10.21608/avmj.2025.346167.1530 Assiut University web-site: www.aun.edu.eg

EFFECT OF NANO-SELENIUM PARTICLES ON REPAIRING DISTAL PART RADIAL BONE FRACTURE IN DOGS: RADIOLOGICAL EVALUATION

MARWAN MAHMOD ALHAMDAN [1] AND OMAR ADIL BADER [2]

[1] Department of Medical Laboratory Techniques, Al-Huwayjah Technical Institute, Northern Technical University, Iraq
[2] Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Received: 8 February 2025; **Accepted:** 18 April 2025

ABSTRACT

Nanotechnology science has significantly changed the field of drug development and alternative therapies because of its great biocompatibility, low toxicity, and antioxidative properties. Nano-selenium is a promising drug delivery vehicle for antioxidant or anti-inflammatory effects, so the objective of the current study is to evaluate the effect of nano-selenium particles on distal transverse radial fracture. Twenty-four adult canine's male which were clinically healthy, weighing (25±0.5) kg and 2±0.5 years old, were used. Dogs were randomly divided into two equal groups. In the first (control) group, a distal part transverse fracture in the radius bone was done and left without adding any material, then reduction and fixation were made. In the second (nano selenium) group, a transverse fracture was made, then treated by adding selenium nanoparticles suspension at the fracture line, then reduction and fixation were done, animals were then monitored clinically and radiographically. Clinically, all experimental dogs showed smooth healing without any complications, but lameness in the nano selenium group subsided more quickly than in the control group. Radiographical results of the nano-selenium group showed good periosteal reaction and callus formation from the second week. Moreover, the fracture line disappeared and returned to normal bone shape in the eighth week. But in the control group, this finding was less clear than in the nano selenium group. The use of selenium nanoparticles accelerated the healing process of distal radial transverse fractures.

Key Words: Fracture Healing, Nano-Selenium, Radial Bone, Dog

INTRODUCTION

A break in the normal structure and continuity of bone is called a fracture. It may be chronic, subacute, or acute, and it can cause damage to nearby nerves, soft tissues, and blood vessels (Dowson *et al*., 2022). In dogs, distal radial fractures have been reported as the third most common type of fractures and the most prevalent type of all radial fractures (Brianza *et al*., 2006). Also, the radius bone is more prone to fracture than the ulna because it bears a greater portion of the forelimb's weight. The low blood supply to the distal radius area and a lack of soft tissues surrounding the fracture site are thought to be the main causes of incomplete or delayed healing of the distal radial fracture (Libardoni *et al*., 2016).

Corresponding author: Omar Adil Bader
E-mail address: omaralbader2003@uomosul.edu.iq
Present address: Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

According to selenium's piezoelectric, photoelectrical, and semiconducting qualities, it is considered an element whose minuscule amounts are necessary for life, so it has attracted a lot of interest in the technical, agricultural, and human sciences. As an antioxidant, selenium is essential for the regulation of reactive oxygen species (ROS) and is known to affect several physiological processes, such as cell differentiation and anti-inflammatory properties (Zoidis *et al*., 2018; Lee *et al*., 2021). Technology that works with materials at the atomic and molecular levels inside the nanoscale range is known as nanotechnology. Compared to traditional materials, nanomaterials have a number of special qualities, such as a greater surface area than their actual size (Brannigan and Griffin, 2016; Qifei *et al*., 2016; Nahi *et al*., 2024). Several scientific fields, including the biomedical sciences, have used and introduced nanomaterials. These materials mimic natural tissue by offering the right extracellular environments for cells to live and proliferate inside them (Zhao-Gui *et al*., 2011). In medicine, selenium nanoparticles (Se NPs) have been used for antibacterial applications to prevent the growth of bacteria (Tran and Webster, 2011). Additionally, it can be employed as an osteo-inductive agent by encouraging mesenchymal stem cells to differentiate into osteoblasts (Fatima *et al*., 2021). Se nanocomposite accelerates the repair of bone fractures by promoting the osteogenic differentiation and migration of bone marrow mesenchymal stem cells (Li *et al*., 2019). This project planned to evaluate the impact of Se NPs on the healing of induced distal radial fractures in dogs radiologically.

MATERIALS AND METHODS

Approval Ethics:
The College of Veterinary Medicine at Mosul University's institutional animal care and use committee approved the research investigation, which was registered according to the number UM.VET.2023.105.

Study Design:
Twenty-four clinically healthy adult male canines weighing 25 ± 0.5 kg and 2 ± 0.5 years old were used in the present study. These animals were maintained in animal husbandry under the same conditions for food and care. Ivermectin 1% (ivermectin, Vapco, Jordan) was injected subcutaneously to all dogs (0.2-0.4 mg/kg BW) to treat them for both internal and external parasites if they present. Random distribution was employed for grouping the trial animals into two equal groups: the control group and the nano-selenium group.

Surgical Procedure:
Throughout general anesthetic protocol of 15 mg/kg BW ketamine hydrochloride 10% (Alfasan, Holand), and 1 mg/kg BW Xylazine hydrochloride 2% (Nita Farm, Russia) (intramuscularly), and 0.04 mg/kg BW Atropine sulfate 1% (Alfasan, Holand) which injected into the muscles approximately ten minutes earlier, the experimental surgical operations were done (Bader and Nahi, 2023; Mohammed *et al*., 2023). Under aseptic operative procedure, all animals had distal complete transverse radial fractures, which was performed by wire saw (Figure 1 and 2), subsequently fiber glass cast tape was used for external immobilization of the broken bone, with leaving a window above the surgical site to allow daily dressing of the wound (Figure 3). In the control group, a complete transverse fracture in the distal part of radial bone was done and left without adding any material, then reduction and fixation were made, while in nano-selenium group a complete transverse fracture was done, then treated by adding 1 mL of selenium nanoparticles (50 nm) suspension at a dose of (125 µg/ml) (El-Sayed *et al*., 2023) at the fracture line (Figure 4), then reduction and fixation were done.

Post-Surgical Follow-up:
For post-operative care following surgery, a daily wound dressing was applied along with five days of intramuscular injection of penicillin at a dose of 10,000 IU and

streptomycin of 20 mg/kg BW (Penstrep, Interchem, Holland), then the thread knots were taken out 10-12 days later.

Evaluations:
All dogs underwent daily physical exams until the last day of the study (60 days). After surgery, a plain radiographic photograph was taken as part of the radiological examination of the fractured bone to ensure that the two ends of the fractured radial fragment were aligned. Then, a mediolateral and craniocaudal follow-up, by X-ray examination, was carried out at 2, 4, 6, and 8 weeks after surgery.

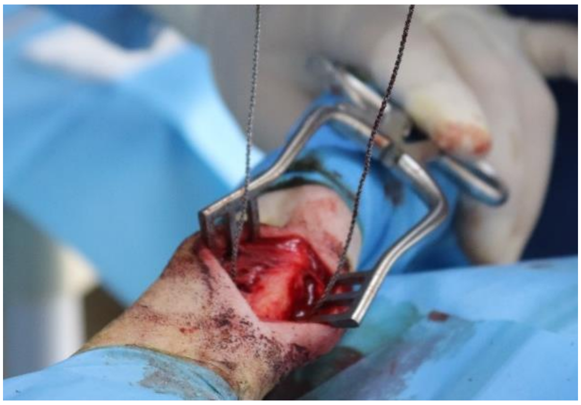

Figure 1: The induced radial fracture using a wire saw.

Figure 2: The distal part radial fracture.

Figure 3: The external fixation of the fractured bone by fiberglass cast tape with a window above the surgical site.

Figure 4: The application of nano selenium at the fracture site.

RESULTS

Clinically, experimental animals in both groups were monitored daily all over the experiment, which demonstrated a partial reduction in appetite on the first and second days, then gradually returned to normal appetite. The skin wound from the fractured limb healed within the first week following the procedure, except for one animal in the control group that experienced mild redness and edema that subsided after two to three days. In the first week animals observed fractured limb disuse and lameness, then began to bear weight gradually in the second, third, and fourth, weeks in the control group, while in nano-selenium group the weight bear started at the beginning of the post operating second week, then ultimately complete weight bear with mild or no lameness.

Radiographic evaluation of the control group at the 2nd week postoperative showed minimal periosteal reaction of the proximal and distal radial fracture segment with a clear fracture line (Figure 5). While at the 4th week post-operation, there was an increased periosteal reaction around the fracture site, with the appearance of a fracture line with a little callus formation (Figure 6). At the 6th week post-operation, periosteal reaction and traces of callus formation were observed in the fracture site, with moderate bridging of the hard callus growth and the fracture line still appearing (Figure 7). While at the 8th week post-operation, the fracture site showed radio-opacity of callus formed at the fracture site, which connected the two fractured ends and increased fracture line disappearance (Figure 8).

Figure 5: Radiographic image at 2nd week post-operation in the control group, showing minimal periosteal reaction with clear fracture line.

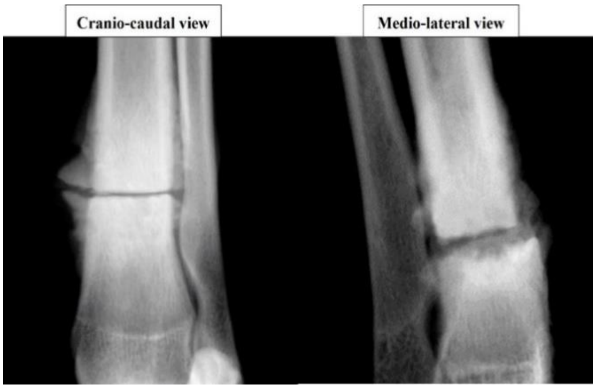

Figure 6: Radiographic image at 4th week post-operation in the control group showing increased periosteal reaction around the fracture site with appearance of fracture line.

Figure 7: Radiographic image at 6th week post-operation in control group showing periosteal reaction and traces of callus formation in fracture site, with moderate bridging of the hard callus growth and the fracture line still appearing.

Figure 8: Radiographic image at 8th week post-operation in control group showing radio-opacity of callus formed at the fracture site, which connected the two fracture ends and with more fracture line disappearance.

Radiographic evaluation of the treated group at the 2nd week post-operation showed clear periosteal reaction around the fractured bone ends, with fracture line appearance and presence of periosteal reaction around the fracture site (Figure 9). At the 4th week post-operation, there was good periosteal reaction around the fracture site, which bridged the line of fracture with blurred fracture line's appearance, and the callus crossed the fracture line partially, with a semi-clear fracture line (Figure 10). While at the 6th week post-operation, there was more prominence of periosteal reaction, an excellent callus formation that connected the two fracture ends with no sign of the fracture line appearance of the fracture site, and semi-normal medullary canal appearance (Figure 11). At the 8th week post-operation, there was a decrease in the callus around the fracture site, with reconnecting of the medullary canal, and the bone took its natural shape (Figure 12).

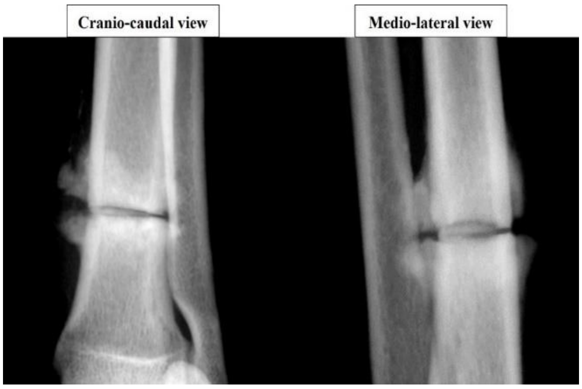

Figure 9: Radiographic image at 2nd week post-operation in the treated group, showing clear periosteal reaction around the fracture site with fracture line appearance.

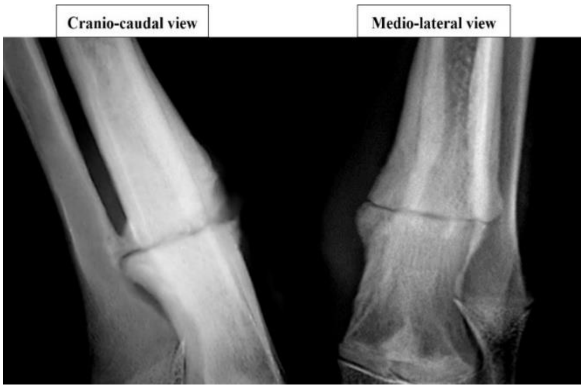

Figure 10: Radiographic image at 4th week post-operation in the treated group showing good periosteal reaction around the fracture site, bridging the line of fracture with a blurred appearance of the fracture lines.

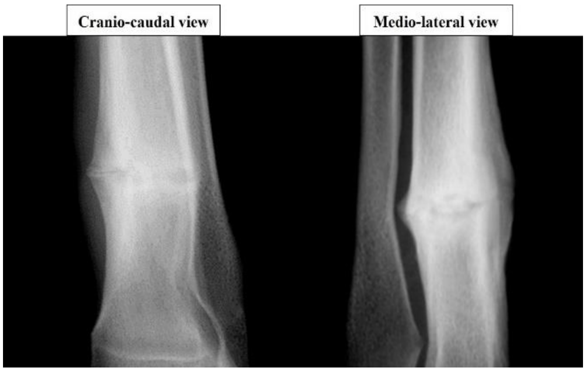

Figure 11: Radiographic image at 6th week post-operation in the treated group showing a more prominent periosteal reaction, an excellent hard callus formation that connected the two fracture ends with no sign of the fracture line

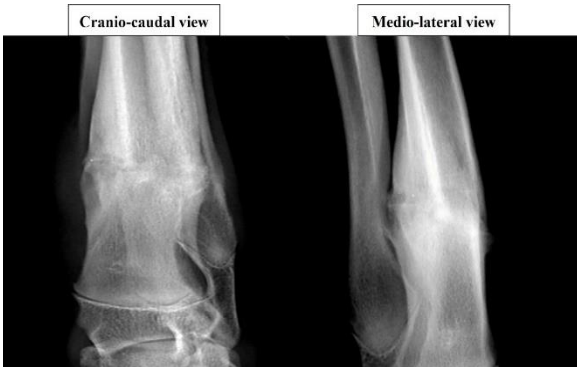

Figure 12: Radiographic image at 8th week post-operation in the treated group showing a decrease in callus around the fracture site, with reconnecting of the medullary canal and the bone took its natural shape.

DISCUSSION

Every experimental dog exhibited a decreased appetite on the first and second day, then gradually returned to the normal appetite. These clinical symptoms disappeared after 4-5 days of the operation. These results agreed with Zheng *et al.* (2020), who mentioned that loss of appetite in animals with bone fracture is more pronounced in the first week after surgery and then over time, the physical activity and feed intake increased gradually

At first week, the control animals showed fractured limb lameness and disuse, then gradually subsided in the second, third and fourth week, these signs agreed with Bader and Nahi (2023) and AlBattat *et al*. (2024), who described that the pathognomonic sign of the fractured limb is lameness which were noticed immediately postsurgical operation then increased during 24-48 hrs. This may be due to pain during the postoperative phase, which might be caused by tissue congestion. It is thought that greater capillary permeability raises intracellular pressures, irritating nearby nociceptors in the process. While in nano-selenium group weight bear began at the beginning of second week post operation with mild or without lameness, this may be due to the nano-selenium effect for reducing the lameness occurrence, and this agreement with Ding *et al*., (2021), who demonstrate that, because nano selenium has a good biocompatibility and antioxidation capabilities, it may be used as a medication delivery vehicle for antioxidants or anti-inflammatory activities.

In the treated group, the radiographical results at the 2nd week post-operation showed clear periosteum reaction around the site of radial fracture and presence of callus formation, while there was minimal periosteal reaction in the control group. At the 4th week post-operation, there was an increase in the callus mass that partially crossed the line of the fracture in the treated group, while in the control group, there was an increase in the reaction of periosteum around the fracture site with little callus formation. At the 8th week in the treated group, there is a decrease in callus around the fracture site and the fractured bone nearly normal shape, but in the control group fracture site showed radio-opacity of the callus with a bridged fracture line. This may belong to the role of nano-selenium particles added to the fracture site and this result coincides with Zeng *et al*. (2013); Li *et al*. (2019); Fatima *et al*. (2021), who pointed out that Mesenchymal stem cells from bone marrow (BMSCs) provide an effective technique for speeding the healing process of bone fractures. Bone fracture healing is significantly influenced by BMSCs' capacity to move to the fracturing site, offer antioxidant defense, and demonstrate osteogenic differentiation. Nanoparticles of selenium have been shown to improve immune surveillance, regulate BMSC proliferation and differentiation, and protect BMSCs from oxidative stress-related damage. Karthik *et al*. (2024) reported that Se NPs' anti-inflammatory and antioxidant abilities resulted in the rehabilitation of catalase (CAT), glutathione peroxidase 1 (GPx1), and Cyclooxygenase-2 (COX-2) mRNA expression, in addition to the normalization of Monocyte Chemoattractant Protein-1 (MCP-1), Interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-α (TNF-α) levels which plays a role in bone healing. Besides that, Zhang *et al*. (2024) explained that by improving bone marrow MSCs' antioxidant capacity in osteogenesis and blocking exuberant intracellular reactive oxygen species (ROS) accumulation, nano selenium can help accelerate fracture healing by controlling ROS levels. Alajmi *et al*. (2024) added that Seleno-proteins containing selenium in the form of selenocysteine are critical for bone remodeling by helping matrix mineralization, osteogenic differentiation, and cellular proliferation and adhesion.

CONCLUSIONS

In conclusion, the use of selenium nano material helped to accelerate the regeneration of bone tissue with improved callus development and a shorter healing period.

Acknowledgments
Authors of the study wishes to convey their sincere appreciation to the University of Mosul's College of Veterinary Medicine for funding this research.

Conflict of Interest
It is confirmed by both authors that they have no conflicting interests.

REFERENCES

Alajmi, K.; Hartford, M.; Roy, N.S.; Bhattacharya, A.; Katiy, S.; Cavagh, B.L.; Roy, S. and Kaur, K. (2024): Selenium nanoparticle-functionalized injectable chitosan/collagen hydrogels as a novel therapeutic strategy to enhance stem cell osteoblastic differentiation for bone regeneration. Journal of Materials Chemistry B, 12, 9268-9282. doi: 10.1039/d4tb00984c

AlBattat, A.H.; Alfaris, A.A. and Saleh, W.M. (2024): Radiological and Clinical Evaluation of Influence of Hydroxyapatite Nanoparticles and Demineralized and Decellularized Bone Matrix on Defect Fracture Healing of Tibia Bone in Goats. Consultant, 64(7), 635-648.

Bader, O.A. and Nahi, H.H. (2022): The effects of thyroidectomy on healing fracture in rabbits model. Iraqi Journal of Veterinary Sciences, 36(3), 563-570. doi: 10.33899/ijvs.2021.130893.1897

Bader, O.A. and Nahi, H.H. (2023): Effect of estrogens level on the fracture healing of tibia bone after ovariectomy and ovariohysterectomy in female dogs Iraqi Journal of Veterinary Sciences, 37(4), 921-928. doi: 10.33899/ijvs.2023.138814.2845

Brannigan, K. and Griffin, M. (2016): An update into the application of nanotechnology in bone healing. Open Orthop J., 10(1), 808-823. doi: 10.2174/1874325001610010808

Brianza, S.Z.; Delise, M.; Maddalena, F.M.; Amelio, P.D. and Botti, P. (2006): Cross-sectional geometrical properties of distal radius and ulna in large, medium and toy breed dogs. J Biomech., 39(2), 302-311. doi: 10.1016/j.jbiomech.2004.11.018

Ding, C.; Yang, C.; Cheng, T.; Wang, X.; Wang, Q.; He, R.; Sang, S.; Zhu, K.; Xu, D.; Wang, J.; Liu, X. and Zhange, X. (2021): Macrophage-biomimetic porous Se@SiO2 nanocomposites for dual modal immunotherapy against inflammatory osteolysis, J. Nanobiotechnol, 19(1), 382. doi:10.1186/s12951-021-01128-4

Dowson, C.; Smith, J. and Brown, T. (2022): Understanding Bone Fractures: A Comprehensive Review. Journal of Orthopedic Research, 45(3), 234-245. doi: 10.1016/j.jor.2022.01.005

El-Sayed, H.; Morad, M.Y.; Sonbol, H.; Hammam, O.A.; Abd El-Hameed, R.M.; Elleth, R.A.; Ibrahim, A.M. and Hamada, M.A. (2023): Myco-Synthesized Selenium Nanoparticles as Wound Healing and Antibacterial Agent: An In Vitro and In Vivo Investigation. Microorganisms, 9(1), 2341. doi: 10.3390/microorganisms11092341

Fatima, S.; Alfrayh, R.; Alrashed, M.; Alsobaie, S.; Ahmad, R. and Mahmood, A. (2021): Selenium nanoparticles by moderating oxidative stress promote differentiation of mesenchymal stem cells to osteoblasts. International Journal of Nanomedicine, 13(6), 331-343. doi: 10.2147/IJN.S285233

Karthik, K.K.; Cheriyan, B.V.; Rajeshkumar, S. and Gopalakishnan, M. (2024): A review on selenium nanoparticles and their biomedical applications. Biomedical Technology, 6(1), 61-74. doi: 10.1016/j.bmt.2023.12.001

Lee S.C.; Lee N.H.; Patel K.D.; Jang T.S. Knowles J.C.; Kim H.W.; Lee H.H. and Lee J.H. (2021): The Effect of Selenium Nanoparticles on the Osteogenic Differentiation of MC3T3-E1 Cells. Nanomaterials, 11, 557.

Libardoni, R.N.; Serafini, G.M.C.; Olivereia, C.; Schimites, P.I.; Chaves, R.O.; Feranti, J.P.S.; Costa, C.A.S.; Amara, A.S.; Raiser, A.G. and Soares, A.V. (2016): Appendicular fractures of traumatic etiology in dogs: 955 cases (2004-2013). Ciência Rural, Santa Maria, 46(3), 542-546. doi: 10.1590/0103-8478cr20150219

Li, C.; Wang, Q.; Gu, X.;Kang, Y.;, Zhanng, Y.; Hu, Y.; Li, T.; Jin, H.; Deng, G.

and Wang, Q. (2019): Porous Se@SiO2 nanocomposite promotes migration and osteogenic differentiation of rat bone marrow mesenchymal stem cell to accelerate bone fracture healing in a rat model, Int. J. Nanomed., 14, 3845-3860. doi: 10.2147/IJN.S202741

Mohammed, F.M.; Alkattan, L.M.; Shareef, A.M. and Thanoon, M.G. (2023): Evaluation the effect of high and low viscosity Nano-hydroxylapatite gel in repairing of an induced critical-size tibial bone defect in dogs: Radiolographical study. Journal of Applied Veterinary Sciences, 8(3), 105-110. doi: 10.21608/ja

Nahi, H.H.; Ashour, M.S. and Bader, O.A. (2024): Study the Effect of Zinc Oxide Nanoparticles on Regeneration of Damaged Sciatic Nerve in Rabbits Model. Egyptian Journal of Veterinary Science, 55(4), 1110-1129. doi: 10.21608/ejvs.2023.250656.1679

Qifei, W.; Jianhua, Y; Junlin, Y. and Bingyum, L. (2016): Nanomaterials promise better bone repair. Mater Today.,19(8), 451-463. doi: 10.1016/j.mattod.2015.12.003

Tran, P.A. and Webster, T.J. (2011): Selenium nanoparticles inhibit Staphylococcus aureus growth. International journal of nanomedicine, 6:1553-1558. doi: 10.2147/IJN.S21729

Zeng, H.; Cao, J.J. and Combs, GF. (2013): Selenium in bone health: roles in antioxidant protection and cell proliferation, Nutrients. 5(1), 97-110. doi: 10.3390/nu5010097

Zhang, M.; Xu, F.; Cao, J.; Dou, Q.; Wang, J.; Wang, J.; Yang, L. and Chen, W. (2024): Research advances of nanomaterials for the acceleration of fracture healing. Bioactive Materials, 31(1), 368-394. doi: 10.1016/j.bioactmat.2023.08.016

Zhao-Gui, Z.; Zhi-Hong, L.; Xin-Zhan, M. and Wan-Chun, W. (2011): Advances in bone repair with nano biomaterials: Mini-review. Cytotechnol., 63, 437-443. doi: 10.1007/s10616-011-9367-4

Zheng, J.S.; Ruann, H.R.; Qiu, S.; Nie, J.; Hou, K.W. and Wu, R. (2020): Therapeutic effects of revascularization on the healing of free bone grafts in dogs. Journal of Veterinary Research, 64, 175-180. doi: 10.2478/jvetres2020-0023

Zoidis, E.; Seremelis, I.; Konotopoulos, N. and Danezis, G. (2018): Selenium-Dependent Antioxidant Enzymes: Actions and Properties of Selenoproteins. Antioxidants, 7(5), 66. doi:10.3390/antiox7050066

تأثير جزيئات السيلينيوم النانوية في إصلاح كسر الجزء القاصي لعظم الكعبرة في الكلاب: تقييم إشعاعي

مروان محمود ، عمر عادل بدر

Email: omaralbader2003@uomosul.edu.iq Assiut University web-site: www.aun.edu.eg

أحدث علم تكنولوجيا النانو تغييرًا كبيرًا في مجال تطوير الأدوية والعلاجات البديلة، نظرًا لتوافقه الحيوي الكبير، وسميته المنخفضة، وخصائصه المضادة للأكسدة. يعد السيلينيوم النانوي وسيلة واعدة لتوصيل الأدوية للتأثيرات المضادة للأكسدة أو المضادة للالتهابات، لذا هدفت الدراسة إلى تقييم تأثير جزيئات السيلينيوم النانوية على الكسر القاصي لعظم الكعبرة.

تم استخدام أربعة وعشرون ذكراً من الكلاب البالغة والسليمة سريرياً بوزن (٢٥ ± ٠,٥) كغم وعمرها ٢ ± ٠,٥ سنة. تم تقسيم الكلاب عشوائيا إلى مجموعتين متساويتين، في المجموعة الأولى (السيطرة)، تم إجراء كسر عرضي في الجزء القاصي من عظم الكعبرة وتركه دون إضافة أي مادة، ثم تم إجراء الارجاع والتثبيت. وفي المجموعة الثانية (النانو سيلينيوم) تم اجراء الكسر المستعرض ثم علاجه بإضافة معلق جسيمات السيلينيوم النانوية عند خط الكسر ثم تم إجراء الارجاع والتثبيت، تم بعدها متابعة الحيوانات سريريا وشعاعيا.

من الناحية السريرية، اظهرت جميع الكلاب التجريبية شفاءً سلسًا دون أي مضاعفات، ولكن العرج في مجموعة السيلينيوم النانوية قد تراجع في وقت أسرع من مجموعة السيطرة. أظهرت النتائج الشعاعية لمجموعة السيلينيوم النانوية تفاعل سمحاقي جيد وتشكل الدشبذ (وهو النسيج الليفي الغضروفي المتكون في منطقة الكسر) من الأسبوع الثاني، كما اختفى خط الكسر عاد العظم إلى شكله الطبيعي في الأسبوع الثامن. ولكن في المجموعة الظابطة كانت هذه النتيجة أقل وضوحًا مما كانت عليه في مجموعة السيلينيوم النانوية.

استخلصت الدراسة الحالية إلى أن استخدام جسيمات السيلينيوم النانوية يعزز ويسرع عملية الشفاء من الكسر الكعبري القاصي.