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ORIGINAL ARTICLE

Evaluation of Post-Surgical Inflammatory and Oxidative Responses in Dogs Undergoing Ovariohysterectomy after Oral Administration of Selenium and Selenium Nanoparticles

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ABSTRACT

Evaluation of oxidative stress status after surgery can be considered as an indicator of any successful treatment after surgery. Therefore, this study investigated the effect of oral administration of selenium and selenium nanoparticles (SeNPs) on surgical oxidative stress status in dogs undergoing ovariohysterectomy (OHE). Twelve female puppies were divided into three equal control, selenium, and nanoselenium groups. In the selenium and nanoselenium groups, capsules containing 50 micrograms of selenium and SeNPs were administered two hours before surgery and followed daily for 4 days after OHE. A significant decrease in SOD activity was observed after OHE compared to before surgery in the control group. While in the nanoselenium group, there was no significant decrease in SOD activity after OHE compared to before surgery. An increase in the activity of SOD, GPx and catalase was observed in the selenium and nanoselenium groups compared to the control group. The lowest MDA concentration was observed in the nanoselenium group. Additionally, high CRP concentrations were observed at 18 and 30 hours after OHE in the control group compared to the two other groups. The increase in the activity of the antioxidant enzymes and the decrease in the serum concentration of MDA and CRP following the oral administration of selenium and SeNPs in dogs that underwent OHE surgery suggest the antioxidant and anti-inflammatory effects of these supplemental treatments after OHE.

Introduction

Ovariohysterectomy (OHE) is considered an elective intervention in the routine sterilization of small animals. However, surgical complications such as hemorrhage, infection, and edema may occur after OHE.[1] On the other hand, it has been stated that OHE causes pain and stress due to tissue damage, inflammation and surgical manipulation.[2,3] Therefore, in addition to performing the correct principles of surgery in OHE, managing of these complications can be optimized by prescribing agents with anti-inflammatory and antibacterial effects. Sometimes, these drug interventions can control the post-surgical inflammatory response in animals undergoing spaying, accelerate wound healing and patient recovery, and reduce unwanted and undesirable side effects of surgery.[4] In fact, the evaluation of inflammatory and oxidative responses after surgery can be considered as a sign of the effectiveness of any therapeutic intervention. Therefore, the measurement of acute phase proteins, including C-reactive protein (CRP), is considered as a positive acute phase protein of inflammation. In other words, serum CRP levels can show the inflammatory response following an

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inflammatory stimulus such as surgery.[5,6] In addition, it is possible to evaluate the oxidative status (response) after surgery by measuring the activity of some antioxidant enzymes including glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT).[7,8] Because surgery as a trauma causes oxidative stress and an uncontrolled increase in the production of reactive oxygen species (ROS), the evaluation of the oxidative response is important for the effectiveness of any treatment after surgery.[9] Selenium is one of the elements that can potentially be considered as a post-operative consideration due to its anti-inflammatory and antioxidant effect.[10] However, despite the administration of selenium after surgery in humans, there are insufficient research results on the use of this element after various surgeries in dogs. Similarly, selenium nanoparticles (SeNPs) have been used as an antioxidant and immune-improving substance in different animal studies.[11] However, no studies were found on the administration of SeNPs after surgery in dogs. Therefore, in the present study, the inflammatory and oxidative response after oral administration of selenium and SeNPs in female dogs undergoing OHE surgery has been investigated.

Materials and Methods

The design and implementation of the current research has been reviewed and approved by the research council of the Faculty of Veterinary Medicine, Shahrekord University, Iran.

Preparation of Selenium and Selenium nanoparticle Capsules

Selenium supplement tablets available in the market (200 micrograms per tablet) were purchased and one tablet was dissolved in 1 ml of distilled water and 0.25 ml of the obtained solution was poured into a 100 mg capsule containing 50 micrograms of selenium.

For preparation of selenium nanoparticle capsules, a 100 ml solution of selenium oxide was made using a high rotation stirrer with a concentration of 5 mM. Then 50 ml of ascorbic acid solution made with a concentration of 44 mM is added drop by drop to the prepared solution to form SeNPs. The final suspension of SeNPs contained 39480 micrograms of selenium in 550 ml. Therefore, 0.190 ml of the obtained solution was poured into a 100 mg capsule containing corn starch yielding a final capsule with 50 micrograms of selenium nanoparticles.

OHE and Groups

In the present study, 12 mixed breed female puppies, 5-6 months old, weighing 7-9 kg were ovariohysterectomized and randomly divided into 3 equal groups of control, selenium and nanoselenium. One day before OHE, a 22-gauge heparinized IV catheter was placed in the cephalic vein of the dogs under aseptic conditions to facilitate blood collection and also to reduce the stress associated with blood sampling on the day of surgery.[4] It was planned to perform OHE of the animals at 11:00 a.m. of the day. Preoperative considerations included intravenous administration of morphine (0.4 mg/kg, IV; Temad Co., Iran) and midazolam (0.2 mg/kg, IV; Caspian Tamin Co., Iran) and subcutaneous injection of meloxicam (0.2 mg/kg; Razak Co., Iran) two hours before surgery. After aseptic preparation and induction of anesthesia by intravenous injection of ketamine (8 mg/kg; Rotexmedica, Germany) and diazepam (0.3 mg/kg; Caspian Tamin Co., Iran), endotracheal intubation was performed to maintain anesthesia using isoflurane (Terrel Isoflurane, USA). Warm lactated Ringer's serum (10 ml/kg/h) was infused until the endotracheal extubation. Prophylactic antibiotics were not used. Spaying of all animals was performed by a surgeon as well as a permanent assistant surgeon. After removing the uterus and ovaries and ensuring no bleeding, the abdominal area was closed routinely.[12,13] All dogs were hospitalized individually during the study and fed the same diet twice a day while having free access to water. Additionally, their surgical wound care and cleaning of their resting area after surgery were carefully considered. Ketoprofen (2 mg/kg, IM; Rooyan Darou, Iran) was administered daily for 2 days to manage postoperative pain.

Administration of Selenium and Selenium Nanoparticles Supplements

Two hours before OHE, the selenium and SeNPs capsules were fed to the animals of the selenium and nanoselenium groups, respectively, and oral administration of these supplements was continued daily for 4 days after the surgery. No supplement was given to control group dogs.

Blood Sampling Times

Blood samples were taken 3 hours before surgery (T0) and then 6 (T1), 18 (T2), 30 (T3), 120 (T4), and 240 (T5) hours after surgery.[14,15] Serum was separated by centrifugation at 2500 rpm for 10 min in a refrigerated centrifuge and was stored at –20 °C until use.

Assessment of Oxidative Markers

Lipid peroxidation was measured using the Buege method. For this purpose, the serum concentration of MDA was measured as an index of lipid peroxidation .The basis of this assessment is the reaction of MDA in the liver or kidney with thiobabituric acid, which results in a pink color in the medium. Therefore, by measuring the intensity of the color created at the wavelength of 535 nm, the concentration of malondialdehyde was calculated.[16] Activity of CAT enzyme is done by evaluating the amount of yellow color created by the reaction of ammonium molybdate with hydrogen peroxide in the presence of serum sample (Goth method). Therefore, the activity of this oxidative marker was read by determining the color intensity at the wavelength of 405 nm.[17] The GPX enzyme activity was measured by Paglia and Valentin method at 340 nm wavelength.[18] Sun technique was used to evaluate SOD activity. In fact, the determination of SOD activity is based on measuring the reduction of superoxide anions. Superoxidase anions are produced by the xanthine oxidase enzyme and interact with the provided water-soluble tetrazolium (WST) dye and create color at a wavelength of 450 nm.[19]

Measurement of CRP as Postsurgical Inflammatory Marker and Stress

The CRP concentration was determined using a commercial ELISA kit specific to dogs (CRP immunoturbidometric kit, Pishtaz Teb, Iran; Device BT1500). The basis of this test is the reaction of the sample with antiserum and the formation of sediment. Therefore, the amount of created turbidity can be measured by the turbidity measurement method at a wavelength of 340 nm. Since the amount of created turbidity has a direct relationship with the concentration of CRP in the sample, the concentration of CRP in the sample can be determined by drawing a standard curve from the amount of light absorption of the standards.[20,21]

Statistical Analysis

Mean (± SD) of recorded data at the same time was compared between different groups. The changes of measured parameters at different times of each group were statistically compared. ANOVA (followed by Tukey's honest significant difference test) was used for statistical comparison at a significance level of $p < 0.05$.

Results

Levels of Systemic Oxidative Stress Markers

Figure 1 shows the statistical comparison of MDA levels in different groups and times. The MDA level increased significantly ($p < 0.05$) in all three control, selenium, and nanoselenium groups at times 6, 18, 30, 120, and 240 of the study compared to time 0 of the same group. A significant decrease ($p < 0.05$) in MDA levels was observed at 6 and 30 hours in the selenium group compared to the control group and between the nanoselenium group compared to the control and selenium groups. The results showed that the concentration of this parameter in the nanoselenium group decreased significantly ($p < 0.05$) at times T2, T4, and T5 compared to the control and selenium groups. As shown in Figure 2, the time of T4, CAT activity increased significantly in both selenium and SeNPs groups compared to the control group ($p < 0.05$), but there was no significant difference at other times of the study .In addition, in the SeNPs group, a significant increase of this parameter was seen at time 120 compared to time. A significant increase in GPx activity was seen at times T2, T3, T4, and T5 in selenium and SeNPs groups compared to the control group. However, it was seen that the

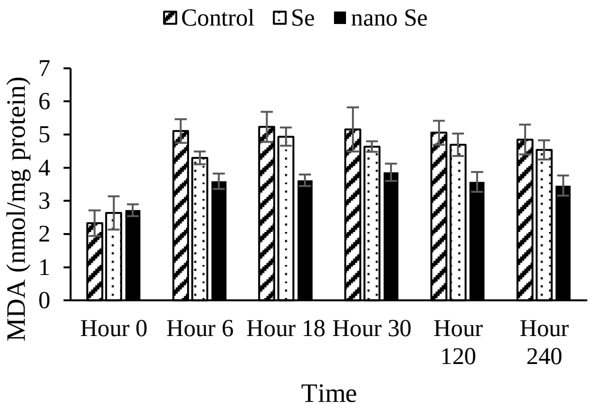

Figure 1. Comparison of mean ± SD of MDA concentration (nmol/mg of protein) in different groups and times ($p < 0.05$).

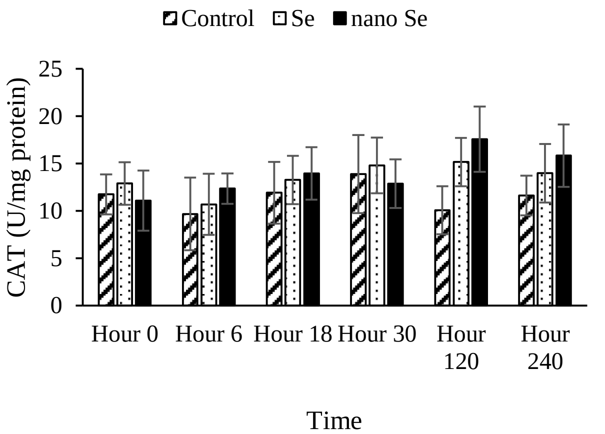

Figure 2. Comparison of mean ± SD of CAT activity (U/mg of protein) in different groups and times ($p < 0.05$).

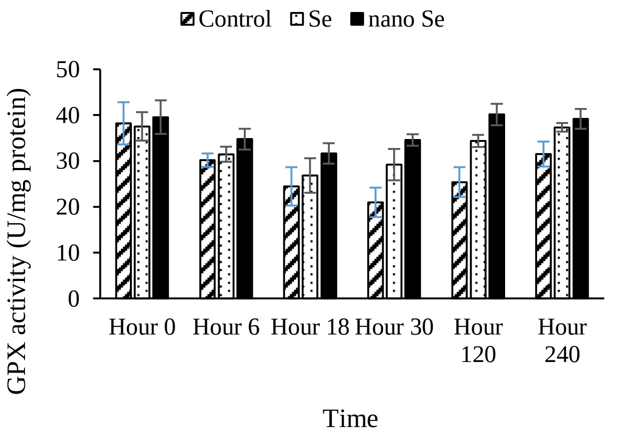

Figure 3. Comparison of mean ± SD of GPx activity (U/mg of protein) in different groups and times ($p < 0.05$).

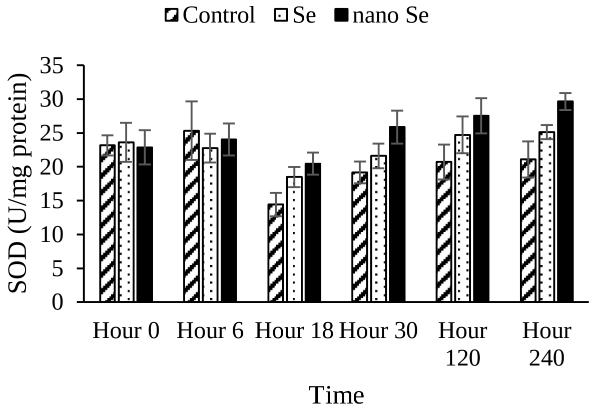

Figure 4. Comparison of mean ± SD of SOD activity (U/mg of protein) in different groups ($p < 0.05$).

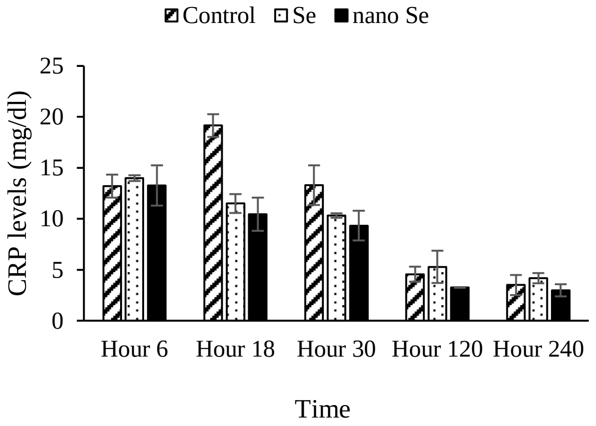

Figure 5. Comparison mean ± SD of CRP levels (mg/dl) in different groups ($p < 0.05$).

concentration of this parameter increased significantly in the SeNPs group at times T2, T3, and T4 compared to the selenium group. Although this parameter did not change at different times in the control group, it increased significantly in the other two groups from time 30 onwards (Figure 3).

The results showed that the SOD acivity at 18, 120, and 240 hours in both selenium and SeNPs groups was significantly higher than the control group, and SOD acivity was significantly higher in the SeNPs group at 30 and 240 ($p < 0.05$, Figure 4). A significant decrease of this parameter was observed at 18 hours in both selenium and control groups compared to time 0 of the study.

Levels of CRP

A significant decrease in CRP was seen in both selenium and SeNPs groups at times T2 and T3 .In the control group, a significant increase in CRP was seen at time T2, while in the other two groups, a significant and continuous decrease in this parameter was seen until the end of the study ($p < 0.05$, Figure 5).

Discussion

In the present study, the oxidative status of dogs undergoing OHE surgery was investigated by evaluating the activity of a number of antioxidants following the oral administration of selenium and SeNPs. Indeed, it is important to consider the management of oxidative stress after OHE, as a routine surgery in canines. Following surgical oxidative stress, antioxidant enzyme defense mechanisms such as SOD, GPx, and CAT are activated.[22] Although there is no precise information on the exact selenium requirement of dogs, the basis of selenium requirement for dogs is reported to be similar to that of cats. The minimum intake of selenium is 8.25 µg Se/kg$^{0.75}$.[10] Therefore, the prescribed dose of selenium for dogs undergoing neutering surgery was confirmed in the present study.

The function of SOD is to catalyze the reduction of superoxide to molecular oxygen and hydrogen peroxide (H_2O_2) .In fact, SOD acts as the first line of defense against free radicals caused by oxygen.[23] With the increase of SOD activity and the accumulation of hydrogen peroxide, GPx and CAT come into action. GPx reduces (neutralizes) reactive oxygen and nitrogen species using selenium as a cofactor.[24] Therefore, administration of selenium to the patient as an adjunctive therapy during oxidative damage can improve the antioxidant activity of GPx .The affinity of GPx for H_2O_2 is higher than that of CAT, but CAT is important in removing hydrogen peroxide, and as H_2O_2 increases, CAT activity also increases.[25]

In the current investigation, evaluation of SOD activity in the control group showed a significant decrease at 18 and 30 hours after the operation compared to before the operation. While in the nanoselenium group, there was no significant decrease in SOD activity after OHE compared to before surgery .Additionally, in the control group, no significant changes were observed in GPx activity before and after surgery. However, the study of Sakundech *et al*. also showed a significant increase in total antioxidant capacity and activity of these two enzymes on day 14 after OHE.[26] Similarly, a significant increase in the activity of these two antioxidant enzymes 14 days after ovariohysterectomy in female dogs was reported by Szczubial *et al*.[27]

Consistent with the previous studies, the results of our study also showed a significant increase in MDA from 6 hours to 10 days after OHE in all dogs. Meanwhile, the concentration of MDA in the nanoselenium group from the 6th hour of the study to the end of the study is significantly lower than the other two groups.[26,27] Although other studies have reported an increase in MDA as an index of lipid peroxidation 24 hours after OHE in dogs,[28,29] but no significant increase in MDA after OHE in cats reported by Torabi-Asl *et al*.[30]

The higher activity of SOD and GPx in the treatment groups of the current study (selenium and nanoselenium groups) from time 30 (compared to time 18 of the study) can indicate the effective antioxidant activity of treatments based on the selenium and selenium nanoparticles .It has been stated that a decrease in GPx activity due to the accumulation of harmful oxidants increases the likelihood of oxidative stress, while an increase in GPx is observed due to the absence of ROS caused by a decrease in oxidative stress.[31] Therefore, the higher activity of GPx and SOD in the nanoselenium and selenium groups can be seen as a sign of the reduction of harmful oxidants in those groups .On the other hand, serum GPx has been proposed as a biomarker of selenium status in dogs as a selenoenzyme that protects cells against oxidative damage.[32] Additionally, a positive and significant correlation between serum selenium level and GPx activity has also been reported.[33] Therefore, in the present study, the increase in GPx activity in the nanoselenium and selenium groups after OHE compared to the control group is a sign of the favorable effect of selenium in these groups. The negative correlation between MDA and GPx levels showed that under oxidative stress control conditions where GPx activity is high, MDA levels are low.[34,35] According to the results of the present study, the lowest level of MDA was observed 6 hours after OHE in the nanoselenium group, which indicated a better control of oxidative stress in this group compared to the other two groups.

It has been found that selenium can reduce lipid peroxidation and MDA levels while increasing the activity of antioxidants, and in addition, it plays a role in controlling oxidative stress by controlling inflammation.[36] In our study, the higher CAT antioxidant activity in both selenium and nanoselenium groups at 120 hours after OHE, as well as the significant higher activity of this enzyme in the nanoselenium group at 120 hours after OHE compared to before surgery, indicate the effectiveness of selenium and especially selenium nanoparticles in increasing CAT activity. Previously, increased activity of SOD and CAT after high serum levels of selenium was reported.[37] An increase in the activity of these two enzymes after the administration of selenium nanoparticles has also been reported.[38] It has been stated that SeNPs have less toxicity and better bioavailability compared to selenium and at the same dose.[39] The results of the present study also confirmed the antioxidant impacts of SeNPs compared to the oral administration of selenium in dogs undergoing OHE surgery.

It has been found that CRP is one of the first proteins whose serum level increases 4-8 hours after surgery and reaches its maximum value after 1-2 days.[40] In our study, the high concentration of CRP at 18 and 30 hours after OHE in dogs compared to the other two groups, as well as the significant increase in the level of this marker in the control group compared to the time before surgery, confirmed the anti-inflammatory effect of SeNPs and selenium. In addition, high CRP level at the time of low serum selenium level by Maehira *et al*.[41] reported. Several mechanisms have been described for the anti-inflammatory effect of selenium. Selenium deficiency appears to indirectly regulate the expression of cyclo-oxygenases and lipoxygenases through the mitogen-activated protein kinase pathway and cyclooxygenase-2, which is a key mediator of immune and inflammatory responses, by downregulating GPx activity.[42] The expression of these enzymes play a role in the production of lipid mediators such as prostaglandins, thromboxanes, and leukotrienes, which are inflammatory biomarkers released in oxidative damage and infection. Therefore, the administration of selenium and SeNPs can modulate the inflammation resulting from surgery.[43] Finally, the increase in the activity of the antioxidant enzymes GPx, SOD, and CAT and the decrease in the serum concentration of MDA and CRP following the oral administration of selenium and SeNPs in dogs that underwent OHE surgery suggest the antioxidant and anti-inflammatory effects of this supplemental treatment in this surgery.

Conflict of Interest

The authors declared is conflict of interest.

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