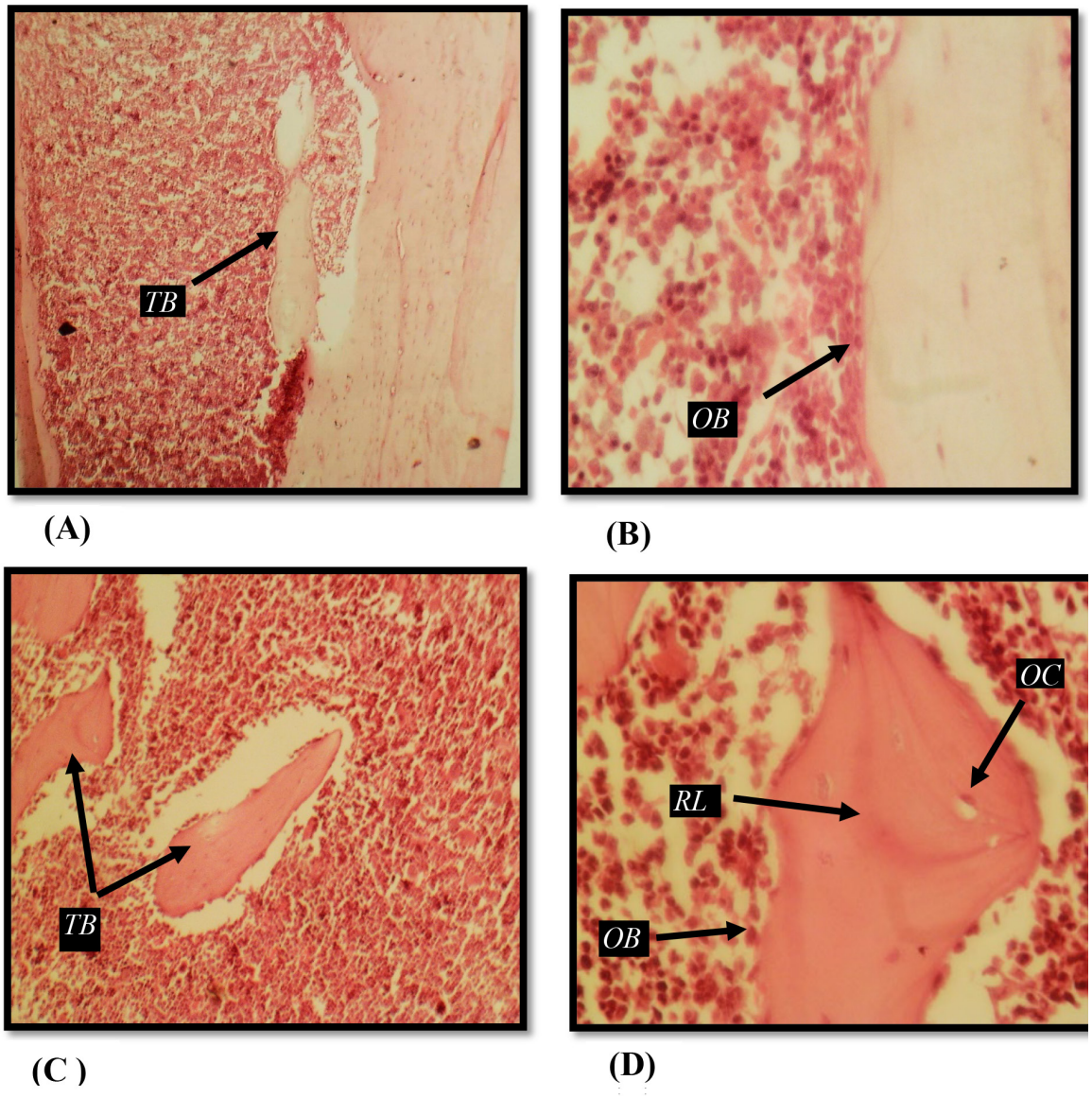

Fig. 1 **(A) Control group TB (trabecular bone) H&E X20. (B) Control group OB (osteoblast) H&E X40. (C): Experimental group showing TB (trabecular bone) H&E X20. (D) Experimental group showing RL (reversal line), OB (osteoblast), OC (osteocyte) H&E X40.**

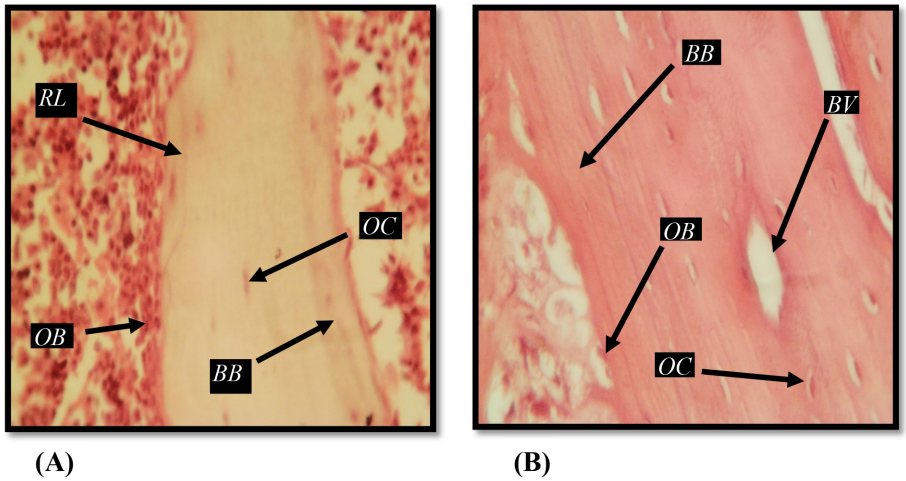

Fig. 2 **(A) Control group showing RL (reversal line), OC (osteocyte), BB (bundle bone), OB (osteoblast) H&E X20. (B) Experimental group showing BV (blood vessel), OC (osteocyte), BB (bundle bone), OB (osteoblast) H&E X20.**

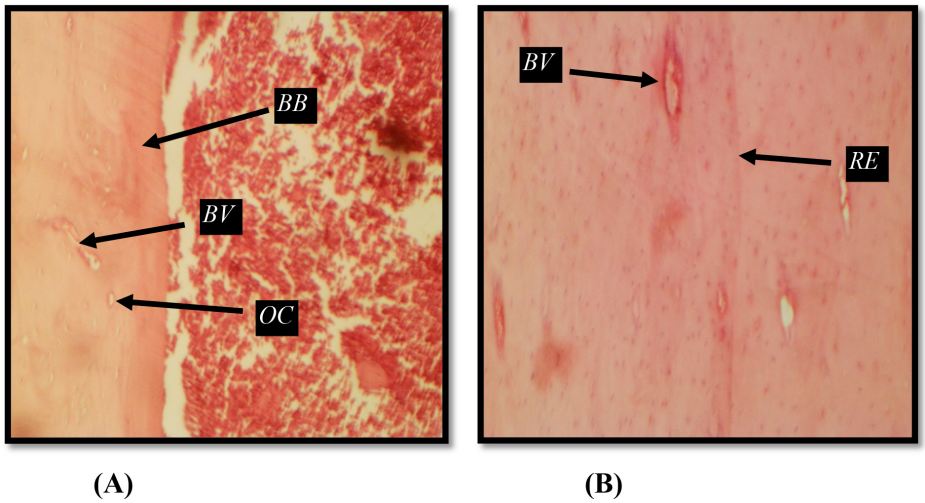

Fig. 3 **(A) Control group showing BB (bundle bone), BV (blood vessel), OC (osteocyte) H&E X20. (B) Experimental group showing RE (resting line), BV (blood vessel) H&E X20.**

Table 1. **Mean and *P*-value of osteoclast cells account**

Period	Group	No	Mean	*P*-value
1 week	Control Experimental	10 10	3.2 2.9	0.09
2 weeks	Control Experimental	10 10	2.8 1.7	0.00
5 weeks	Control Experimental	10 10	1.3 0.56	0.00

Table 2. **Mean and *P*-value of osteoblast cells account**

Period	Group	No	Mean	*P*-value
1 week	Control Experimental	10 10	25.1 45.8	0.00
2 weeks	Control Experimental	10 10	31.3 20.6	0.07
5 weeks	Control Experimental	10 10	23.43 12.95	0.03

Osteocyte

Table 3 reveled that there is a significant difference at the second and the fifth week in comparison with the control group in account of osteocyte cells.

Blood vessel

Table 4 showing that an Artichoke oil group recorded a significant difference at first week and a high significant difference at the fifth week than control group.

Trabecular Bone

As shown in Table 5 there is a significant difference at first and fifth week in experimental group in comparison with control group.

Discussion

Herbal therapy is still essential for about 75% to 80% of the world, especially in the developing countries. This is because herbal therapy is without any side effects, cheap and available.[14]

Table 3. **Mean and *P*-value of osteocyte cells account**

Period	Group	No.	Mean	*P*-value
1 week	Control	10	21.5	0.08
	Experimental	10	28.65	
2 weeks	Control	10	26.32	0.03
	Experimental	10	43.26	
5 weeks	Control	10	37.81	0.02
	Experimental	10	54.3	

Table 4. **Mean and *P*-value of blood vessel formation**

Period	Group	No.	Mean	*P*-value
1 week	Control	10	0.1	0.04
	Experimental	10	0.9	
2 weeks	Control	10	0.45	0.06
	Experimental	10	1.1	
5 weeks	Control	10	1.2	0.00
	Experimental	10	3.6	

Table 5. **Mean and *P*-value of trabecular bone formation**

Period	Group	No.	Mean	*P*-value
1 week	Control	10	12.15	0.02
	Experimental	10	18.33	
2 weeks	Control	10	8.9	0.09
	Experimental	10	10.64	
5 weeks	Control	10	6.3	0.04
	Experimental	10	1.9	

The Artichoke is a plant. Its leaves, roots and stem are usually used to make "extracts." "Extracts" contain high concentrations of some chemicals that are found in the plant naturally. The Artichoke leaf extract can be used alone or in combination with many other herbs. It contains little amount of fat and a higher levels of minerals like (sodium, potassium and phosphorus), fibres, vitamin C, polyphenols, inulin, hydroxycinnamates and flavones. Polyphenols and Inulin possesses anticarcinogenic, hepatoprotective and antioxidant activities.[15]

Artichoke leaf extract used in current study because it has an antioxidant that is accelerate healing by increased cell division,[16] also Artichoke oil has an anti-microbial effect due to phenolic contant,[17] anti-inflammatory effect that is important to prevent inflammation further more accelerate healing.[18]

The current study showed that blood vessel formation is more accelerated in the experimental group than the control group due to the stimulation and the improvement of endothelial cells growth by using artichoke oil[19] and the antioxidant effect due to the phenolic content.[17]

Present study revealed that the trabecular bone formation was more accelerated in artichoke oil group than the control group because of the artichoke oil anti-inflammatory effect due to its polyphenolic and the verbascoside compounds that had been reported in many reports to show a potential spectrum of several activities including anti-inflammatory and antioxidant effects.[20]

Osteoblast and osteocyte cells in the current study showed an early formation in the experimental group than the control group and this may be caused by the anti-inflammatory and antimicrobial effects of artichoke leaf extract[17] and also because of the mineral composition which include calcium, phosphorous and sodium that lead to early maturation and formation of osteocyte.[21]

Osteoclast cells had a shorter effect in the experimental group than the control group and this may be due to the cynarin content of artichoke leaf extract that has an immune modulator activity.[22]

Conclusion

Artichoke leaf extract oil, which is a cheap and easy to get product, can accelerates bone healing by enhancing the osteoblast formation, early blood vessel formation and can reduce the osteoclast effect.

Conflicts of Interest

None.

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