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Radiological evaluation for effect of NAC on tibia puncture fracture in dogs

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***Abstract*---**The present study used N- acetyl cysteine NAC to study its effects on tibia bone defects in dogs. This study aimed to evaluate the effective use of N- acetylcysteine (NAC) on tibia puncture fracture in dogs. The dogs were divided into three groups (5 dogs for each one): Control group (treated with autogenous cartilage only), treated group 1(treated with autogenous cartilage mixed with NAC), and treated group 2 (treated with autogenous cartilage and NAC orally). Radiological assessment fourteen days postoperatively, there is no evidence of periosteal reaction in the radiological examination in the control group. While the radiological findings revealed evidence of periosteal response at the ridges of the bone gap in treated group one and radiological results showed evidence of more periosteal interaction at the edges of the bone gap in treated group two compared with the group one and control group, and the gap appeared cloudy in color. Twenty-eight days postoperatively showed that the bone gap seemed dark in the shade of bone gap in control groups. Twenty-eight days postoperatively in treated group one, radiographs showed a narrowing in the bone gap diameter as showed by radiological examination. After Twenty-eight days, the smaller diameter gap was compared with the control group and the treated group postoperatively. Forty-two days postoperatively radiological study showed closure of the bone gap in treated group two. In comparison, forty-two days postoperatively, radiological examination in treated group one showed a (transparent) smaller diameter gap which appeared gray. As demonstrated after Forty-two days postoperatively, a radiological study in control groups revealed that the gap was smaller in diameter. Conclusion: NAC was

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safe, with no complications, and using NAC increased bone healing in treated groups compared with control groups.

***Keywords*---**N-acetylcysteine, Bone fracture, tibia, dog, radiology.

Introduction

Bones are a component of the skeletal system responsible for giving form, mechanical support, and protection to the body and aiding mobility. Under certain stressful and constant compression situations, the bone tissue's capacity to bear strength declines (Abdalbari A. Alfaris and Muhammad Jassim Muhammad. 2017). Ten to twenty percent of all fractures are tibia fractures in dogs and cats, with 73 percent involving the tibia shaft. Approximately fifty percent of tibia fractures in young dogs and cats arise from trauma (Beale et al., 2020). (Hayashi, K. 2018). The vast majority of tibia fractures are diaphyseal. Ten to twenty percent of tibia fractures are open fractures, with the distal tibia of adult animals being the most frequent for an open fracture. (Virani, S. R., et al., 2016).

For tibial fractures, immediate immobilization of the crus is suggested, and surgical treatment is often necessary. The choice of repair method depends on many factors, such as the fracture's type and location, the animal's age, the presence of associated soft tissue defects and infection (especially in open fractures), economic considerations, and the surgeon's preference. The prognosis for tibia and fibula fractures is typically favorable when adequate treatment is given (Vidal et al., 2020). Hayashi, K. (2018). (K. C. McInnis, 2016). N-acetylcysteine (NAC) is often used to treat paracetamol overdoses and as a mucolytic agent. It has a well-established safety profile, and its toxicity is rare and dose-and route-dependent. (Tardiolo et al., 2018).

NAC's exceptional antioxidant and anti-inflammatory activity is the biochemical foundation for treating various disorders associated with oxidative stress and inflammation. The capacity of NAC to enhance the intracellular concentration of glutathione (GSH), the most critical bio thiol responsible for cellular redox imbalance, is the principal antioxidant function of NAC. As an anti-inflammatory chemical, NAC inhibits the function of nuclear factor kappa B (NF-B) to lower levels of tumor necrosis factor-alpha (TNF-_) and interleukins (IL-6 and IL-1_). Despite NAC's substantial therapeutic potential, its success in clinical trials addressing various pathological disorders remains restricted, according to much experimental research. (Aldini et al., 2018). N-acetylcysteine (NAC) is a drug that supplies bioavailable cysteine for glutathione replenishment and prevents oxidative damage as well as inflammation. It also leads to glutathione (GSH) formation in the body.(Hiba m. Abd Alrahma et al., 2020)

Aim of Study

This research aimed to determine the efficacy of N-acetyl cysteine (NAC) in treating tibia puncture fractures in dogs

Materials and Methods

Animal

The experiment was conducted on 15 canines weighing between 15 and 25 kg and aged between (1-3) years of age. Animals were kept in separate cages in the animal house of the College of Veterinary Medicine/University of Basrah for one week to acclimate and adjust to the setting, which included the same temperature, management, and food.(Jasim, Mohammed et al., 2020) *Ivermectin (200 milligrams per kilogram of body weight subcutaneously) was supplied to the dogs after a clinical examination to guarantee their wellbeing (Paradies et al. 2019).

Equipment and instruments

	Instruments	Origin
1	Electric drill	China
2	Suture material nylon No 0	Hamburg, Germany
3	Catgut 3/0	Cyprus
4	X-Ray machine	
5	Ketamine	Belgium
6	Xylazine	Belgium
7	Surgical orthopedic site	Germany
8	Antibiotic (penicillin)	UK
9	Medical cotton, gauze and iodine	Iraq
10	N- acetyl cysteine	USA

Experimental design

The dogs were placed into three groups of five animals each: the control group (treated with autogenous cartilage alone), group 1 (treated with autogenous cartilage mixed with NAC), and group 2 (treated with autogenous cartilage and NAC orally).

Surgical procedures

The medial side of the distal left tibia was prepped aseptically for surgical operation by clipping and shaving the medial and lateral portions of the tibia, followed by the application of cotton soaked with 70% ethyl alcohol to the surgical site for 20 minutes before the procedure (Tollefson et al., 2018). The surgical procedure was performed under general anesthesia by injecting 10 mg/kg of acepromazine maleate intramuscularly as a tranquilizer. Inject a combination of (30–40) mg/kg body weight of ketamine hydrochloride* and (5-8) mg/kg body weight of xylazine intramuscularly** after 10 minutes to induce anesthesia (Kosenko et al., 2020). The animal was placed in a lateral recumbent posture, and a skin incision was made in the medial portion of the distal third of the tibia. Using Metzenbaum shears, the subcutaneous tissues were separated with minor trauma.

After exposing the distal tibial bone, a 4 mm piece was taken from the bone with an electric drill and local administration of sterile standard normal saline to prevent necrosis and clean the site of bone marrow debris. (Figure1). The autogenous ear cartilage was made by removing one centimeter from the base of the dog's ear, separating the skin from the cartilage, and placing it in normal saline. For the control group, the bony space was filled with cartilage produced directly from the ear of the same animal (Alaa, 2018). (Figure 2). In addition to 140 mg/kg of NAC, cartilage was used to fill the bone defect in the second group (treatment 1 group). In the third group (treatment group 2), the bone defect was filled with cartilage, and the subjects were administered 140 mg/kg of NAC orally for five days. (Ashraff waleed Abduleazaq et al., 2020). The skin was then closed with a direct interrupted suture using surgical silk 3.0 usp*. The last stage was treating the wound. Observe animals till they have fully recovered from anesthesia.

Preparation of autologous cartilage grafts

The dog's ear cartilage was used to prepare the autologous cartilage transplant (Alaa.,2018). The base of the ear is surgically prepped using an aseptic method. The ear cartilage was sliced intraoperatively using a knife and blade and then placed in a sterile saline solution (figure 3). The ear wound was surgically sutured.

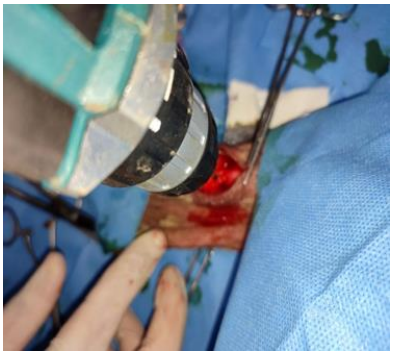

Figure 1. Use an electric drill to make a defect in the bone

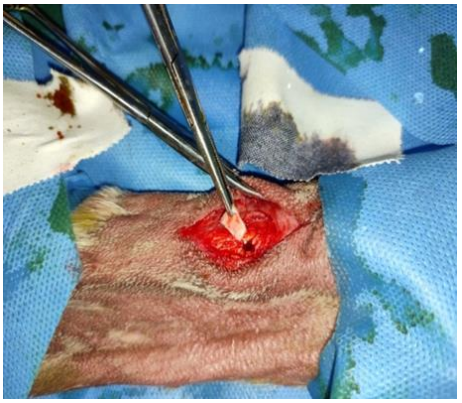

Figure 2. Cartilage in bone gap

Figure 3. Fill a defect in bone with autogenic Cartilage

Postoperative management

This contained a once-daily intramuscular injection of penicillin (10000 IU/kg body weight) and streptomycin* (10 mg/kg body weight (PS) for 3 to 4 days. After 7–10 days, suture materials are removed.

Parameters examined
Clinical evaluation

Throughout the trial, the surgical sites of the animals were evaluated for swelling, redness, discomfort, and tenderness. In addition, the clinical indications of lameness were assessed using a numerical rating scale (NRS) with the inter-observer agreement (Nganvongpanit et al., 2013). The scale is between 1 and 5 tables (1).

Table 1
Numerical rating scale (NRS) to assess locomotion in dogs

Scale number	Details
1	Walks normally
2	Slightly lame when walking
3	Moderately lame when walking
4	Severely lame when walking
5	Reluctant to rise and will not walk more than five paces

Radiographic examination

A radiographic examination was conducted on the fourteenth, twenty eighth, and fortieth days following surgery, utilizing a mediolateral view of the tibia bone to evaluate the callus development and periosteal response at the defect location. Under precise settings, the operated right tibia was radiographed utilizing an MX-20 cabinet X-ray system (35 kV, 300 mA, and 240 s). (Alaa., 2018).

Statistical investigation

The results were presented as mean values with standard deviations. Using statistical software, data were statistically examined using One-Way ANOVA with

multiple comparison tests (SPSS for Windows version 22, USA). At (P 0.05), differences were judged significant.

Results and Discussions

Clinical observation

Due to the consistency of the lesions, all dogs had the same degree of lameness, which was practically identical, and lameness was often noticeable on the first day after surgery but considerably improved in treated group 1 and treated group 2 three days postoperatively. Five days after surgery, the lameness was significantly reduced in group 2 compared to group 1 and the control group table (1).figure (4)

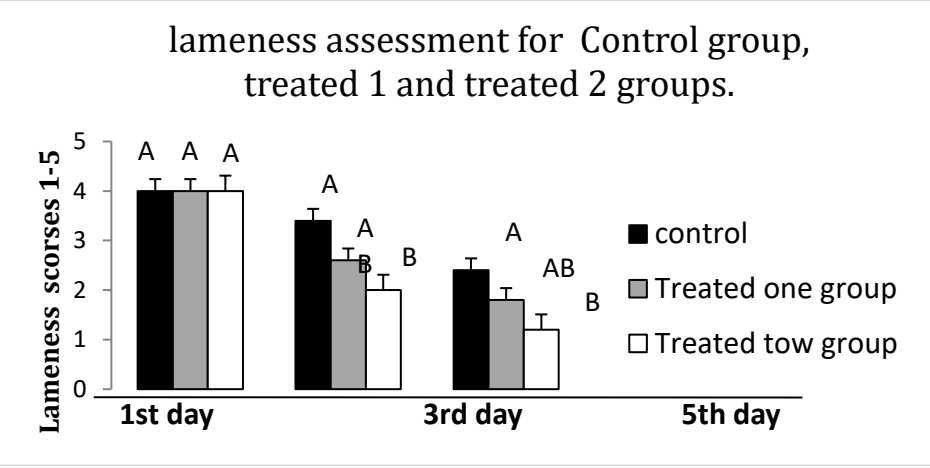

Figure 4. clinical study showed lameness for control, treated 1, treated 2 groups during different periods (1,3,5 days post surgery). ^{AB} Different letters among groups indicates significant differences (P< 0.05)

Table 2
The lameness scores for control, treated 1, treated 2 groups during different periods (1,3,5 days post surgery). (means and standard errors)

Groups	1st day	3rd day	5th day
Control	4.00± 0.00^{A}	3.40±0.24^{A}	2.40±0.24^{A}
treated 1	4.00± 0.00^{A}	2.60±0.24^{AB}	1.80±0.37^{AB}
treated 2	4.00± 0.00^{A}	2.00±0.31^{B}	1.20±0.20^{B}

AB Different letters within each column indicates significant differences (P<0.05).

Radiological Assessment

Radiological findings of the control group

- Radiographic examination fourteen days postoperatively reveals no signs of periosteal response. (Figure 5).

- Twenty-eight days after surgery, the bone gap seemed to have a dark tint (Figure 6).
- Forty-two days after surgery, the radiological evaluation indicated that the width of the gap had shrunk (Figure 7).

Radiological findings of the one treated group

- A radiographic examination fourteen days after surgery showed no periosteal reaction. (Figure 5).
- Twenty-eight days following surgery, the gap in the bone seemed to be stained with a black hue (Figure 6).
- Forty-two days following surgery, a radiological examination revealed that the gap's diameter had diminished (Figure 7).

Radiological findings of the treated two group

- Fourteen days after surgery, radiographic data showed higher periosteal contact at the bone gap's margins than in the second group, and the gap seemed murky (Figure 5).
- Radiological evaluation at 28 days postoperatively revealed a decreased diameter gap compared to the control and second groups (Figure 6).
- A radiological test forty-two days postoperatively revealed closure of the bone gap (Figure 7).

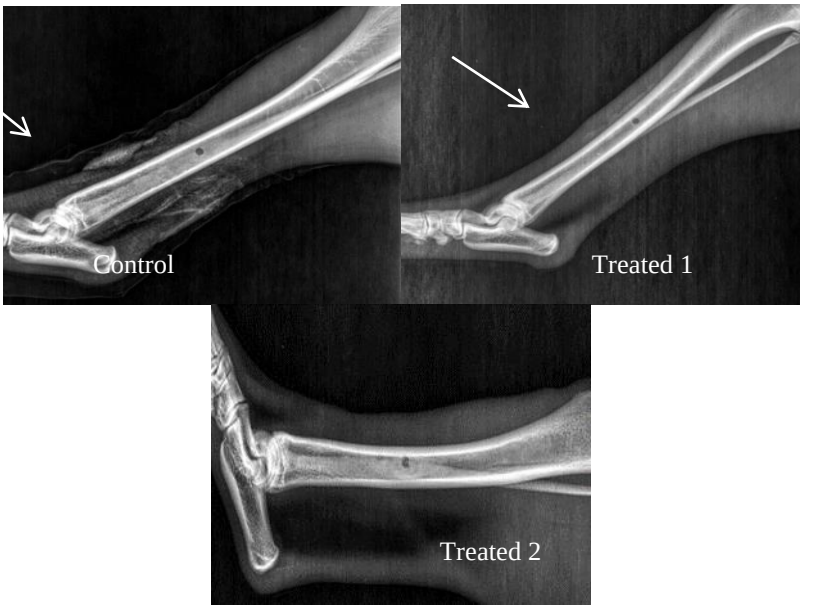

Figure 5. show control, treated 1 and treated 2 groups 14 days postoperatively

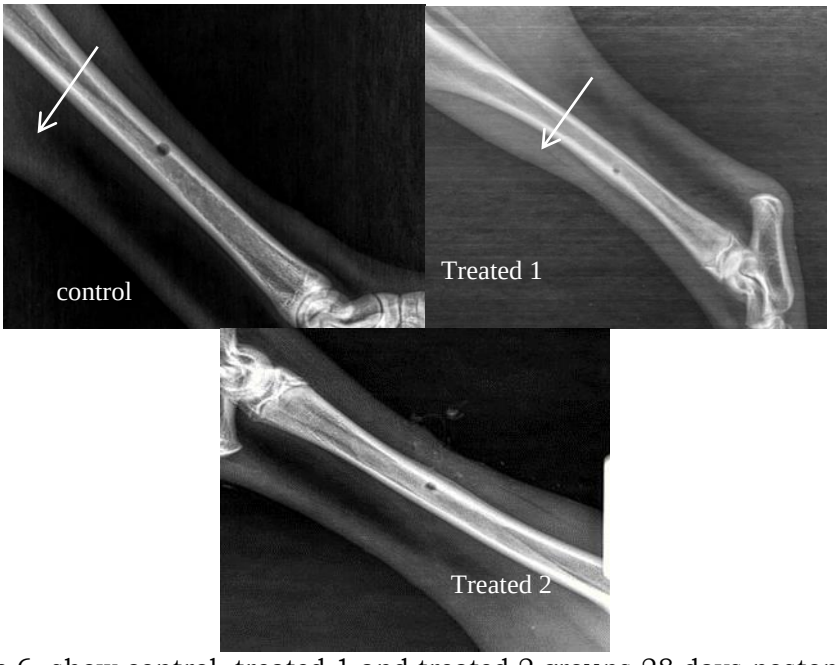

Figure 6. show control, treated 1 and treated 2 groups 28 days postoperatively

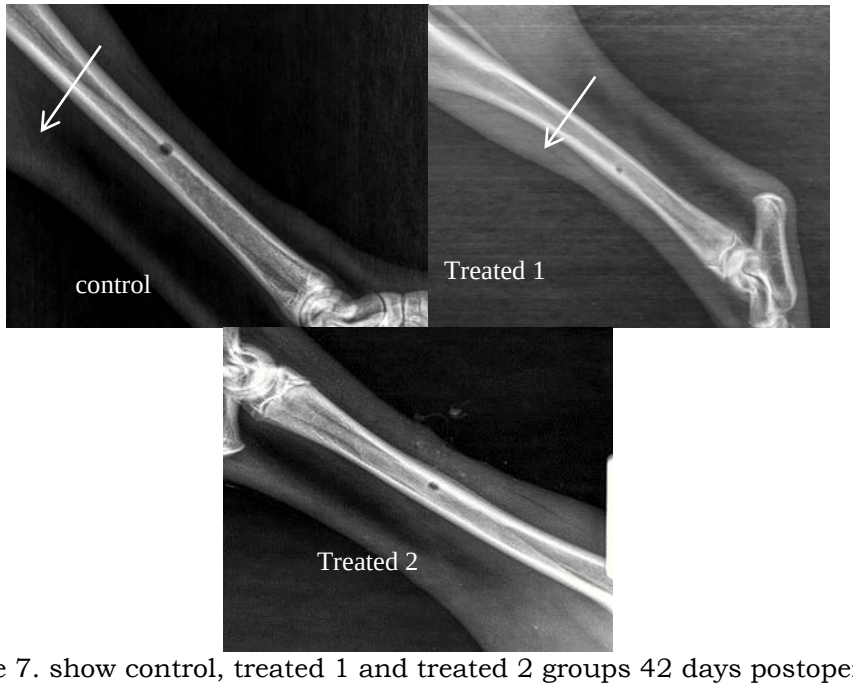

Figure 7. show control, treated 1 and treated 2 groups 42 days postoperatively.

Table 3
Radiological examination

Groups	14 days	28 days	42 days
Control	no evidence of periosteal reaction	the bone gap appears dark in color	the gap smaller in diameter
Treated 1	periosteal reaction at the ridges	showed narrowing in the bone gap diameter	The gap smaller in diameter and gray in color
Treated 2	periosteal reaction at the ridges	narrowing in the bone gap diameter	closure of the bone gap

Discussion

In the present study, NAC was used topically in group two, orally in group three, and not in group one, which served as the control. Compared to the control group, fracture recovery was gradual, and new bone formation occurred within a reasonable time frame in treatment groups one and two. In the radiological examination of the control group fourteen days postoperatively, there is no sign of periosteal reaction. Radiographic data indicated evidence of periosteal response at the ridges of the bone gap in group one. They increased periosteal interaction at the borders of the bone gap in group two compared to group one and the control group. The gap looked hazy in color in group two. These findings concur with those of previous research. (Min-Ling et al., 2014 Young-Hee et al., 2017; Guangqi et al., 2020.

Jay and Matthew (2014) show that NAC tremendously affects cell proliferation and healing when utilized. After twenty-eight days postoperatively, the bone gap in the control groups seemed to have a dark hue, by the findings of this research. Radiographs of the first treatment group 28 days postoperatively revealed a reduction in the bone gap diameter. After twenty-eight days postoperatively, the radiological evaluation revealed a decreased diameter gap compared to the control and treated groups. Radiological examination 42 days postoperatively revealed closure of the bone gap in treated group two. In comparison, radiological examination 42 days postoperatively revealed a (transparent) smaller diameter gap that looked gray in treated group one. As shown by radiological testing in control groups 42 days postoperatively, the width of the gap decreased. These findings concur with (Sameh et al., 2020) in that the use of NAC was risk-free, there were no problems, and the addition of NAC to the bone defect led to a significant increase in bone width and bone density assessments.

Due to the consistency of the lesions, all dogs had the same degree of lameness, which was practically identical, and lameness was often noticeable on the first day after surgery but considerably improved in treated group 1 and treated group 2 three days postoperatively. Five days following surgery, the treated group 2

demonstrated a substantial improvement in lameness compared to the treated group 1 and the control group. These findings thoroughly disclose a novel use of NAC in the therapeutic field of inflammatory bone disease and suggest a prospective therapeutic method for treating inflammatory bone disease. These results are consistent with Guangqi et al., 2020; Min-Ling et al., 2014 found that the use of NAC was safe and, that there were no problems and that the use of NAC accelerated bone repair in treated groups compared to controls.

Conclusion

use of NAC was safe and there were no complications and used of NAC increased bone healing in treated groups compare with control groups.

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