INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

CLINICAL AND RADIOLOGICAL STUDY OF THE EFFECTS OF NANO ZINC OXIDE ON FRACTURE HEALING IN RABBITS

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ABSTRACT
The aim of this study was to evaluate the effects of Nano Zinc oxide on fracture healing. Twelve healthy adult rabbits were used. The animals were divided into two equal groups (control and treated groups). Rabbits were anesthetized by receiving acepromazine as premedication at dose rate of 0.1mg/kg B.W intramuscular and 10 min. later (xylazine hydrochloride 6mg/kg and ketamine hydrochloride 35 mg/kg) was injected intramuscularly. In the control group a transverse mid-shaft femoral fracture was done, and fixed by intramedullary pinning, while in the treated group, same as in the control group, but after fixation liquid solution of Nano Zinc oxide was applied on fracture site. The results of radiological examinations at the end of 2nd, 4th, and 8th weeks post-operation revealed that, periosteal reaction at the end of 2ed week, invisible of fracture line at the ends of 4th weeks, and incorporation of external callus with cortical bone at the end of 8th weeks in treated group compared with control group.

KEYWORDS: Fracture, Bone, Nano Zinc oxide.

INTRODUCTION
A fracture is a medical condition in which there is a break in the continuity of the bone[1]. Bone fracture repair is a complex process of bone regeneration, that is, reconstruction of bone defects and nonunion, including the structural and functional reconstruction of bones[2,3]. Besides conventional fixation, bone grafts combined with appropriate physical therapy, like electrical stimulation, ultrasound, and gradient low oxygen environment, has been studied for bone repair[4]. Materials used for bone fracture healing include autografts, allografts, xenografts, and synthetic bone materials[5].
Nanomaterials were defined as having at least one dimension between 1 and 100 nm, they present unique physical and/or chemical properties that were different from conventional materials and have emerged as promising breakthroughs to bone fracture repair [4, 6]. A novel application of nanotechnology to achieve this goal is the application of specific nanoscale surfaces to produce specific cellular responses[7]**.** Zinc oxide nanoparticles (nano-ZnOs) are widely used and possess great potentials in agriculture and biomedicine[8]. Nano-ZnO show widely application in orthopaedics, were demonstrated that the ZnO nanoparticles can enhance the proliferation, adhesion, and differentiation of osteoblasts by affecting on factors that facilitate the recruitment of mesenchymal stem cells which then start differentiating into specialized cells to build new bone tissue [9,10]. The aim of the experimental study was to evaluate the effects of Nano ZnO on fracture healing.

MATERIALS & METHODS
Twelve clinically healthy adult male local breed rabbits were used; their weights between 1.5-2 Kg. Rabbits were housed in a special housing room belonging to College of Veterinary Medicine/University of Baghdad. They received antiparastic drug (ivermectin 1mg/kg) for prevention and treatment from most internal and external parasite. The rabbits were anesthetized by receiving acepromazine as premedication at dose rate of 0.1mg/kg BW intramuscular [11] and 10 min. later (ketamine hydrochloride 35 mg/kg and xylazine hydrochloride 6mg/kg) was injected intramuscularly. The experimental animals were randomly and equally divided into two groups: In the control group, under aseptic technique, skin incisions extending from the major trochanter to the lateral condyle of the femur were induced. Thereafter, the fascia was incised and underlying muscles were separated bluntly between biceps femoris and quadriceps femoris muscles, allowing the femur to be reached (Figure 1). A transversal osteotomy was done in the mid shaft of the femoral diaphysis used a Gigli saw (Figure, 2), and the fracture was fixed by Steinman intramedullary pin (ø=2.5 mm), which was inserted by retrograde method (Figure, 3). The same procedure was used in the treatment group but after fixation of fracture added liquid solution nano-zinc oxide (*Sigma*-Aldrich, U.S.A) in fracture site in a dose 5 µg (0.5ml) and concentration 10 % (Figure, 4). After this, muscles and fascialata were sutured by simple continuous pattern using 3/0 absorbable polyglactine suture. The skin was closed with simple interrupted pattern using 2/0 non absorbable silk suture. Animals were observed for detection of clinical signs which include, swelling, redness of the fracture site, unusual movement, dehiscence of wound and stitch abscesses up to 14 days post operation. Radiographic examination of the fracture site was done for all animals at (2nd, 4th and 8th weeks) post operation. The radiographic imaging was done after giving light anesthesia with a Medio-lateral view. Post-operative

care, skin incision was daily dressed, and skin stitches were removed after 7 -10 days post operation.

FIGURE 1: Shows the mid shaft of femur exposed, after curved forceps introduced beneath it.

FIGURE 2: Shows Gigli wire saw was introduce to induced a transverse fracture of the mid-shaft of femoral bone.

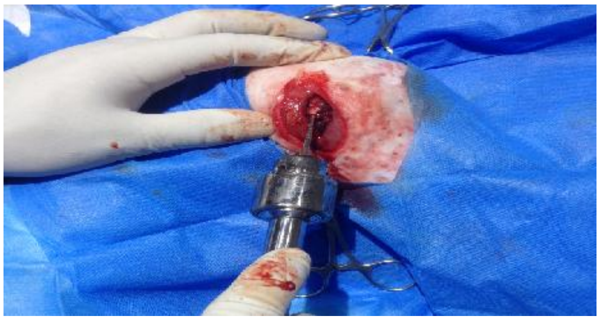

FIGURE 3: Shows intramedullary pin inserted into the proximal part of fractured bone.

FIGURE 4: Shows approximate of fractured ends, liquid solution of Nano-ZnO infusion at the fracture site.

RESULTS & DISCUSSION

Clinical observations of both groups revealed that there is swelling at the fracture site, red in color, inability to bear of his weight on fractured limb, and limited movement. These signs gradually disappeared after 5 to 7 post-operative days, in which, its disappeared in the treated group before control group. The swelling of surgical site might be due to, there is oozing of fluid from blood vessels at the surgical site as results of increase permeability of blood vessels and escape of fluid outside of this vessels causing edema and swelling of area with pain. This pain might be due to pressure on nerve ending. These observation were coincided with others, whom explained that, fractures, at any site, were characterized by pain, swelling and loss of function, in some cases it may be possible to hear or feel the broken bone ends moving against each other (crepitus), deformity due to muscle spasm, displacement at the fracture site and other sings that result from damage to adjacent tissue [12].

Radiological examination, at the end of 2nd weeks post operation, showed no evidence of periosteal reaction around the fracture site as well as a clear fracture line in control group, (Figure, 5), while in the treated group, during the same period there is evidence of periosteal reaction around fracture site, and visible fracture line (Figure, 6). In the control group at 4th weeks post-operation, there is periosteal reaction around the fracture site, but the external callus shows no complete bridge fracture line (Figure, 7). In the treated group the radiograph shows increased external callus formation in the bridge fracture line (Figure, 8). At the end of 8th weeks post- operative, the external callus, bridge fracture site, invisible fracture line are seen in the control group, (Figure, 9), while in treated group, the radiographic show, limitation of the callus formation around the fracture site, with the signs of remodeling through incorporation of the callus with the cortical bone (Figure, 10).

The radiographic findings of two groups revealed that, the periosteal reaction around the fracture site at two weeks post-operative, were superior in the treated group compared with the control group. This fact might be due to the action of Nano zinc oxide to enhance the proliferation of osteoblasts for callus formation. These events are accordant with other researcher, studying on Nano zinc oxide, in orthopedic surgery, bone replacement, bone regeneration scaffolds, bone grafts and promote healing [10]**.** Also Nano zinc oxide (ZnO) can induce osteogenic properties from stem cells[13]. The mechanism of action of nano-zinc oxide to promoting of osteoblast proliferation and differentiation occurs due to enhanceing collagen synthesis, alkaline phosphatase activity and calcium mineral deposition by osteoblasts[14]. This occurs due to enhanced the expression of growth factors, like fibroblast growth factor (FGF) and VEGF, IGF-I which, in turn, promoted cell proliferation and angiogenesis[9] .

The healing processes were more advanced radiographically at 4 and 8 weeks post-operative in treated group than control group, in which represented by callus bridge the fractured site, invisible fracture line and incorporation of external callus with cortical original bone. This phenomena consisted by other workers, explaining that zinc metallic could have significant impact on the healing of bone[15].

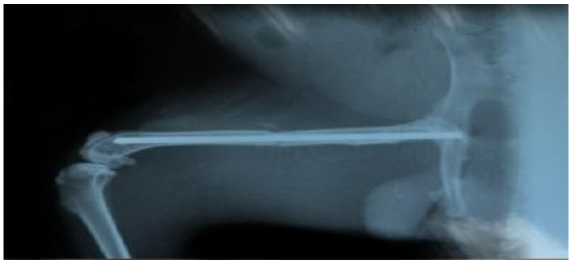

FIGURE 5: Radiograph, shows no evidence of periosteal reaction around the fracture site, clear fracture line, at the end of 2nd weeks post-operative, in control group.

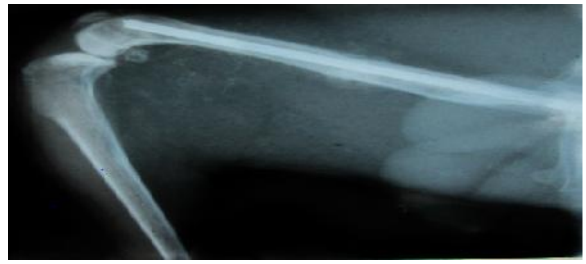

FIGURE 6: Shows, evidence of periosteal reaction around fracture site, visible fracture line, at the end of ^{2}nd week post -operative in treated group.

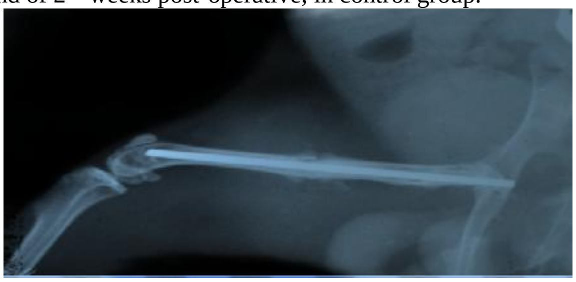

FIGURE 7: Shows periosteal reaction around the fracture site, but the signs of bony callus bright formation and not complete, at the end of ^{4}th weeks post-operative, in control group.

FIGURE 8: Show increased external callus formation, around the fracture and bridge fracture site, invisible fracture line, at the end of ^{4}th week's post-operative, in treated group.

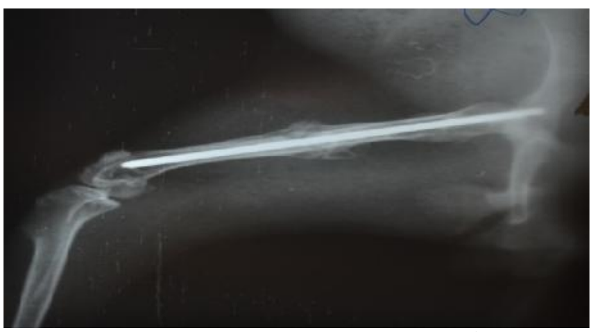

FIGURE 9: Shows the external callus, bridge fracture site, invisible fracture line, at the end of 8th week's post-operative, in control group.

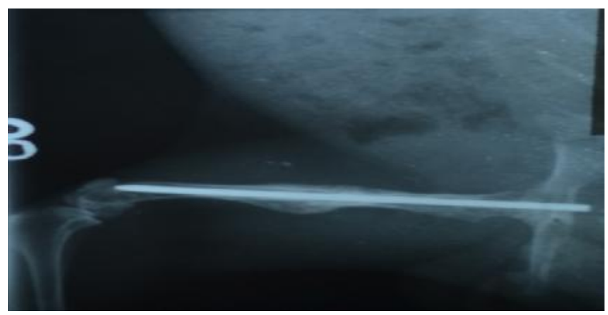

FIGURE 10: Shows, limitation of the callus formation around the fracture site, with the signs of remodeling through incorporation of the callus with the cortical bone, at the end of 8th weeks post-operative in treated group.

REFERENCES

[1]. Witmer, D.S., Marshall, S.T. and Browner, B.D. (2016) "Emergency Care of Musculoskeletal Injuries". Chapter 18. In: *Sabiston Textbook of Surgery E-Book*, 20th Ed. By: Townsend, C.M., Beauchamp, R.D., Evers, B.M. and Mattox, K.L. Elsevier Health Sciences: Pp. 462–504.

[2]. Perán, M., García, M.A., López-Ruiz, E., Bustamante, M., Jiménez, G., Madeddu, R., and Marchal, J.A. (2012) Functionalized Nanostructures with Application in Regenerative Medicine. Int. J. Mol. Sci., 13(3): 3847-3886.

[3]. Tautzenberger, A., Kovtun, A. and Ignatius, A. (2012) Nanoparticles and their potential for application in bone. Int. J. Nanomedicine, 7:4545-4557.

[4]. Zhang, Z-G; Li, Z-H.; Mao, X-Z., and Wang, W-C. (2011) Advances in bone repair with nanobiomaterials: mini-review. Cytotechnology, 63(5): 437-443.

[5]. Oryan, A., Alidadi, S. and Moshiri, A. (2013) Current concerns regarding healing of bone defects. Hard Tissue, 2(2):13.

[6]. Wang, Q., Yan, J., Yang, J. and Li, B. (2016) Nanomaterials promise better bone repair. Materials Today, 19 (8): 451 – 463.

[7]. Boverhof, D.R., Bramante, C.M., Butala, J.H., Clancy, S.F., Lafranconi, M., West, J. and Gordon, S.C. (2015) Comparative assessment of nanomaterial definitions and safety evaluation considerations. Regulatory Toxicology and Pharmacology. 73(1):137-150.

[8]. Baena, R.R., Rizzo, S., Manzo, L. and Lupi, S.M. (2017) Nanofeatured Titanium Surfaces for Dental Implantology: Biological Effects, Biocompatibility, and Safety. Journal of Nanomaterials. 2017: 18.

[9]. Augustine, R., Dominic, E.A., Reju, I., Kaimal, B., Kalarikkal, B. and Thomas, S. (2014) Investigation of angiogenesis and its mechanism using zinc oxide nanoparticle-loaded electrospun tissue engineering scaffolds. RSC Adv., 4: 51528-51536.

[10]. Foroutan, T. (2014) The effects of zinc oxide nanoparticles on differentiation of human mesenchymal stem cells to osteoblast. Nanomed. J., 1(5): 308 – 314.

[11]. Khalaf, F.H., AL-Zuhairi, A.H. and Al-Mutheffer, E. A. (2014) Clinical and hematological effect of acepromazine, midazolam, ketamine as general anesthetic protocol in rabbits. I.J.S.N., 5 (2): 328-331.

[12]. Johnson, A.L. and Huls, D.A. (2002) Fundamentals of orthropedic surgery and fracture management, decision making in fracture management. In: Fossum, T.W. (ed.): *Small Animal Surgery*, 2nd ed. St. Louis, Mosby.Pp:10-14.

[13]. Liu, W., Su, P., Gonzales III, A., Chen, S., Wang, N., Wang, J., Li, H., Zhang, Z. and Webster, T. (2015) Optimizing stem cell functions and antibacterial properties of TiO_2 nanotubes incorporated with ZnO nanoparticles: experiments and modeling. Int. J. Nanomedicine, 10(1):1997-2019.

[14]. Webster, T.J. and Zhang, L. (2009) Nanotechnology and nanomaterials: Promises for improved tissue regeneration. Nano Today, 4: 66—80.

[15]. Balasubramanian, P., Strobel, L. A., Kneser, U. and Boccaccini, A.R. (2015) Zinc-containing bioactive glasses for bone regeneration, dental and orthopedic applications. Biomed. Glasses, 1:51–69.