Original Article

The Pathological and Ultrasonographic Evaluation of the Chemical Castration in Dogs using Calcium Chloride Injection

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ABSTRACT

Many researchers have been curious about the chemical sterilization method, which may be a choice of castration. The 20% calcium chloride ethanolic solution can prevent animals from some tumors and control the side effects of surgical castration. This experiment divided 12 male mixed-breed dogs into sham and chemical groups (n=6). Normal saline and 20% calcium chloride (20 ml/testis) were injected in the sham and chemical group's testis, respectively. Ultrasonography and related scoring were operated at 0-, 7-, 14-, and 2-days post-injection to evaluate echogenicity and measure the left testes' dimensions. Blood samples were taken on days 0, 7, 14, and 21 of the experiment evaluating the superoxide dismutase (SOD), glutathione peroxidase (GPx), and testosterone levels. The semen in the left epididymis of the chemical group was aspirated on day 21 post-injection for counting the sperm numbers. The testes of all dogs were surgically removed at 21 days post-injection, and the left one was put in formaldehyde for tissue processing. The intertubular edema, necrosis of the seminiferous tubules, neutrophil infiltration, and calcification was scored. The average dimensions of the chemical groups' left testes significantly decreased 7, 14, and 21 days after injection. The echogenicity of the testes decreased in the chemical group. A significant echogenicity difference was observed between the first day and the 7th and 14th day in ultrasonography. Calcium chloride injection failed to reduce the mean testosterone levels on all experimental days compared to day zero. Otherwise, the sperm number in the left testes of the chemical group decreased on day 21 post-injection. The degree of intertubular edema with neutrophil infiltration and severe tubular necrosis in the chemical group was significantly higher than in the sham group on the experimental days, including 7, 14, and 21. The mild calcification in the chemical group is likely the reason for higher echogenicity on day 21. The scrotum was swelled and ulcerated in the chemical group. Ultrasound is effective in demonstrating the castration ability of calcium chloride in the chemical method. Due to the inflammatory clinical effects, the chemical method is recommended in dogs only when surgical methods are unavailable.

Keywords: Chemical castration, Dog, Pathology, Ultrasonography

1. Introduction

Castration is a general convention in domestic animals to control tumors and breeding (1). Low circulating testosterone levels are the main side effect of removing the testes (2). Castration can occur due to immunological, chemical, and surgical techniques. Physical castration can be performed mainly through surgery (orchiectomy). The immunological method of castration is based on decreasing testosterone levels through immunocontraception. It means using an animal's immune system to prevent it from fertilizing offspring. Chemical castration is based on the burning or osmotic mechanisms of chemical compounds that provoke the seminiferous destruction of testes. Some chemical castrators are CaCl2, lactic acid, and NaCl (3-5). Surgical castration could be extremely painful and accompanied by side effects. For example, it is operated in cattle despite inadequate requirements, resulting in considerable pain and microbial contamination (6). The calves suffered from various disorientations, decreased feed intake, and agitation. Such distress conditions can be set by traits capable of determining anxiety, discomfort, and inflammation methods, such as serum cortisol levels and acute phase proteins (paraoxonase 1) (7). Currently, in the cattle breeding industry, there are many management difficulties with pain and distress on the animals. Thus, using castration techniques to improve animal interest benefits the said industry (8). The intracellular antioxidant levels decreased in the chemical-castrated animals by elevating reactive oxygen species (ROS) levels. The antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which are necessary for neutralizing of ROS (9). The injection to testes is an efficient minimally interfering castration method. However, the effects of NaCl injection to testes on the castration of calves are not as practical as CaCl2 for chemical castration (10). The present study aimed to examine the results of injection to testes with 20% CaCl2 on characteristics of oxidative tension and inflammatory responses in dogs for up to two weeks.

2. Material and Methods

2.1. Study Animals

In this experiment, 12 adult and clinically healthy male mixed-breed dogs weighing approximately 15 kg were divided into sham and chemical groups (n=6). Normal saline was injected into the sham group's testis, while the chemical group's testis received a single bilateral intratesticular injection of 20% calcium chloride (20 ml/testis/Inoxia, UK). In the chemical group, calcium chloride was injected into both testicles. The injection sites were forwarded from the testis's abdominal surface towards the rostral. Blood samples were taken on days 0, 7, 14, and 21 of the experiment.

2.2. Molecular Examination

Half of the tubes were contained and half free of Ethylenediaminetetraacetic acid (EDTA) for each dog blood sample. In the serum samples, testosterone hormone was analyzed by the ELISA (human kit, ZellBio, Germany) assay. Blood-containing tubes were evaluated to detect GPx and SOD activity (Randox, UK).

2.3. Ultrasonografical Analysis

Before taking blood samples, the ultrasonografical evaluation was operated on the left testes to measure dimensions and echogenicity. The echogenicity of the left testes was measured before and after the chemical injection and on the 7th, 14th, and 21st days after the injection. A micro-convex probe studied the dogs on 7.5 MHz. The echogenicity brightness was scored as 0: No, 1: low, 2: medium, 3: high, and 4: very high.

2.4. Histopathological Analysis

All testes were surgically removed at the end of the experiment on day 21, and the left testes put in 10% buffered formaldehyde. Tissue was processed for investigating pathological changes. The microscopic phenomena were scored, including the intertubular edema, necrosis of the seminiferous tubules, neutrophil infiltration, and calcification was scored from 0-4 based on the absence - observation of low, medium and high intensity of elements under different

microscopes (11).

2.5. Statistical Analysis

All data were explored by operating the GraphPad Prism software (version 9). Values were defined as mean ± standard errors. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Ultrasonografical Findings

3.1.1. Dimensions of the Testes

All dogs under calcium chloride injection into the testis exhibited no distress behavior, unusual fatigue, or lack of appetite. In the chemical group, the ultrasound revealed that the testis' dimensions were significantly reduced according to the time passing. However, no significant size change was seen in the sham group (Fig 1, 2).

3.1.2. Echogenicity of the Testes

The echogenicity of the testes decreased in the chemical group, and it was significant during the investigation 7, 14, and 21 days post-injection compared to day zero, both before and after injection. The 14th day after the injection showed the lowest echogenicity (Fig. 2).

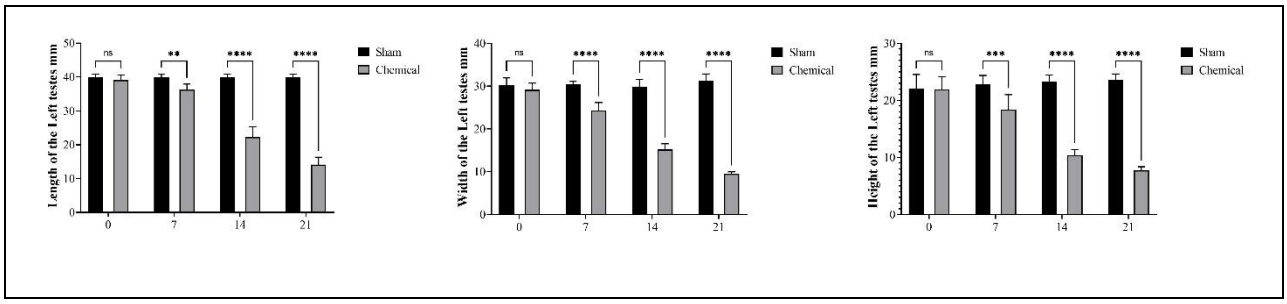

Figure 1. Comparative analysis of the left testes' dimensions of dogs at various days of experiment in sham and chemical groups using sonography (ns: Non significant; *: *P*<0.03; **: *P*<0.002; ***: *P*<0.0002; ****: *P*<0.0001)

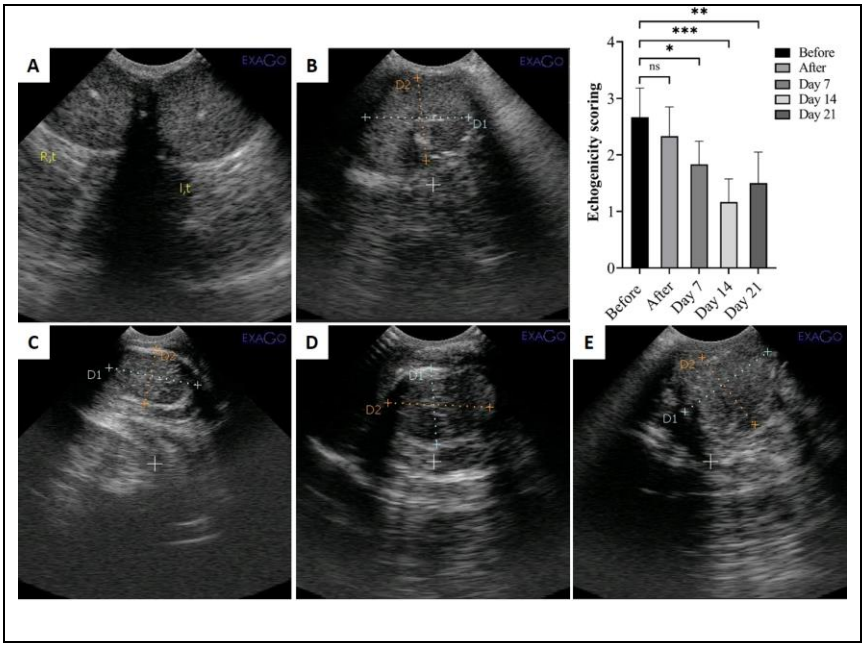

Figure 2. B-mode ultrasound of the left testicles of chemical group dogs. A: Measuring the depth and width before injection. B: Immediately after injection. C, D and E: First, second, and third weeks after injection, respectively. The diagram shows the comparison of the echogenicity in the 0, 7th, 14th, and 21th days post injection

3.2. Molecular Findings

3.2.1. Superoxide Dismutase (SOD) and Glutathione

Peroxidase (GPx) Findings

Regarding SOD and GPx, we should mention that the concentration of SOD in the blood of animals receiving calcium chloride was significantly lower than that of the control group. This finding was seen on all the study days except day zero (Fig. 2). Regarding GPx, this reduction was observed on days 14 and 21 but not on days 0 and 7 (Fig. 3).

3.2.2. Testosterone Findings

Calcium chloride injection could not reduce the mean testosterone levels on all days of the experiment (Fig. 4). The sperm number of the testis of the chemical group was significantly reduced (Fig. 4).

3.3. Histopathological Findings

In the microscopic evaluation of the tissues, there were edema and severe tissue necrosis without significant leucocyte infiltration and calcification. The pathological findings were higher than the chemical group (Fig 5, 6).

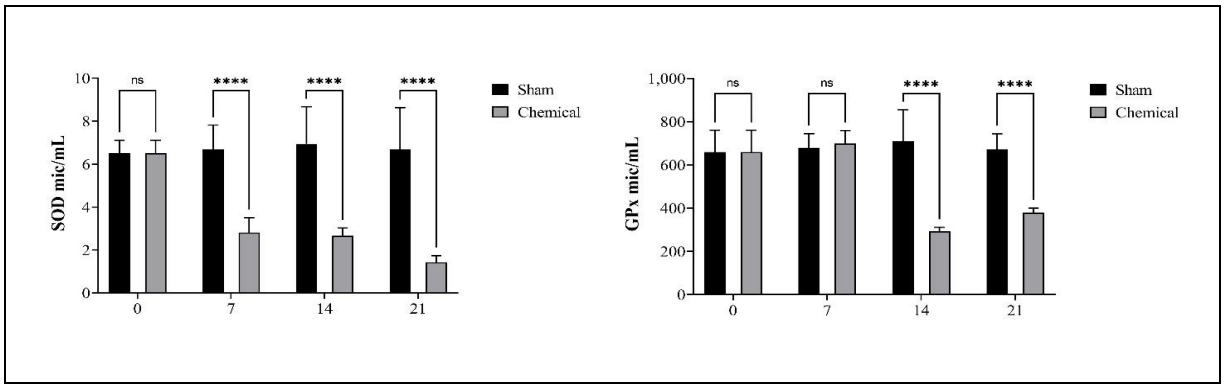

Figure 3. Comparative analysis of the SOD and GPx activity in blood samples of dogs at various days of experiment in sham and chemical groups (ns: Non significant; *: $P<0.03$; **: $P<0.002$; ***: $P<0.0002$; ****: $P<0.0001$)

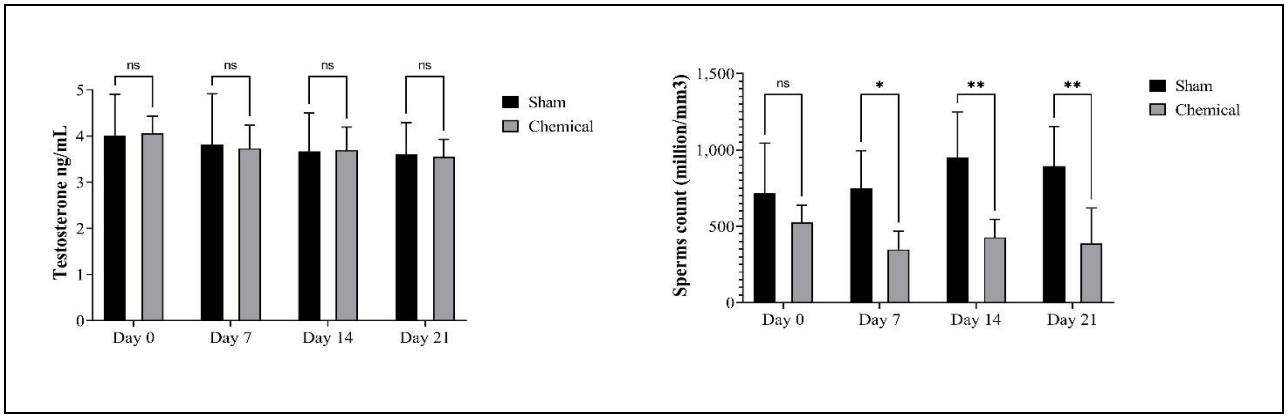

Figure 4. Comparative analysis of the testosterone levels and sperm counts of dogs at various days of experiment in sham and chemical groups (ns: Non significant; *: $P<0.03$; **: $P<0.002$; ***: $P<0.0002$; ****: $P<0.0001$)

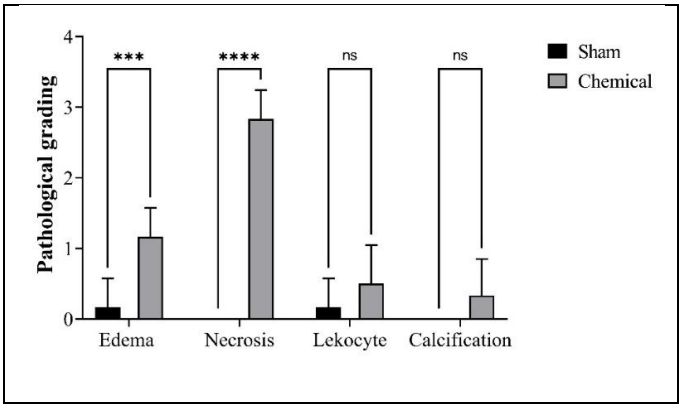

Figure 5. Comparative analysis of the pathological findings in dogs at various days of experiment in sham and chemical groups (ns: Non significant; *: $P<0.03$; **: $P<0.002$; ***: $P<0.0002$; ****: $P<0.0001$)

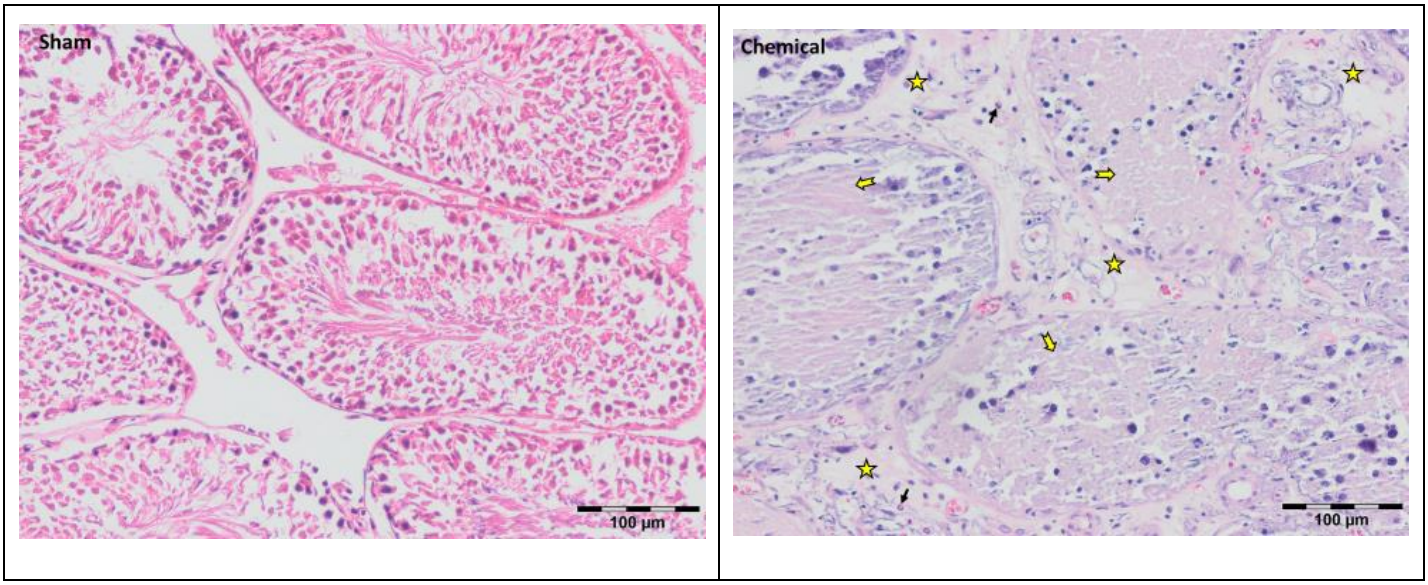

Figure 6. Comparison of the histopathological lesions of the testes from sham (UP) and chemical (DOWN) groups in dogs on 21th day of experiment (H&E×100)

4. Discussion

The behavior of animals treated with calcium chloride was mild discomfort with pain in the present study, similar to a few prior investigations (3, 5, 6). Chemical irritation could induce ROS, which is the cause of more pathological lesions. In the normal condition of physiologic metabolism, various cellular antioxidant mechanisms could reduce the amount of ROS (3, 9). The significant reduction of SOD and GPx was evaluated in the blood samples of chemical groups. Then, it is suggested that the oxidative stress increased after injecting 20% calcium chloride (9). Abu Khalil et al., in a study similar to the current research, but on donkeys, found that the reduction of antioxidants and the increase of stress indicator due to the effect of 20% calcium chloride solution for animal castration was more than surgical castration. They reported that serum cortisol level as a stress indicator in donkeys with calcium chloride castration was higher than in animals with the surgical method (4). In a study on the chemical castration of dogs, the antioxidant parameters were reduced on day 14 post-castration (12), which was similar to our research. The higher the calcium chloride dose, the more GPx and SOD levels are reduced in testicular tissue (9). In a report by Jana et al., calcium chloride injection into rat testes could not reduce GPx and SOD levels in castrated testis tissues with 2.5 mg/kg. In contrast, the SOD level was decreased, and the malondialdehyde was increased in testes treated with a higher dose of calcium chloride (13). Jana et al. in their study evaluated the oxidation or ant oxidation that occurred in the testes, whereas the present study attended to the blood circulatory parameters. In another study on cattle castration comparing chemical and surgical methods, the oxidative stress and inflammation were mild (6). Another study on rats revealed that calcium chloride for chemical castration could not reduce the level of corticosteroid (13). Evaluation of the testosterone levels in the present study revealed no significant reduction in serum of various days post-injection compared to day zero. Another study revealed that using calcium chloride in donkeys for chemical castration could not decrease testosterone levels in plasma (5). Similarly, the present study shows that using calcium chloride in dogs could not reduce testosterone levels (13). The saturated saline solution in rats could reduce testosterone plasma levels (14). Furthermore, the chemical castration of calves with 20% sodium chloride, or 20% calcium chloride injection in dogs (15 and 20 mg/kg), has reduced testosterone levels (12). in contrast to the present study. In contrast to our research, degeneration of testis interstitial cells (Leydig) due to chemical castration could reduce plasma testosterone levels. This investigation revealed that the sperm numbers in the dogs under chemical castration were decreased than in the sham. In a previous investigation, low quantity injection of 20% calcium chloride could not reduce sperm numbers in rat epididymis, in contrast to the high quantity of chemical materials that can do (13). A study on the injection of calcium chloride in the testes of dogs revealed that it could reduce sperm count in the epididymis (12). This finding is interconnected with the testosterone level (15). However, we did not show that this relation between testosterone and sperm count may be due to the short study duration. Pathologically, calcium chloride can create necrosis, Leydig cell atrophy, infiltration of leukocytes, and fibrosis in the testes (13). Calcium chloride injection in the bull testes led to tubular and Leydig cell necrosis, leukocyte infiltration, fibroplasia, and edema (16). The 30% calcium chloride injection into buffalo ameliorated and induced fibrosis (17). Some research revealed that 20% and 30% calcium chloride created severe necrosis of tubules with Leydig cells in the lumen of the seminiferous tubules in puppies, mature dogs, and 5-month-old calves (6, 18). The recent pathological findings from the research were similar to previous studies except for mild calcification on day 21 post-injection. Another study showed that the chemical castration had a few observable clinical side effects on general health, such as testes swelling with pain (5). In present study, the related

adverse effects of the chemical agents were low, similar to other reports (16). Then, chemical castration may be associated with clinical appearance, pain, and inflammation. According to the outcomes of this analysis, chemical castration methods could lead to tissue degradation. Chemical castration should not be used alone due to high oxidative burst, ROS production, pain, edema, and clinical appearance. Administration of antioxidants in addition to NSAIDs is suggested to decrease the side effects.

Authors' Contribution

NK: data collection, drafting the manuscript and supervised the study process; AV: study design and conducting the study; AA: supervised the pathology slides, SMR: supervised the castration process; PM: review of the manuscript and consulting on biochemical analysis.

Ethic

The research project was approved by the Ethics Committee of Science and Research Branch Islamic Azad University (code: IR.IAU.SRB.REC.1397.215).

Conflict of Interest

The authors declare that they have no conflict of interest.

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