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Histological Effects of Adding Gold to (Eggshell and Seashell) Nanohydroxyapitite on Rabbits bone Healing

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الخلاصة

الاهداف :تهدف الدراسة الى تقييم التأثير النسيجي للذهب لوحده اوعند خلطه مع هيدروكسيباتيت النانوي المحضرمن (قشور البيض والصدف) على شفاء العظام في الفك السفلي للأرانب. **المواد وطرائق العمل**: تم تعيين اثني عشر أرنبا من الذكور لتشكيل اربعة مجموعات تجريبية وكل مجموعة تضم ثلاثة حيوانات، تم عمل أخدود صغير في عظمة الفك السفلي للأرانب. المجموعة الاولى :مجموعة التحكم)لم يتم ملء الأخدود بأي شئ) ،المجموعة الثانية: مجموعة الذهب لوحده (ملئ الأخدود بالذهب)، المجموعة الثالثة: مجموعة الذهب مع هايدوكسي اباتايت المحضر من قشور البيض (الأخدود ملئ بالهايدروكسي اباتايت المحضر من قشور البيض مع الذهب بتركيز ٥%) والمجموعة الرابعة: مجموعة الذهب مع الهايدروكسي اباتايت المحضر من الصدف (الأخدود ملئ بالهايدروكسي اباتايت المحضرمن الصدف مع الذهب بتركيز٥%). **النتائج**: أظهرت الدراسة النسيجية لإضافة الذهب مع الهايدروكسي اباتايت النانوي المحضر من(قشور البيض والصدف) أنه عند إضافة الذهب يزداد في التئام العظام مثل ملء الفراغ والعظام المشكَّلة حديثًا وملئ عدد كبير من الثغرات مقارنة مع مجموعات التحكم ، واضافة الى ذلك انه عند إضافة الذهب مع هيدروكسيباتيت النانوي المحضرمن (قشور البيض والصدف) أكثر تعزيزًا في التئام العظام مقارنةً بالذهب وحده. **الاستنتاجات**: توصلت هذه الدراسة إلى أن إضافة الذهب لوحده او عند اضافته مع الهيدروكسيباتيت النانوي المحضرمن (قشور البيض والصدف) قد زاد بنجاح من التئام العظم مقارنة بمجموعة التحكم.

ABSTRACT

Aims:To evaluate the histological effect of the gold alone and gold with nanohydroxyapitite(nHA) of (eggshell and seashell) on healing in the rabbit's mandibular bone . **Materials and Methods**: twelve male rabbit were assigned to form four experimental groups and three animals in each groups, small groove was done in the rabbits mandibular bone: Group 1: control groups (groove was not filled by anything), Group 2: gold alone (groove was filled by gold), Group 3: eggshell nHA with 5% from gold (groove was filled with eggshell nHA with gold 5%) and Group 4: seashell nHA with gold 5% from (groove was filled with seashell nHA with 5% from gold).**Results**: Histological study of adding the gold showed increasing in bone healing like space filling and newly formed bone trabeculi and highly number of lacuna compare with control groups while when adding the gold with nHA (eggshell and seashell) showed more enhancing in bone healing compare with control and gold alone. **Conclusion**: this study concluded that the addition of gold on nHA (eggshell and seashell) was successfully increasing in bone healing compare with control group.

Key words: Gold, Eggshell, Seashell, nanoparticles hydroxyapatite, Bone healing.

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INTRODUCTION

Metallic and gold compounds have long been used for medical uses . Gold, usually as the metal, is perhaps the most anciently administered medicine [1]

Gold nanoparticles have been in active use in the identification of chemical and biological agent, Gold has been exploited for its putative medical properties throughout the history of civilisation. [2] Gold has many application in Jewellery, Electronics ,Cuisine Miscellanea and medicine [3]

One of the new application of the gold is consider as a Methods to enhance fracture healing which is an important way to ensure the patients rapid recovery, as Autologous bone grafting, the current "gold-standard" in the enhancement of fracture repair, is costly, time-consuming, and associated with morbidity including pain, injury, hematoma, and fracture .[4] Hydroxyapatite (HA), is an important inorganic biomaterial consisting of calcium and phosphorous which are important minerals found in human bone [5]

Hydroxyapatite can either be synthesized from the natural organic based materials or inorganic components. The classical way of obtaining synthetic HA is working with expensive reagent chemicals, those synthetic routes are expensive and time consuming [6] Natural HA is believed to have better metabolic activity and more dynamic response to the environment than the synthetic [7] Seashell and the eggshell are mostly composed of calcium. Thus, they may be good candidates as sources of HA. One form of processed egg shell HA has shown promising results in bone regeneration. [8] Therefore the present study aimed to investigate effect of gold alone and with combination to natural prepared nanoparticles hydroxyapatite from egg shell and sea shell on bone healing in rabbits.

MATERIALS AND METHODS

Experimental Animals

twelve clinically healthy local male rabbits, 4-6 months-old, weighted (1-1.5Kg) were used in this study. A case sheet containing the rabbits' number, weight, dose of anesthesia, date of operation, and date of sacrifice was recorded for each rabbit . The study was carried at the chemistry and pharmacology laboratory of department of dental basic science.

Surgical Procedure:

General anesthesia was achieved using a mixture of an intramuscular injection of ketamine (40 mg/kg) and xylazine (8mg/kg) and waiting for a few minutes until the rabbit lose its consciousness. After the animals had been anesthetized within 5 minutes, A small incision (about 5 cm in length) was made in the skin over the submandibular area running with the lower border of the mandible starting from the symphysis area extended posteriorly along the inferior border of mandible, blunt dissection was reflected the periosteal and the mandibular bone was exposed **.** small groove (2) mm in

diameter and 6mm depth) was drilled in heavy duty dental engine of low speed hand piece by using 2.3mm round carbide bur. The bony defects were dried from blood with cotton pellets. The groove was filled by small spoon excavator and condensed using amalgam condenser The wound closed with 3.0 black silk suture using simple interrupted technique and rinsed with povidine iodine 4%, and aluminum numbering tag was placed in the rabbit ear for identification .

According to material filled in grooves animals were divided in the following groups:

1- **Group 1:**serve as control group consisted from 3 rabbits no treatment.
2- **Group 2:** serve as gold alone group consist from 3 rabbits. The animal were treated with gold nanoparticles
3- **Group 3**: Prepare eggshell nanoparticle HA with gold 5%. This group consisted from 3 rabbits.
4- **Group 4**: Prepare seashell nanoparticle HA with gold 5%. This group consisted from 3 rabbits.

All these rabbits were sacrificed after 30 days.

Histologic Procedures:

Sacrificed was performed after 30 days post operatively for each group successively. Bone formation was evaluated histologically by a histologist. The bony mandibles were dissected from the head and immediately immersed in formalin 10% solution for fixation.

For bone biopsies, mandible was harvested and put initially in formalin 10% for 2-3 days, then decalcified by keeping in 30% formic acid for 5-9 days, the acid was replenished every two days until the bone was become soft then washing by the water at minimum 24hr, then located in the Natural Buffered Formalin (NBF). dehydrated through a graded series of xylene and ethanol (70-99%), embedded in paraffin wax, sectioned by a microtome (4µm thick) and stained by hematoxylin and eosin. The regeneration process of a bone injury occurs by means of different steps, including the development of granulation tissue, combined with the differentiation of osteoprogenitor cells and osteoblasts, which are responsible for bone matrix deposition [(9).]

RESULTS

Histological examination was performed by three examiner in a blind manner . Histological examination exposed that all groups showed healing and osteogenesis but with variation it included description of specimens, evaluation of space filling, formation of connective tissue, bone cells, lamellar bone trabeculi and assessment of vascularity and blood vessels.

1-Control groups: At 30 days in the site of defect showed in complete filling of the space with newly bone formation, dense connective tissue and ossified callus containing lamellar bone trabeculi **(**Figure 1).

Figure (1): Light micrograph of rabbits mandibular bone from control group at 30 days showed newly bone formation, lamellar bone (⟶) trabeculi. Staining H&E . Magnification 4 X .

2-Gold alone: At 30 days the defect sites showed that the bone which is highly dense, thick lamellar and appear in continuity with the original bone compare with control groups, the size of osteoclast is bigger with few number of osteoblast cells, blood vessels and bone marrow compare with control groups. filling of space with maturation of bone newly formed bone trabeculi and highly number of lacuna **(**Figure 2,3).

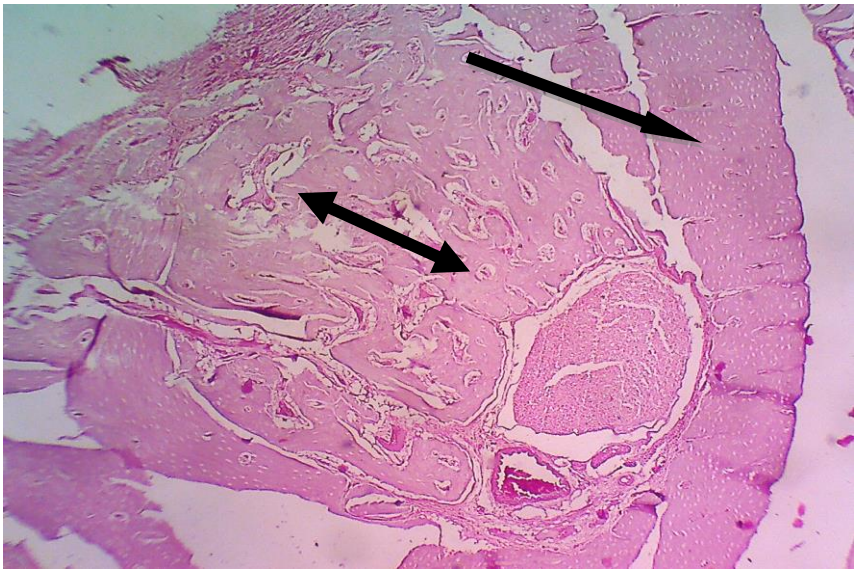

Figure (2): Light micrograph of rabbits mandibular bone from Gold alone group at 30 days showed that granulation tissue (⟷), osteocyte cells(⟶) and few number of osteoblast cells . Staining H&E . Magnification 4X

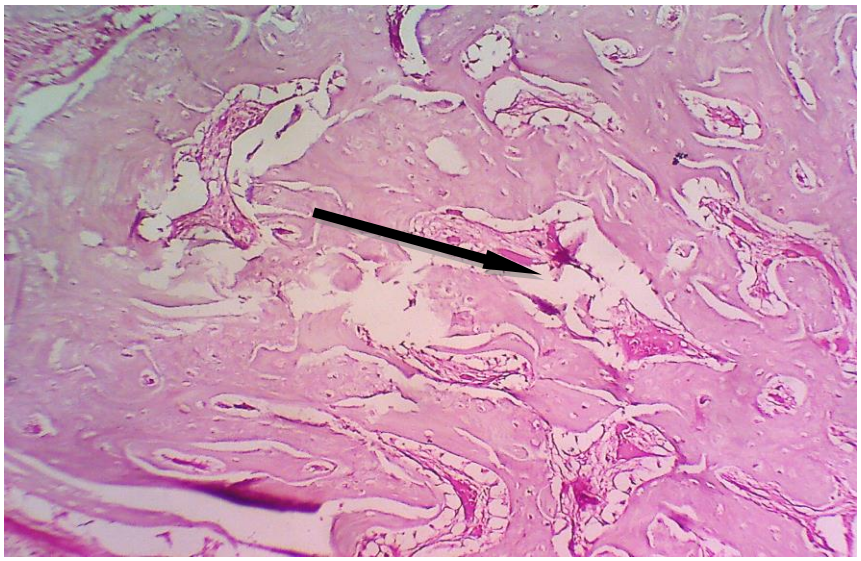

Figure (3) : Light micrograph of rabbits mandibular bone from Gold alone group at 30 days showed osteoclast cells in the remodeling phase (⟶) with new bone formation and maturation of bone. Staining H&E . Magnification 10X.

3-Eggshell nHA with gold 5%: The groups of Eggshell nHA in gold 5% showed increasing in healing and osteogenesis. the bone which is highly dense, the size of osteoclast is bigger compare with control and gold alone also few number of osteoblast cells ,few amount of granulation tissue, blood vessels and bone marrow . filling of space was highly in gold 5% with eggshell nHA compare with control and gold alone and showed more bone maturation filling of newly formed bone trabeculi and highly number of lacuna in comparison with control group and gold alone (Figure 4).

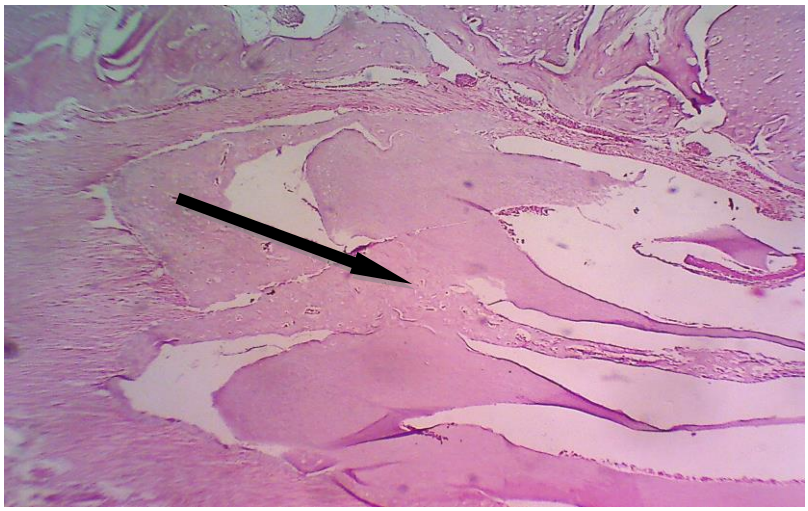

Figure (4): Light micrograph of rabbits mandibular bone of 5%Gold with Eggshell nHA group at 30 days showed filling of space, mature osteocyte cells (⟶) and less amount of granulation tissue. Staining H&E . Magnification 4X.

4-Seashell nHA with gold 5%: The groups of Seashell nHA with gold 5% showed increasing healing and osteogenesis. The bone which is highly dense, collagen fibers embedded with variable amount of osteoid mineralized osteon islets and gaps within areolar tissue , also showed few amount of granulation tissue, blood vessels and bone marrow compare with eggshell nHA with gold 5%. Moderate filling of space with bone maturation, border between the regeneration and original bone was not clear in comparison with eggshell nHA with gold 5% and gold alone. filling of space with maturation of bone. Newly formed bone trabeculi and highly number of lacuna compare with gold 5% to eggshell nHA and gold alone (Figure 5,6)

Figure (5): Light micrograph of rabbits mandibular bone of 5% Gold with Seashell nHA group at 30 days showed space filling with granulation tissue and new bone formation (⟶) Staining H&E . Magnification 10X.

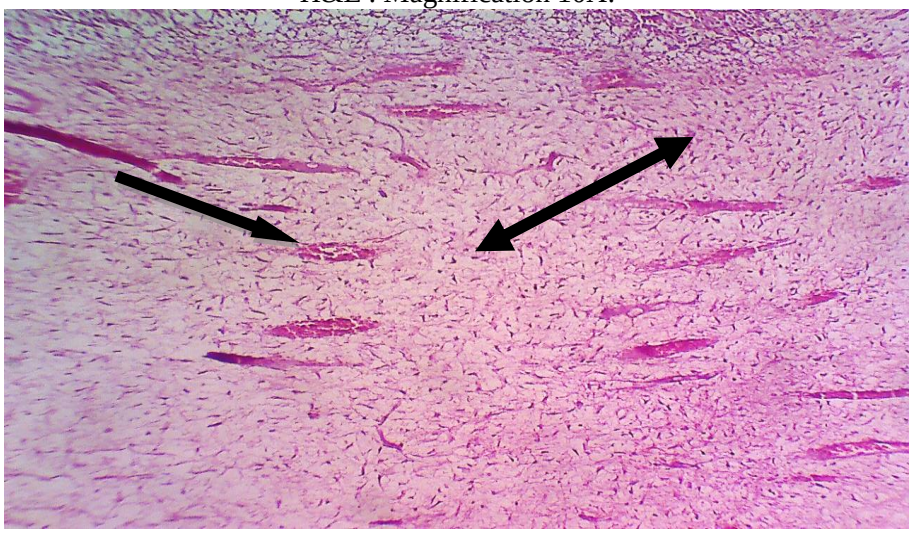

Figure (6): Light micrograph of rabbits mandibular bone of 5% Gold with Seashell nHA group at 30 days showed lacuna (⟷) and collagen fiber (⟶ with fibroblast cells to convert to bone tissue Staining H&E . Magnification 10X.

DISCUSSION

Histological examinations were performed to evaluate bone regeneration. The histological findings in the present study indicated that the defect areas of the experimental animals in all groups showed various amounts of new bone formation by gold this result may be explained by opinion mentioned by [10] that the scaffolds from the gold may represent an innovative paradigm in bone tissue engineering by inducing osteogenesis as a means of remodeling and healing bone defects). The histological finding of gold groups at 30 days post-operation filled the bone defect compared to the control groups, nHA increasing bone formation, this result may be explained by opinion mentioned by [11] that the process of bone formation onto the bone surface using a hydroxyapatite/collagen bone. Egg shell nHA forming a new bone formation this result may be related to the egg shell which is accelerated the bone formation this result may be explained by opinion mentioned by [12] that the eggshell-based graft powder, , is a biocompatible material which has the potential to enhance new bone formation. The egg shell HA which used calcium phosphate in both rabbits and humans this result may be explained by opinion mentioned by [13] that the eggshell-derived multiphasic calcium phosphate scaffold displayed improvement in the mechanical properties with higher porosity and osteoinductivity in bone-graft application. Sea shell nHA also enhanced bone formation and this result may be explained by opinion mentioned by [8] that the seashell HA has ideal pore size and properties supporting bone tissue growth and cell proliferation. Therefore, it can be a good candidate for clinical applications owing to low production cost and natural–biological origin.
The histological finding of the gold with egg shell nHA in treated groups at 30 days post-operation filled the bone defect and well fitted to the bone cut ends, compared to the gold alone and control group.

Results from histological examination of inflammatory cell infiltrate at the site of the bony defect were found only on the control group while there is no infiltration of inflammatory cells in all groups of gold and gold with HA. The inability to detect inflammatory cells in the HA group could be due to the anti-inflammatory effect of the material used, thus it didn't evoke any unexpected inflammatory response and consequently, no excess inflammatory cells accumulated at the site of the operation. this result may be explained by opinions mentioned by [14] that the HA can stimulate release of anti-inflammatory cytokines that indicates the presence of potential anti-inflammatory properties, also showed the anti-inflammatory effect of the gold, This result may be explained by opinion mentioned by [15] that the gold nanoparticles has the anti-inflammatory, analgesic and anti-tumor effects .

However, histological finding that osteoid tissue forming in seashell nHA with gold 5%. Such a trend seems to increase with follow up time post-surgery. These results actually showed that the healing process in the groups is faster in gold with seashell , gold with egg shell nHA and gold alone respectively with greater amount of bone formation compared to the control group.

CONCLUSION

The present study were conclude that the gold increases bone healing and increases more when it is added to the nanohydroxyapatite from eggshell and seashell.

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