How to Cite:
Malik, Z. J., & Eesa, M. J. (2022). Effect of magnesium oxide nanoparticles locally or systemic with hydroxyapatite on regeneration of radial fracture in rabbits: Radiological study. *International Journal of Health Sciences*, *6*(S1), 10273–10282. https://doi.org/10.53730/ijhs.v6nS1.7425

Effect of magnesium oxide nanoparticles locally or systemic with hydroxyapatite on regeneration of radial fracture in rabbits: radiological study

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***Abstract*---**Study aimed to develop and test effect of magnesium oxide nanoparticles (MgONPs) and hydroxyapatite (HA) with gel on repair of distal radius transverse fractures. MgONPs concentrations 200 µg /ml were dissolved in 1 ml distilled water, stirred for 10 minutes, 0.5 mg HA in 1 ml gel, shaking for 15 minutes. These materials were tested in vitro. Forty adult male rabbits, at 1.5 to 2 years old and weighing 1.7-2.3 kg. Rabbits were divided into 2 groups: Rabbits were given intramuscularly 40 mg/kg B.W ketamine hydrochloride, 5 mg/ kg B.W xylazine to anesthetic before operations. A 5 cm incision was made in the skin of forelimb cranio-medially, transverse fracture was induced. MgONPs/HA/gel applied locally group (A). While HA/gel was applied locally, MgONPs were intraperitoneally injected group (B). Clinical examination revealed excellent apatite with minor swelling at the fracture site. At 2nd, 4th , 6th , and 8th weeks, Radiographic evaluation revealed group A had periosteal reaction within fracture site, at 2nd week post-operatively, group B had active periosteal reaction at fracture site. At 4th week, callus bridge connected two ends of fracture in group A. At same time, a visible callus crossed two ends of fracture in group B. After 6th week, fracture site of group A was completely connected, while in group B, fracture was connected and beginning of remodeling. Fracture site in group A had remodeling through 8th week, while fracture site in group B was semi-normal in shape. Study concluded with better results in osteoinductive, and osteoconductive.

***Keywords*---**Fracture, MgO NPs, HA, Rabbit.

Introduction

Fractures of the forelimb are the most common site in animals (28.68%) (1). Fractures of both the radius and ulna have a higher risk of late union or sometimes nonunion. Radial and ulnar fractures are most common in the distal part of the radial region (2). Long bones, such as the radius and tibia, are known to have surgical problems, leading researchers to experiment with a variety of special materials (3). Magnesium oxide nanoparticles (MgONPs) have a major role in antimicrobial effects, proliferation in osteoblast cells, and osteoblast cell adhesion. MgONPs have been shown to suppress gram-negative, gram-positive, and spore-formation, leading to faster healing and less pathology at the surgical site (4, 5). It's been proven that osteoinductive and osteoconductive. As a result, hydroxyapatite (HA) is an excellent choice when compared with MgO in bone applications (5). Using gel compositions, Carbopol 934 had good stability, homogeneity, and an anti-inflammatory effect, as well as a good dispersion (6).

Materials and Methods

Experimental Animals

Forty normal, healthy male adult rabbits, ranging in age from 1.5 to 2 years and weighing 1.7 to 2.3 kg. They were placed in special kennels. The study was approved by the University of Baghdad/Veterinary Medicine College's ethics committee. Rabbits were supplied with pellets and green grass for two weeks before surgery, and all animals were given ivermectin as an anti-helminthic (alfamec-Holland). The dose of MgONPs was 200 µg /ml chosen after the study was conducted, which was mixed in 1 ml of distilled water and stirred for 10 minutes to ensure that the mixture was well diluted. On the other hand, MgONPs are safe when administered intraperitoneally at a concentration of 200 µg/ ml (7). Hydroxyapatite was 0.5 mg/1 ml of gel, stirred at the vortex for 15 minutes to ensure a well-mixed. (8).

Experimental Design: The experimental rabbits were randomly separated into two groups (20 animals for each) as follows: Group A (MgO NPs locally group): Rabbits in this group had a full transverse fracture at the distal third of their radius (right forelimb). Locally, a mixture of HA (Avonchem, UK)/gel and MgONPs was treated. Group B (MgO NPs systemic group): Rabbits in this group were given a mixture of hydroxyapatite with gel locally at the fracture site and MgO NPs "suspension" which was injected intraperitoneally.

Biosynthesis of MgO using Aloe vera extract: According to the approach described by (9), Aloe vera is used as a reducing agent in the biosynthesis of MgO.

Characterization of Magnesium oxide nanoparticles: X-ray diffraction (XRD) analysis: The solution of magnesium oxide nanoparticles was centrifuged for 30 minutes at 10,000 rpm. The solid residues of MgONPs were rinsed two times with deionized distilled water and dried at 80 °C. To obtain MgONPs powder, it must be measured by X-ray powder Diffraction (XRD). The XRD patterns were acquired using a system (Shimadzu XRD-6000) and set at 30 mA and 40 kV with copper radiation (1.5406). The XRD used a copper tube radiation line at a wavelength of 1.54o A in 2è range from 10^{o} to 80^{o}) (10). The grain size was determined from the

width of the XRD patterns, and the calculation of the crystallite size was according to "Scherer's equation" (11):

D =, k λ/, β cosθ..... (1) Where:-

D: the crystallite size (nanometer; nm), K: 0.9 (the Scherer's constant), λ: X-ray wavelength (1.54060 Å), β: Full Width at Half Maximum (FWHM) of the diffraction line, and θ: the Bragg diffraction angle of the XRD peak.

Field Emission Scanning Electron Microscope (FESEM) analysis: Field emission scanning of electron microscopy (JEOL JSM 6390, USA Inc.) was used to analyze the size and morphology of magnesium oxide nanoparticles (11).

Gel preparation: Carbopol concentration (0.125 g) was disseminated in distilled water at 6.25 w/w, spinning at 800 rpm for 60 minutes, and then corrected with NaOH 10% dropwise (neutralization agent). To avoid contamination, methanol dropwise was used. The chemical substances were mixed until a translucent gel formed and an estimated pH of neutral (12).

Characterization of Gel:

Spreadability: In an in vitro experiment, the spreading width of 1 g of gel was determined after one minute; the gel was diluted five times and spread on wax paper with a spreader to the spreading level by adding one drop of eosin stain to the gel.

Surgical operation: From the mid-shift of the humerus to the mid-shift of the metacarpus bones, the operating area was aseptically prepared for surgery. The experimental animals were given intramuscularly 5 mg/ kg xylazine and 40 mg/ kg B.W ketamine hydrochloride. A *cranio-medial* incision (5 cm) was created in the skin of the forelimb. The radius and ulna were exposed after dissecting between *extensor carpi radialis longus* and *flexor carpi radialis* (Figure- 1.A). The distal radius was fractured using osteotomies (Figure- 1.B). A mixture of HA and MgONPs gel was applied to the fracture site in the group (A), while HA and gel were applied locally and MgONPs were intraperitoneally administered in the group (B) (Figure- 1.C and D). The muscle, subcutaneous tissue, and the skin were closed in distinct layers with polyglactin 910 (3-0) sutures before the fracture was externally fixed with a modified aluminum plate (8). After surgery, patients received daily wound treatment and dressing, as well as a 5-day course of penicillin/streptomycin injections at 10,000 IU and 10 mg IM, respectively (13).

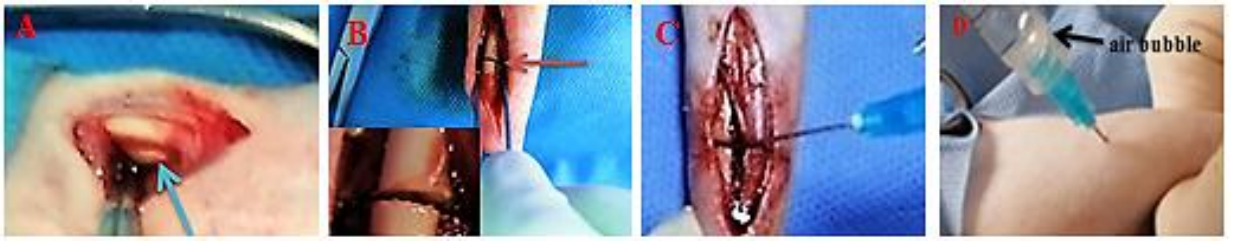


Figure- 1: (A) Expose the radius and ulna. (B) The distal radius was fractured using osteotomies. (C and D) HA and gel MgONPs gel was applied to the fracture site in group (A), while HA and gel were applied locally and MgONPs were intraperitoneally administered in group B.

Radiographic Evaluations: Radiography was done on two different views, anteroposterior and mediolateral. At the 2nd, 4th, 6th, and 8th weeks, the animals

were examined with an X-ray machine (ENIE Radiologie®). The X-ray machine was set to 70 kV and 8 mA.s to look at periosteal reactivity, new bone formation, and bridging.

Results

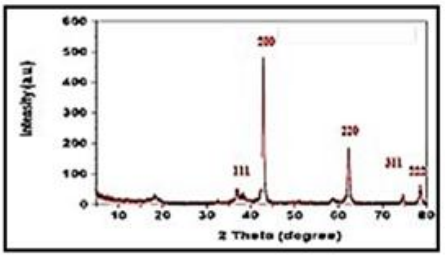
Laboratory Evaluation of Magnesium Oxide Nanoparticle

X- ray Diffraction (XRD): The samples ranged from 10^0 to 80^0 in the theta (20) range, with intensity peaks ranging from 0 to 600^0. There are no meaningful peaks from undesirable components. In the X-ray diffraction pattern of MgO nanoparticles produced by the biosynthetic technique, the higher diffraction peaks indicated smaller particle sizes. Peaks were identified at values (111), (200), (220), (311), and (222) that correlate to reflections with 2 degrees of the Bragg angles at 36.93°, 42.93°, 62.27°, 74.77°, and 78.69°, respectively (Figure- 2).

Scanning Electron Microscope (SEM): It was used to examine the appearance and content of biologically produced MgONPs with alovera extract. The field emission scanning electron microscopy view of MgONPs in (Figure- 3 A, B) revealed a flake-like structure due to the presence of thousands of MgONPs.

Laboratory Evaluation of hydrogel

Physical appearance and spreadability: The spreadability of the gel was estimated by measuring the distributing diameter of 1 g of gel after one minute at 24 hours after dissolving (Figure- 4).



igure- 2: The XRD pattern of the esultant MgO NPs showed 5 strong eaks. In the full spectrum of 2 degrees extending from 10° to 80°.

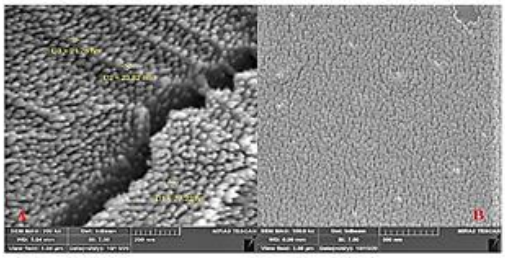


Figure- 3: SEM Microphotograph: A at 200nm and B at 500nm

:igure- 4: The spreadability of
ɟel at six dilutions. refers
o the dilution we'll be using in
our experiment.

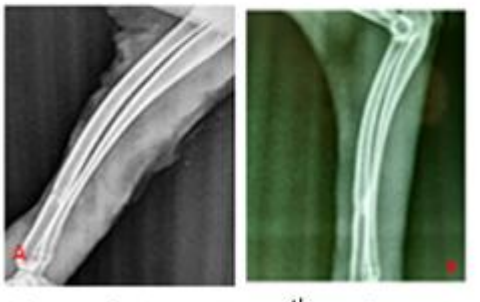
Clinical Evaluation: All experimental animals' operating sites showed no infection, swelling, or abscess after surgery. However, animals in all groups had a normal appetite for the first 2 to 4 hours after surgery.

Radiological Evaluation: To assess the degree of fracture healing, radiographs of the fracture sites of two groups were performed at the 2nd, 4th, 6th, and 8th weeks after the operation. Group A showed a clear periosteal reaction within the fracture line (Figure- 5), whereas in the 2nd week after surgery, group B had a little clearer periosteal reaction within the fracture line (Figure- 6). In the 4th week, radiological signals in group A show callus bridges at the fracture line (Figure- 7). At the same time, callus formed on both sides of the fracture in group B, connecting the two fractured ends (Figure- 8). After the 6th week after the operation, group A had completely reconnected the fracture site with the fracture remodeling (Figure- 9). However, in group B, the fracture location was linked to the remodeling of the fracture at the same time (Figure- 10). The fracture site in group A begins to remodel in the 8th week (Figure- 11). At the same time, remodeling occurs in group B, and the bone returns to its normal shape (Figure- 12).

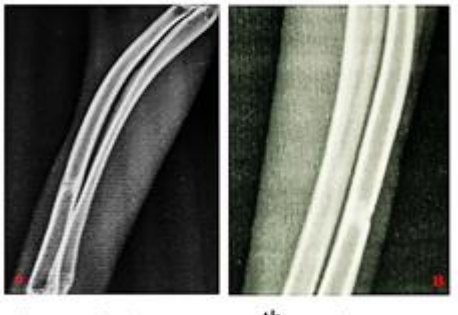
Figure, 5: Group A at second week p.o, Mediolateral B: anterioposterior views visible fracture line with minimal periosteal reaction within fracture site

Figure, 6: Group B at second week po .: Mediolateral B: at anterioposterior iews show periosteal reaction at the fracture site

Figure, 7: Group A at 4th week po, A: Mediolateral B: anterioposterior views ppear with callus connected the acture site and end of it, visible :allus bridge connected two ends of the fracture site

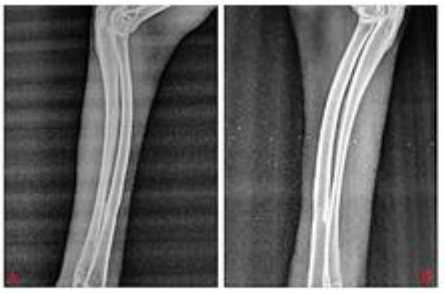


igure, 8: Group B at 4th week po, :Mediolateral B: at anterioposterior iews appear callus bridge connected he fracture site, callus cross the two ends of the fracture site. .

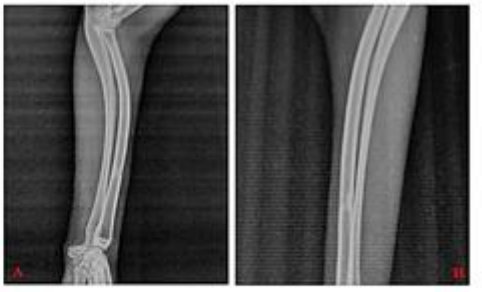


Figure, 9: Group A at 6th week po, A: ediolateral B: at anterio-posterior ews appear connected the cortex acture site, remodeling of the acture with remodeling and bone taken semi normal in shape .

Figure, 10: Group B at the 6th week po, at two views appear complete connected ie fracture site with remodeling the fracture site.



igure, 11: Group A at the 8th week o, at two views (A and B) appear isible remodeling of the fracture site



igure, 12: Group at the 8th week o, at two views (A and B) appear the bone taken normal shape.

Laboratory Evaluation of Magnesium Oxide Nanoparticle

Discussions

X- ray Diffraction (XRD): MgONPs resulted excellent purity; the high peak broadening indicates that the MgONPs have very tiny particle sizes. These result findings confirmed that the substance examined was high-purity magnesium oxide nanoparticles. These facts are in line with (14).
Scanning Electron Microscope (SEM): These findings have been accepted by one study (11) from morphology and structure.

Laboratory Evaluation of hydrogel
Physical appearance and spreadability: The experiment required firm substance because osteoblast cells need more hardness. However, it was not firm enough to allow HA/ MgONPs to reach the fracture site and function as an extracellular matrix to stimulate cell growth and tissue repair, as described by (15).
Clinical Evaluation: All experimental animals' showed this results, which could be because MgONPs have an analgesic, sedative, and anti-inflammatory impact, all of which correspond with the findings of (16). According to scientists who examined MgONPs, they were discovered to reduce pain and anxiety in animals, MgONPs' impact on hormonal pathways and glutamate, and neurotransmitters like Gamma Amino Butyric acid (GABA), Magnesium affects glutamatergic neurotransmission modulation and reduces the effect of glutamate as an excitatory amino acid in anxiety-like activity (17). Inorganic antibacterial agents have been studied using metal oxide nanoparticles like MgO, ZnO, and CaO (18).
Radiological Evaluation: In the current study, a transverse fracture was generated in the rabbit model to evaluate repair utilizing MgONPs with HA mixed with gel to improve fracture healing. Many studies have suggested that nonunion of radius-ulna fractures is caused by instability and mini-movement at the fracture region, as well as diminished vascularity (19). The results of the present study of two groups demonstrated that using MgONPs and HA improves fracture healing by forming a callus that connects the two fracture edges at the 4th week after surgery. According to the pervious report, Mg is particularly important for bone formation and meniralization, it increases the activity of osteoblasts and phosphatase enzymes, both of which are important in bone formation and development (20). However, this is excellent regeneration since MgONPs combined with HA promote mineral

metabolism, cell adhesion, and crystallization processes, which agrees with (21). MgONPs considered angiogenesis, osteogenesis, and precipitated calcium in bone as well as hydroxyapatite like an osteoinduction factor. According to the findings of (22) the combination of the two resulted in improved bone repair, they noted that doping Mg with hydroxyapatite improved bone regeneration. This evidence is in agreement with (23) that states that high biocompatibility and hydrophilicity when mixed with HA provided a cohesive, adhesive, and flexible network for the distribution of MgONPs that were both osteoinductive and osteoconductive, and HA was added to extend the time of stability.

Conclusion

MgONPs are easily prepared from Alovera at a low cost, and gel from carpobol is likewise simple to make at an economically cost, and easy to apply to the fracture site. The combination of MgONPs and HA with gel produced better results in the osteoinductive, osteogenesis, and osteoconductive activity over a longer period of time.

Acknowledgments

I'd like to thank the College of Science at the University of Baghdad in Iraq for their assistance of this project, especially Dr. Laith Ahmad Yaaqoob, and Dr. Mohanad A. Al- Bayati at the pharmacology department of the University of Baghdad/ College of Veterinary Medicine.

تأثير جزيئات اوكسيد المغنيسيوم النانوية موضعيا وجهازيا مع الهيدروكسيابيتيد على اصلاح كسر عظم الكعبرة في الارانب: دراسة شعاعية

الخلاصة

) وماده الجل كما تقييم ماده الهيدروكسي ابيتيد MgONPS هدفت هذه الدراسة تصنيع وتقييم جزيئات اوكسيد المغنيسيوم النانوية (واعطائهم مخلوطين موضعيا على موضع الكسر(مجموعه ا) كما اعطاء الجل مع الهيدروكسي ابيتيد موضعيا و جزيئات اوكسيد المغنيسيوم النانوية في تحويف البريتون (مجموعه ب) للمقارنة بين تأثير كلتا الطريقتين على شفاء كسر الكعبرة المستعرض. تركيز جزيئات اوكسيد المغنيسيوم النانوية 200 مايكروكرام/مل مذاب في 1 سيزي ماء مقطر اما الهيدروكسي ابيتيد بتركيز 0.5ملغم لكل 1 مل من الجل. حيث تلك المواد قيمت مختبريا. بعد ذلك تم استخدام 40 ارنب ذكر بالغ باعمار 1.5- 2 سنه وباوزان تتراوح بين 1.7- 2.3كغم. حيث قسمت الحيوانات الى مجموعتين متساويتين حيث خدر الحيوان باعطاءه 40ملغم لكل كغم من الكيتامين هيدروكلورايد يسبقه 5 ملغم لكل كلغم من الزايلازين ثم تم عمل شق بحدود 5 سم بجلد المنطقه الانسيه الاماميه لليد اليمنى ليتم فيما بعد تقييم المجموعيتين سرائريا حيث اظهرت تلك النتائج شهية ممتازه للمجموعتين وتورم قليل في موضع الكسر في كلتا المجموعتين كما اظهرت الدراسة الشعاعية التي اجريت في الاسبوع الثاني, الرابع, السادس و الثامن على التوالي اسبقية مجموعه (ب) في ظهور علامات الشفاء من وجود تلاحم سمحاقي. مميز مقارنة بمجموعة ا. في الاسبوع الرابع نلاحظ جسور عظمية تربط نهايتي الكسر في المجموعة ا كما تتخلل موقع الكسر في المجموعة ب اما الاسبوع السادس يظهر التحام كلي وشبه عوده العظم الى الطبيعي خاصة المجموعة ب و في الاسبوع الثامن نلاحظ اختفاء تام للكسر ليظهر العظم شبه طبيعي خاصة المجموعه ب.

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