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Chemical castration using an intratesticular injection of mannitol: a preliminary study in a rat model

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Abstract: This study was aimed to evaluated chemical sterilization using an intra-testicular injection of mannitol 20% in rats. Fifty male Wistar rats were divided into five groups, including 1) intact, 2) surgical castration and, 3 to 5) intra-testicular injections of normal saline, hypertonic saline, and mannitol, respectively. The baseline testosterone levels were measured. Surgical castration was performed in group 2. Intra-testicular injections were performed in groups 3, 4, and 5. On day 60, the testosterone level was evaluated. Then, sperm count, viability, motility, morphological abnormalities and DNA damage were studied. Gonadosomatic index was recorded on day 60. Serum testosterone level was significantly decreased in hypertonic saline and mannitol groups compared to the intact and normal saline groups. Severe testicular atrophy, tubular depletion, vacuolization, the formation of multinucleated giant cells, and testicular congestion were noted in the mannitol group. No live spermatozoa were found in groups 4 and 5. Significant reduction in sperm count and morphologic abnormalities were observed in the mannitol group. Gonadosomatic index in groups 4 and 5 were significantly decreased. Severe dehydration and osmotic shock due to the intra-testicular injection of mannitol resulted in testicular degeneration and spermatogenesis disturbance. It could be concluded that intra-testicular injection of mannitol could effectively induce infertility in male rats.

Key words: Chemical castration, mannitol, rat, sperm, testosterone

1. Introduction

Sterilization aims to control animal over-population and eliminate unwanted secondary sexual behaviors [1]. In this regard, different surgical and nonsurgical methods have been recruited in animals. For decades, surgical castration has been considered the standard method of male sterilization. However, several shortcomings have been reported with this method such as small-scale application, medical equipment, the requirement of anesthesia, a trained veterinarian, a sterile surgical suite, high cost, time consumption, need for postoperative care, and risk of post-operative complications [2].

Nonsurgical approaches are intended to be less expensive and less labor-intensive options for sterilization, allowing mass-scale sterilization quickly, and safely [3]. Chemical castration includes an intra-testicular injection of a sclerosing or toxic agent to induce irreversible damages and/or dysfunction to testicles [4], which leads to azoospermia type of infertility. It has been shown that various agents such as formalin, chlorhexidine, and ethanol resulted in complete infertility [5].

Calcium chloride and zinc gluconate have been advocated to induce complete contraception and approved to be used in some countries [6–9]. However, there are a few studies illustrating severe complications after chemo-sterilization with these two agents in various domestic animals. The most frequently reported complications included scrotal self-mutilation due to testicular inflammation, necro-suppurative orchitis, scrotal necrotizing dermatitis, scrotal sloughing, and scrotal fistula in which either medical management such as antibiotic- and anti-inflammatory therapy, or surgical intervention like scrotal ablation were performed on these cases [10–13].

Recent studies in rats, tried to use an intratesticular injection of hypertonic saline, which has been proven more useful and safer compare to previous agents [14,15]. It is believed that the injection of hypertonic saline instantly causes a local osmotic shock, severe dehydration, and extensive necrosis [16]. However, this method was incapable of providing sterilization in adult canine model [17].

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Mannitol, a sugar alcohol with osmotic properties, is primarily recommended to treat increased intracranial pressure and glaucoma. The diuretic effect of mannitol is also known and is used to force urine production in patients suffering from acute kidney failure [18]. Mannitol is a relatively benign and nontoxic agent and has beneficial effect when it is administered intravenously. However, in case of extravasation it causes soft tissue damage via capillary collapse, local hypoxia, and necrosis [19,20]. An in vitro study revealed that mannitol exposure induces vascular endothelial cell apoptosis leading to tissue necrosis [21].

The presented study was undertaken to attempt that intra-testicular injection of mannitol could potentially induce a state of severe dehydration and osmotic shock which may lead to testicular degeneration and spermatogenesis arrest in a similar manner to hypertonic saline. The purpose of this research was to evaluate and compare the efficacy of mannitol with hypertonic saline solution as chemo-sterilizing agent in male rats.

2. Materials and methods

2.1. Animals

Fifty mature male Wistar rats (180–220 g) were used in this study. The rats were housed in plastic cages and acclimated at 25 ± 2 °C and relative humidity of 55 ± 10% under natural light/dark conditions for one week before the experiment. Rats were fed a commercial pellet diet twice daily and water was provided ad libitum. The experiment was carried out according to the guidelines of the Institutional Ethics Committee for Animal Research (No. IR-UU-AEC-3/PD/512-587). Following a week acclimation period, the animals were divided into five groups (10 rats in each) including, 1) intact, 2) surgical castration, 3) normal saline (0.9%), 4) hypertonic saline (20%) and 5) mannitol (20%). Intact rats were not manipulated in any way to calculate the gonadosomatic index (GSI) at the end of the study. They were also kept to measure the baseline serum testosterone and its concentration on day 60.

2.2. Hormone assay

For serum isolation, blood tubes were centrifuged at 2000 g for 5 min. Serum samples were separated and kept at –20 °C until the analysis. To assess testosterone concentration, enzyme-linked immunosorbent assay kit (Monobind Inc., Lake Forest, USA) was used according to the manufacturer's instruction. Briefly, control, and serum samples (10 µL) were dispensed into the respective wells. 100 µL testosterone-HRP conjugate was applied to each well. Substrate blank was dispensed into well A1. Then, the wells were covered with aluminum foil. At room temperature, incubation was carried out for 1 h. The cover was removed and the contents aspirated. Then, the wells were washed three times with diluted HRP wash buffer, 300 µL per well per wash. Soaking time between each wash cycle was about 5 s. The remaining fluid was removed by tapping the strips on a paper towel. TMB (tetramethylbenzidine) substrate solution (100 µL) was added to all wells. In a dark place, the wells were incubated for 15 min at room temperature. Stop solution (100 µL) was applied to all wells in the same order and the same rate as for the substrate. The absorbance was read at 450 nm within 20 min after adding the stop solution.

2.3. Surgical castration

The animals in the second group underwent anesthesia with an intraperitoneal injection of 90 mg/kg ketamine (10%, Bremer Pharma GmbH, Bremerhaven, Germany) and 5 mg/kg xylazine (2%, Alfasan, Woerden, Netherlands). Castration was performed through two scrotal incisions. The spermatic cords were double ligated using Dexon (3-0, Covidien, Mansfield, USA), and the scrotal incision was sutured using Silk (3-0, Ethicon, Somerville, USA). The testicles were weighed using a digital weight scale to calculate the baseline gonadosomatic index (GSI).

2.4. The preparation of solutions and intra-testicular injection

To prepare a 20% hypertonic saline, 2 g sodium chloride was sterilized using an oven at 160 °C for 2 h. Then, it was dissolved in 10 mL sterile distilled water. Intravenous infusion solutions of mannitol 20% (Polifarma, İstanbul, Turkey) and 0.9% normal saline (Polifarma, İstanbul, Turkey) were provided from a local drug store. For intra-testicular injections, the rats were anesthetized with intraperitoneal injection of 40 mg/kg ketamine and 5 mg/kg xylazine, and the scrotum was prepared aseptically. Using a 27 gauge needle, 0.5 mL of normal saline, hypertonic saline, and mannitol were injected into each testicle in multiple directions in the concerned groups.

2.5. Sampling

Sixty days after surgery/injections, the rats were anesthetized with intraperitoneal injection of 90 mg/kg ketamine and 5 mg/kg xylazine, then tail blood samples were collected using a 23G needle to determine baseline and post-intervention serum testosterone levels and then, serum testosterone levels were measured. The hCG stimulation test is known as a reliable dynamic test for evaluation of testicular steroidogenesis. After the first blood sample collection, 100 IU human chorionic gonadotropin (hCG; Intervet, Boxmeer, Netherlands) was injected intramuscularly, 120 min later, cardiac blood samples were obtained using a 21G needle. At the end of day 60, the rats were sacrificed using a CO_2 chamber, and the testicles and epididymis were dissected out. Then, body weights and testicular weights were measured for GSI using the following formula:

GSI = [Gonads weight (g)/Body weight (g)]×100.

2.6. Clinical evaluation

All rats were examined for routine clinical observations including scrotal swelling and dermatitis, fistula formation and discharge, and testicular pain on palpation at weekly intervals.

2.7. Sperm parameters

By mincing, epididymal spermatozoa were collected from the caudal region of the epididymis into small pieces in 1 ml of human tubal fluid + 4 mg/ml bovine serum albumin and incubated for 10 min at 37°C and 5% CO_2 to allow sperms to swim out of the epididymal tubules. The standard hemocytometer method was used for epididymal sperm count. After dilution of epididymal spermatozoa to 1:20 in HTF medium, 10 µL of the diluted specimen was transferred to the hemocytometer. The spermatozoa were counted with a light microscope (Olympus, Tokyo, Japan) at 400×. The sperm count was expressed as the number of sperm per milliliter. The sperm motility (%) was estimated visually using a light microscope at 400× magnification. A drop of sperm suspension (10 µL) was placed on a clean prewarmed microscope slide and a coverslip was placed over the droplet. At least 10 microscopic fields were observed. The percentage of motile spermatozoa was assessed microscopically within a few min of their isolation from the epididymis and recorded as a percentage of motile sperm of the total counted spermatozoa. For sperm viability, a 20 µL of sperm suspension was mixed with a volume of 20 µL of 0.05% eosin-nigrosin. After 2 min of incubation at room temperature, a bright field microscope with 400× magnification was used to assess the slides. Dead sperms with altered plasma membrane appear pink and live sperms with intact plasma membrane are not stained. 200 sperms were counted in each sample and sperm viability (%) was recorded (Figure 1A). To assess the sperms DNA damage, the acridine-orange staining was used. Thick smears were fixed in Carnoy's fixative (methanol: acetic acid 1: 3) for 2 hours. Then, the slides were stained for 5 min and gently rinsed. About 200 sperms were evaluated, among which green-staining sperm have intact DNA, whilst red-staining sperms have denatured DNA. All slides were prepared and analyzed, using an Epi-fluorescent microscope (GS7; Nikon Co., Tokyo, Japan) (Figure 1B).

2.8. Histopathological analyses

For analysis of spermatogenesis, each testicular biopsy was evaluated using the Johnsen score under 200× magnification. In total, 100 tubules were examined per slide and each slide was scored on a scale of 1–10 based on the level of spermatogenesis. Accordingly, a score of 10 represents maximum spermatogenesis activity, while a score of 1 represents the complete absence of germ cells [22]. The testes were fixed in 10% buffered formalin and embedded in paraffin and five µm thick sections were stained with hematoxylin and eosin. Seminiferous tubules (n = 30) were randomly examined per section. The testicular biopsy score (TBS) indicates the overall state of spermatogenesis in the seminiferous tubules. The spermiogenesis index (SPI) was defined as the ratio of the number of seminiferous tubules with spermatozoids to the depleted seminiferous tubules [23].

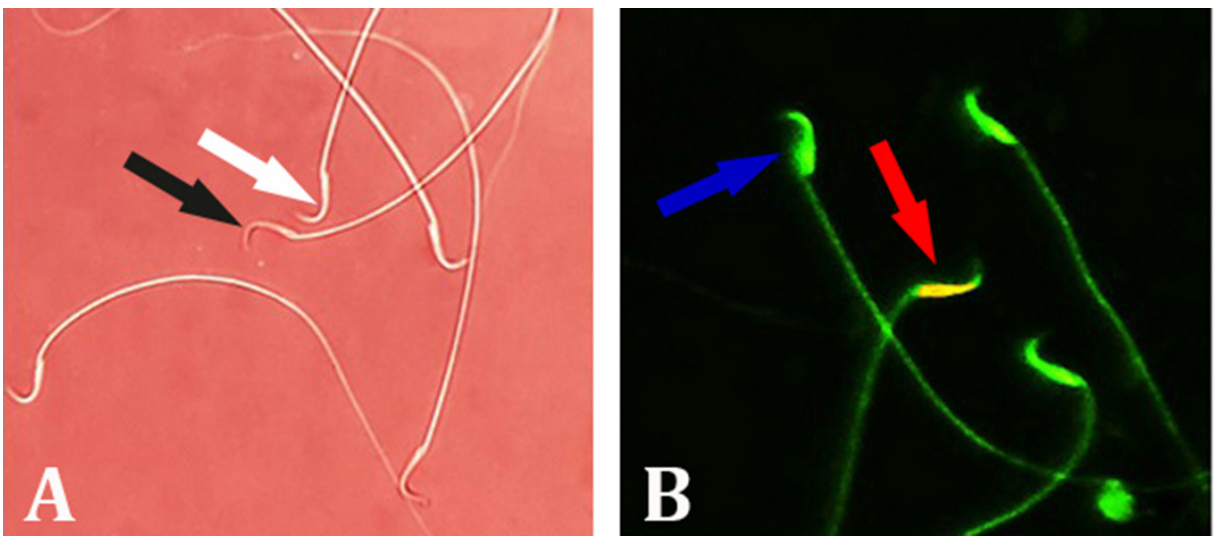

Figure 1. A) The black arrow indicates the dead sperm in which the eosin stain has penetrated the cytoplasm and appears pink and the white arrow shows the live sperm, which is not stained (Eosin-nigrosin staining, 1000×); B) Blue arrow indicates a normal sperm with undamaged DNA showing green head and red arrow shows a sperm with damaged DNA indicated by yellow or orange head (Acridine orange staining, 1000×).

2.9. Statistical analysis

The Kolmogorov–Smirnov and Levene's tests were used for data normality and homogeneity of variance, respectively. The results are presented as mean ± standard deviation. Data were analyzed with one-way ANOVA, followed by Tukey's test, using Minitab software (version 16.0; Minitab Inc., Boston, USA), and $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Clinical findings

No adverse effects including scrotal inflammation, fistula formation or discharge were observed following intra-testicular injections. Mild testicular swelling was noted due to the injections which subsided at the end of the first week. Self-mutilation due to testicular pain was not observed in any of rats after the intra-testicular injections and scrotal palpation was well tolerated without signs of discomfort or aggressive response. No complications were noted in surgically castrated rats.

3.2. Gonadosomatic index

The animals in intra-testicular injected groups exhibited reduced testicular weights versus intact rats (Figure 2). The reduction in the normal saline group was not significant when compared to the intact group ($p > 0.05$). While a significant reduction was observed in both hypertonic saline and mannitol groups in comparison with the intact group on 60th day ($p < 0.05$). No significant difference was observed between hypertonic saline and mannitol groups in terms of gonadosomatic index ($p > 0.05$).

3.3. Sperm parameters

The rats in all intra-testicular injection groups exhibited a significant reduction in sperm count compared to the intact rats ($p < 0.05$). Normal saline injection significantly reduced the count to about one half of that in the intact rats. Also, a notable decrease in sperm count was observed as a result of hypertonic saline and mannitol injections ($p < 0.05$) with no significant difference between these two groups ($p > 0.05$) (Figure 3).

Sperm motility examination revealed that any intra-testicular injections were significantly reduced the percentage of sperm motility when compared to the intact group on day 60 ($p < 0.05$). No significant difference was observed between hypertonic saline and mannitol groups ($p > 0.05$) (Figure 4A).

The results of sperm viability were similar to the sperm motility and a significant reduction in sperm viability was observed due to the intra-testicular hypertonic saline and mannitol injections ($p < 0.05$). In hypertonic saline injected rats, no live sperm was detected using eosin-nigrosin staining (Figure 4B).

In hypertonic saline and mannitol injected rats, increased percentages of sperm with DNA fragmentation were determined when compared to the intact and normal saline samples ($p < 0.05$). Also, a significant increase in sperm DNA fragmentation was observed in the normal saline group in comparison with the intact samples ($p < 0.05$) (Figure 5).

3.4. Histopathological findings

According to Johnsen's score system, there were significant reductions in tubular quality and maturation following intra-testicular injections compared to the intact rats ($p < 0.05$) (Figure 6). Normal saline injection resulted in disruption of the germinal layer and exfoliation of spermatogenic cells into the lumina associated with testicular congestion. Severe intratubular vacuolization and spermatogenic cells maturation arrest were observed

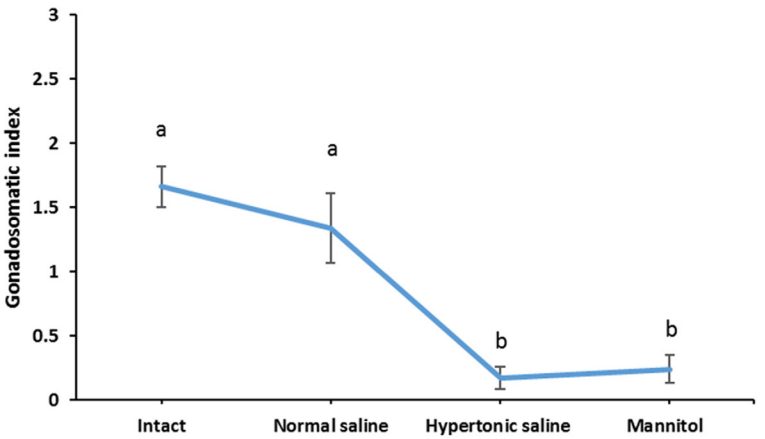

Figure 2. Gonadosomatic index of the rats in different experimental groups on day 60.
[a,b] Different letters indicate significant differences among the groups ($p < 0.05$).

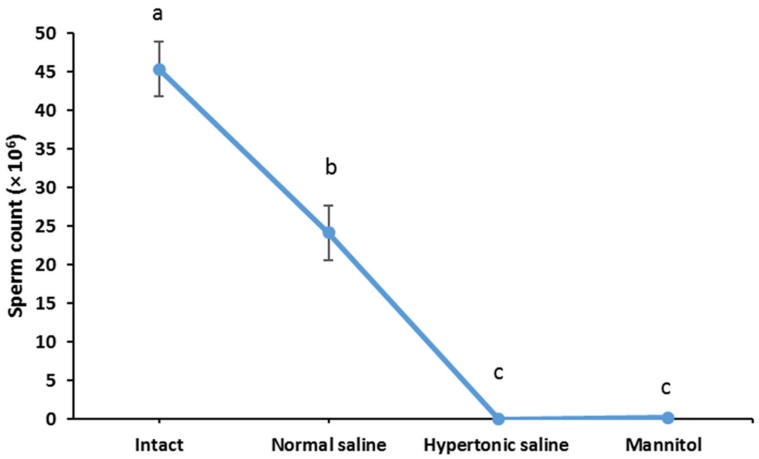

Figure 3. Sperm count of the rats in different experimental groups on day 60.
[a,b,c] Different letters indicate significant differences among the groups ($p < 0.05$).

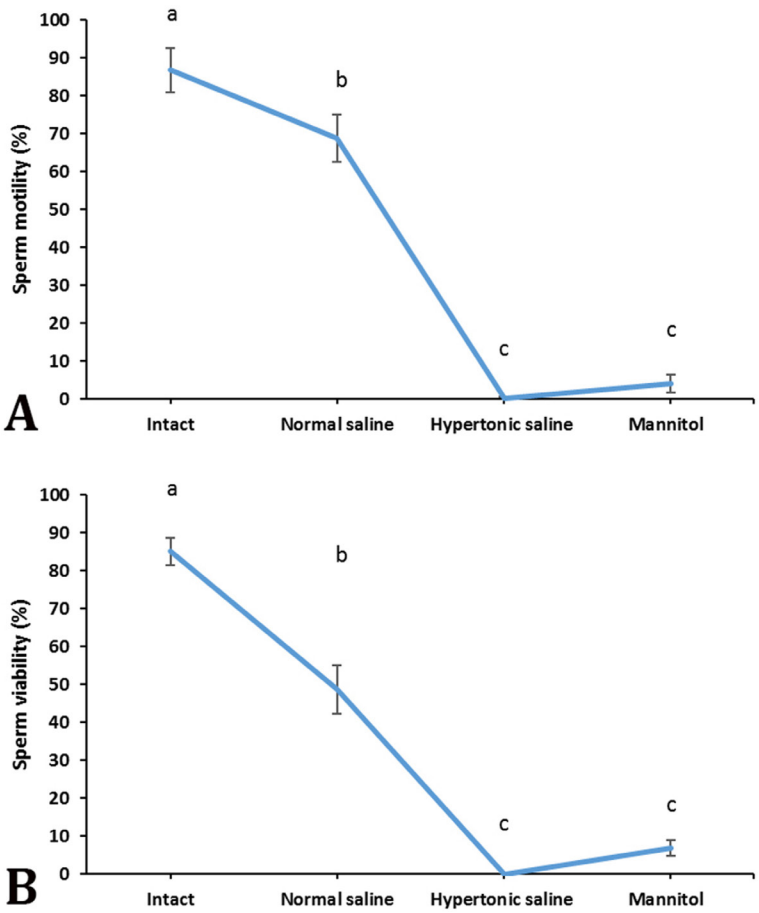

Figure 4. A) Percentages of rat sperm motility in different experimental groups on day 60. B) Percentages of rat sperm viability in different experimental groups on day 60.
[a,b,c] Different letters indicate significant differences among the groups ($p < 0.05$).

after hypertonic saline injection. Following mannitol injection, tubular depletion, atrophy, vacuolization, the formation of multinucleated giant cells, and testicular congestion were noted. Statistical analysis revealed no significant differences between the hypertonic saline and mannitol groups ($p > 0.05$) (Figure 7).

According to testicular biopsy scoring, all intra-testicular injections resulted in a significant decrease in the quality of seminiferous tubules and overall scores when compared to intact samples ($p < 0.05$). The observed difference between hypertonic saline and mannitol groups was not significant ($p > 0.05$) (Figure 8A).

A significant reduction in SPI was observed due to hypertonic saline and mannitol injections compared to the intact and normal saline-injected rats ($p < 0.05$). The difference between the two latter groups was not significant ($p > 0.05$) (Figure 8B).

3.5. Testosterone concentration

Hormonal evaluations revealed that there were significant changes in testosterone levels due to intra-testicular injections when compared to the baseline and end value in intact rats ($p < 0.05$). No significant differences were found between the castrated and intra-testicular injected rats in terms of testosterone concentration at the end of the study. hCG stimulation resulted in a remarkable elevation in testosterone concentration in intact and normal saline-injected rats ($p < 0.05$). However, the observed increases in both hypertonic saline and mannitol groups were not significant after hCG stimulation ($p > 0.05$) (Figure 9).

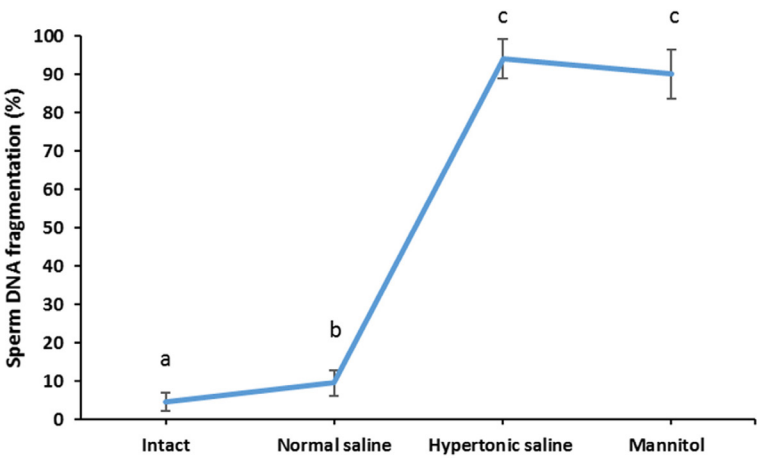

Figure 5. The percentages of sperm with DNA fragmentation of rats in different experimental groups on day 60.
[a,b,c] Different letters indicate significant differences among the groups ($p < 0.05$).

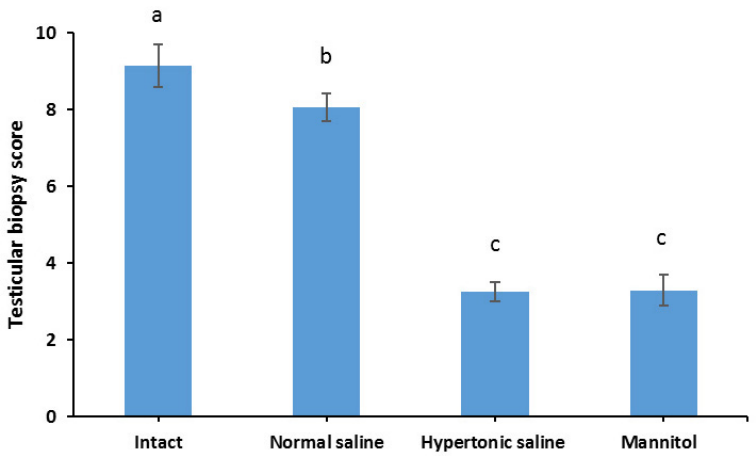

Figure 6. Testicular biopsy score of rats according to Johnsen's score system in different experimental groups on day 60.
[a,b,c] Different letters indicate significant differences among the groups ($p < 0.05$).

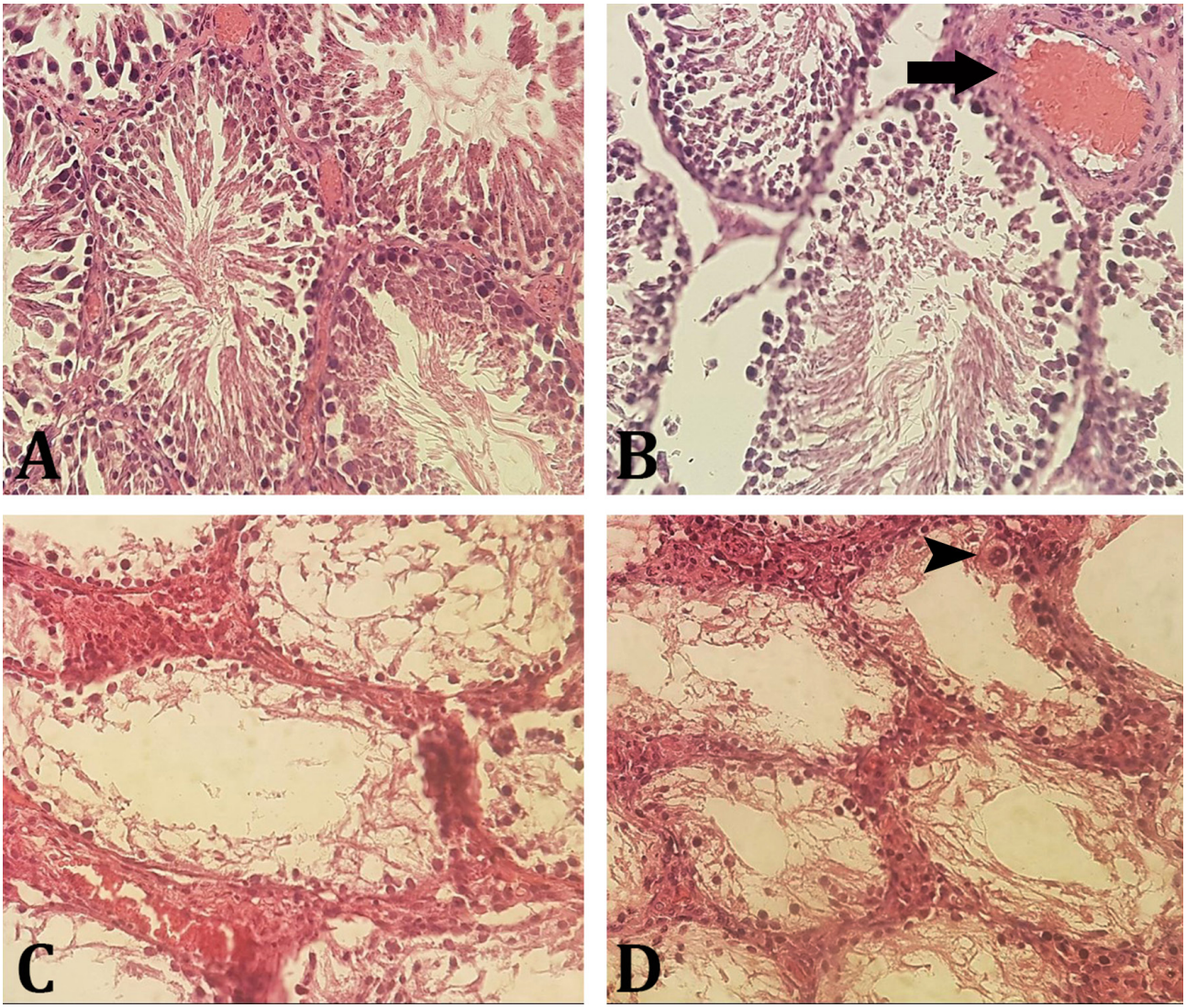

Figure 7. The effects of different treatments on testicular histoarchitecture. A photomicrograph of a section in rat testis of A) Intact group exhibiting active spermatogenesis, characterized by well-organized spermatogenic cells distribution in the germinal epithelium of seminiferous tubules; B) Normal saline-treated group showing small degrees of spermatogenic cells disarrangement and detachment as well as testicular hyperemia (arrow); C) Hypertonic saline group showed spermatogenic cells maturation arrest along with severe intratubular vacuolization; D) Mannitol group exhibited tubular depletion, atrophy, and vacuolization. In the latter, multinuclear giant cell formation (arrowhead), (Hematoxylin and eosin, 400×).

4. Discussion

Overpopulation of free-roaming dogs and cats cause major public-health problems in urban areas. These problems include the prevalence of zoonotic and parasitic diseases [3], the incidence of biting injuries and secondary infections [24], noise nuisance [25], increased risk of a road traffic accidents [26], and fouling in public places [27] have been reported. Therefore, a potential preventive strategy should be devised to control this issue.

Surgical castration is considered the most effective, safe, and humane procedure to induce male sterilization [28]. However, several disadvantages have been associated with this technique such as the high cost, the risk of surgical and anesthetic complications, and the need for post-operative care [29].

Male chemical castration is an effective alternative, because it eliminates the disadvantages of surgical sterilization. It also provides the possibility of large-scale sterilization in a short period. An ideal chemical sterilant should effectively arrest both spermatogenesis and androgenesis and reduce libido without provoking systemic toxicity or serious side effects [2].

Several chemical agents have been studied in this regard and different results have been obtained [5–9]. For

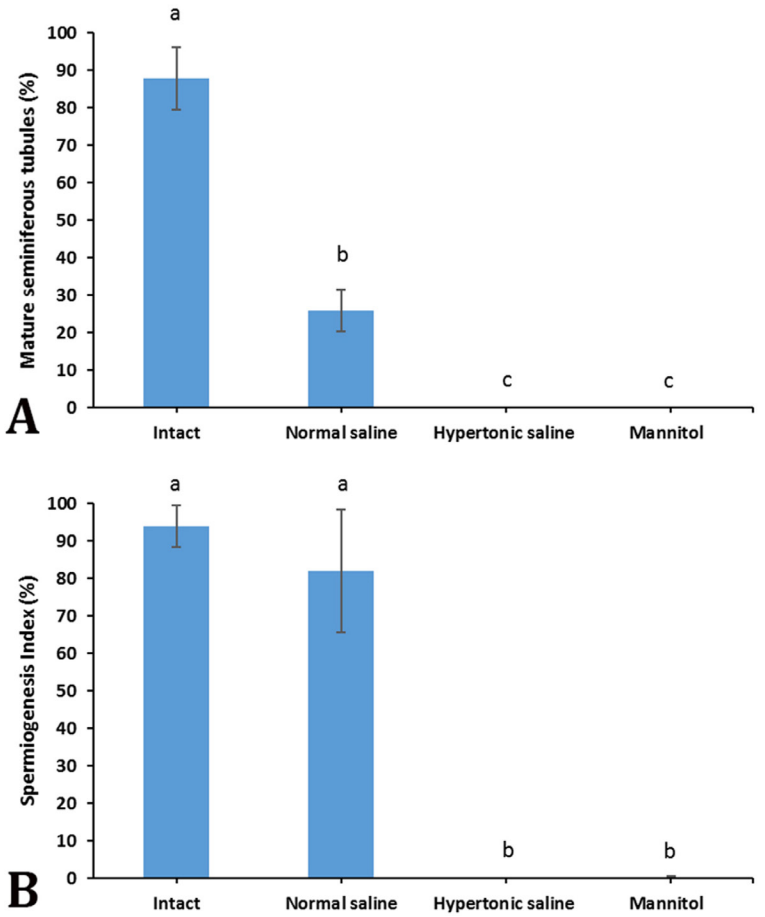

Figure 8. A) The percentages of mature seminiferous tubules in rats in different experimental groups on day 60. B) Spermiogenesis Index (%) of rats in different experimental groups on day 60.
[a,b,c] Different letters indicate significant differences among the groups ($p < 0.05$).

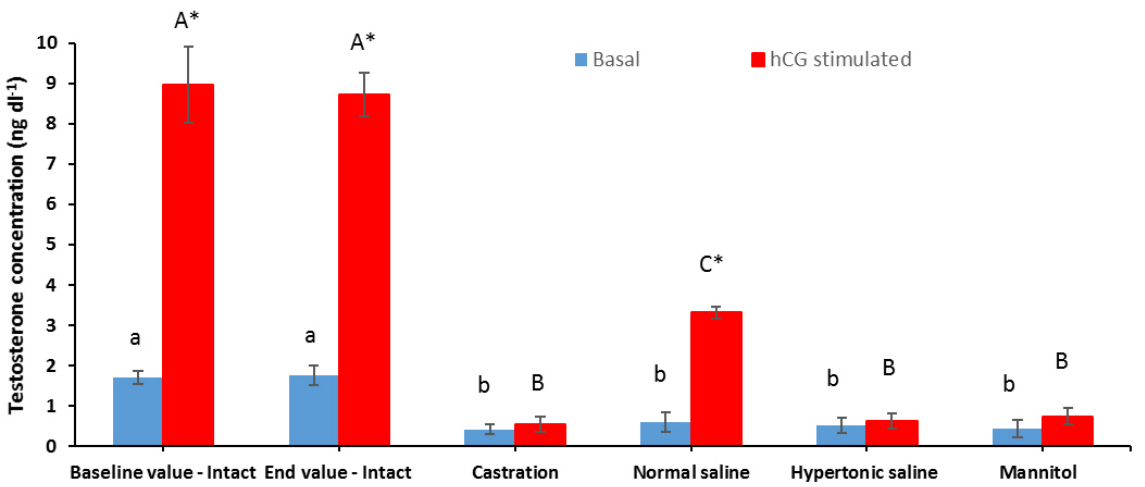

Figure 9. Testosterone concentration of rats in different experimental groups.
[a,b,c] Different lowercase letters indicate significant differences in testosterone level before hCG injection among the groups ($p < 0.05$).
[A,B,C] Different uppercase letters indicate significant differences in testosterone levels after hCG stimulation among the groups ($p < 0.05$).
* Significant differences between pre- and post-hCG injection in each group ($p < 0.05$).

complete sterilization, a specific chemical has not been yet recommended due to the severe side effects.

Although 20% has been successfully used as chemical sterilant in dogs and cats, it should be taken into account that calcium chloride needs to be diluted in 95% ethyl alcohol to provide maximum contraceptive effects. Yet, there is no commercially available mixture to be used for this purpose. Reportedly, lower concentrations may fail to sterilize the animal and in contrary higher concentrations may develop scrotal ulcer and testicular fistula after injections which require anti-inflammatory drugs and antibiotic therapy, even surgical intervention in severe cases [7]. Also, according to Leoci et al., one-fifth of the dogs receiving 20% calcium chloride had regained some testicular activity by 12 months after injection which is questioning its effectiveness in canine chemo-sterilization [7].

Kutzler and Wood (2006) reported that zinc-gluconate injection does not produce a decline in testosterone levels and long-lasting enough to decrease the nuisance behaviors in the treated dogs. Also, local and systemic reactions including scrotal dermatitis and ulceration, preputial swelling, scrotal self-mutilation, diarrhea, vomiting, lethargy, anorexia and leukocytosis have been reported after intra-testicular injection of zinc-gluconate [30]. Another study revealed that about 4% of dogs treated with zinc gluconate developed necrotizing reactions at the injection site necessitating extensive surgical repair [11]. In addition, the commercial preparation of zinc-gluconate is not currently available worldwide and it is not approved by regulatory agencies to be used for mass-scale chemo-sterilization of dogs and cats in some countries [28].

In 2008 and 2013, the effect of intra-testicular injection of 20% sodium chloride solution (hypertonic saline) was studied in male Wistar and Sprague-Dawley rats in which similar results to surgically castrated rats were obtained in terms of decreased serum testosterone levels. Also, severe degeneration of seminiferous tubules and infiltration of inflammatory cells into the testicular tissue was observed in the histopathology [14,16].

It is believed that hypertonic saline causes severe osmotic shock leading to necrosis due to physicochemical stress [31]. In a recent experimental study in 2016, the effects of intra-testicular injection of hypertonic saline for chemical sterilization of adult and immature male dogs were evaluated. According to the results, the injection led to infertility only in half of the immature dogs and all adult dogs maintained androgenesis [17].

In the presented study, mannitol 20%, was evaluated for chemical sterilization of male rats. Mannitol, when injected intravenously, causes elevated vascular osmotic pressure and thus intracellular water transfer to the intravascular space. Mannitol is used to treat cerebral edema and glaucoma and accelerate the urinary excretion of toxins [32]. Due to the hypertonic nature of mannitol, it seems that with a similar mechanism to 20% sodium chloride, it owns the potential for chemical sterilization.

The histological evaluations of the presented study showed that intra-testicular injection of mannitol 20% resulted in depletion of testicular seminiferous tubules, atrophy, and vacuolization of the tubules. Besides, the quality and maturity of testicular seminiferous tubules in the hypertonic saline and mannitol groups showed a significant decrease, similarly. In consistent with previous studies [14,33], significant decrease in the spermiogenesis index was observed in hypertonic saline and mannitol groups. In another study, similar tissue changes were obtained using a hypertonic saline injection in immature dogs. However, atrophy was associated with severe swelling and fistula formation at the injection site and scrotal sloughing [17]. While, in the presented study, no clinical complications were observed in the rats.

Physico-chemical changes like sudden osmotic change induce water efflux from the cells and tissue necrosis [34].

In the presented study, it seems that intra-testicular injection of mannitol caused a severe change in tissue osmotic pressure and through cellular dehydration, it led to degenerative changes and eventually atrophy. Previously, endothelial cell apoptosis in the presence of mannitol has been demonstrated in an in vitro study [21]. Zhang et al. reported the apoptotic effect of different concentrations of mannitol on cardiomyocytes and renal glomerular cells of rats. They showed that mannitol induced apoptosis and cell death via oxidative stress and cytoskeletal damage [35].

Spermatogenesis and Leydig cell steroidogenesis are both vulnerable to oxidative stress [36]; therefore, the mannitol-induced oxidative stress could lead to degeneration of testicular tissue, germinal cell death, azoospermia and eventually infertility, in the presented study.

The significantly decreased gonadosomatic index observed in hypertonic saline and mannitol groups are consistent with the histopathological findings in this study. The degenerative changes and atrophy caused severe testicular shrinkage and testicular weight loss in these two groups. It has been illustrated that the testicular weight significantly correlates with spermatogenesis and testosterone biosynthesis [37].

According to AL-Megrin et al., oxidative stress and apoptosis in testicular tissue result in serious complications such as testicular weight loss, defective spermatogenesis, and germ cell death along with altered levels of testosterone secretion [38].

Reduced sperm concentration and poor sperm motility (asthenospermia) are considered as male infertility factors

[39]. In the presented study, according to sperm count, viability and motility which dropped to zero or near zero in hypertonic saline and mannitol injected rats, respectively.

On the other hand, high levels of sperm DNA fragmentation in these groups were observed when compared to the intact and normal saline groups using acridine orange staining. Reportedly, DNA damages like fragmentation and denaturation can affect fertilization adversely and can result in male infertility [40].

Kwak and Lee evaluated the effects of intra-testicular injection of hypertonic saline in the rat model [16]. Using agarose gel electrophoresis, they failed to confirm DNA fragmentation in all samples. Subsequently, Canpolat et al. tried to induce chemical sterilization using an intra-testicular injection of hypertonic saline in adult dogs without any success [17].

Testosterone has critical roles in different aspects of spermatogenesis, including meiosis and differentiation of haploid germ cells [41]. Destruction of testicular parenchyma results in low testosterone production and infertility [42]. In the presented study, a significant reduction in testosterone levels was observed in all intra-testicular injected groups. The level of reduction was similar to that observed in surgically castrated rats on day 60. Although such a decrease was found in normal saline received rats, the hCG stimulation resulted in a significant increase in testosterone concentration in this group after 120 min. However, hypertonic saline and mannitol received rats responded poorly to hCG administration with no significant changes versus their basal levels. The hCG stimulation is an essential test to determine the function of Leydig cell, and normal response (i.e. testosterone elevation) indicates a normal Leydig cell function and presence of viable testicular tissue [43]. The results of hormone assay in the presented study revealed that the degenerative changes after injection of hypertonic saline and mannitol were severe enough to irreversibly impair the interstitial tissue and suppress the steroidogenesis.

This study confirmed the effectiveness of single, bilateral intra-testicular injection of mannitol as a promising chemo-sterilant in a rat model. The commercially available 20% solution of mannitol resulted in comparable effects to hypertonic saline solution without clinical complications. The mechanism of infertility may be related to the severe osmotic shock and dehydration leading to testicular necrosis. Based on the results of this study, it may be considered as an alternative to surgical castration. Further studies are recommended to assess the effectiveness of intra-testicular injection of mannitol in different species.

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