Explor Anim Med Res.
Vol. 14, Issue 1, 2024
DOI: 10.52635/eamr/14.1.59-67

ISSN 2277-470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

IMPLICATIONS OF INTRATESTICULAR ADMINISTRATION OF ZINC GLUCONATE AND DIMETHYL SULFOXIDE ON TESTICULAR MORPHOMETRY IN DOGS

Omkar Kulkarni, Satya Nidhi Shukla*, Shivika Chouksey, Jitendra Kumar, Dileep Arya

Received 02 March 2024, revised 16 June 2024

ABSTRACT: A study was performed to investigate the potential contraceptive impact of intra-testicular injection of zinc gluconate with dimethyl sulfoxide (DMSO) with and without local anesthetic in eighteen male mongrel dogs. Animals of Group I and II were treated with 2.62% zinc gluconate plus 0.5% DMSO and 2.62% zinc gluconate plus 0.5% DMSO along with 1% lignocaine hydrochloride solution, respectively. In contrast, Group III animals were maintained as control. The efficacy of combinations was examined by morphometry of testis, scrotal circumference (on day-0, 7, 15, 30), and ultrasonographic examination (on day-0 and 30). The testicular morphometry of both testicles revealed that the testicle size increased significantly on day 7 in the treatment groups and then significantly reduced on days- 15 and 30. The ultrasonographic examination revealed decreased testicular dimensions and increased parenchymal lesions in both the testicles of treatment groups which consisted of heterogenous echotexture, mild evidence of mediastinal line, presence of hypoechogenic areas, and heterogenicity in parenchyma. However, incorporating 1% lignocaine hydrochloride into the zinc gluconate and DMSO did not reveal any appreciable changes during the post-exposure period.

Keywords: Dogs, Chemical sterilization, Zinc gluconate, DMSO, Testicular morphometry.

INTRODUCTION

The overpopulation of stray dogs is a global problem that harms the ecosystem and raises the possibility of outbreaks of zoonotic diseases [18]. Rabies is a serious zoonotic illness and dogs are also clearly involved in the 75-99% of cases of rabies transmission that occur in people and animals. A huge number of canines can also be a major danger to biodiversity and wildlife since they can be rivals, predators, carriers of disease, and interbreeding partners for native species [7].

It needs to sterilize males for control of the canine population. Surgical, hormonal, immunological, and pharmacological castration are the primary techniques used in castration. The most successful approach is thought to be surgery (vasectomy or orchidectomy), but it is also the most expensive. Conversely, the chemical procedure is preferable to other castration techniques as it just needs a small number of people with less experience [3]. The advantages of chemical sterilization include the apparent decrease of discomfort and tension as well as the removal of surgical sequelae such as myiasis, bleeding, hernia, infection, and others [4].

Chemical sterilization has been proposed as a quick and affordable substitute that may be used for a variety of dog populations, particularly in underdeveloped areas where the issue appears to be more severe [13]. Certain male animal species, including goats [10], bulls [2], rats, and dogs [9] have been subjected to chemical castration.

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India.
**Corresponding author. e-mail: snshukla2@gmail.com*

Sclerosing agents when introduced into the ductus deferens and epididymis, induce fibrous occlusions that prevent the transportation of sperm cells which leads to azoospermia. Additionally, the substance results in testicular shrinkage and inhibits spermatogenesis and androgenesis, which lowers the risk of androgen-related illnesses such as prostatic disorder, undesired behavior (urine marking, mounting, and aggressiveness), and gonadal disorders [12].

At the ideal quantity, zinc is necessary for spermatogenesis and is a major component of semen, however, at excessive amounts, zinc inhibits germ cell division and replication and fragments the cell membrane and nucleus. The first chemical sterilizing agent to meet all three of the essential requirements of the perfect chemical sterilization method was zinc gluconate, which included a high margin of safety with no negative environmental effects, large-scale application of the sterilizing agent in male animals, and permanent and irreversible effects after just one treatment [5]. Testicular degeneration, a decrease in germ cells, increased atrophy, disturbance of the architecture of the seminiferous tubule, loss of germ and Sertoli cells, and focal regions of intratubular calcification and vasculitis are all brought on by zinc gluconate in combination with DMSO [13].

Zinc gluconate administered intra-testicularly in conjunction with lignocaine was not previously reported. It is evident from a review of earlier papers that there has never been any documented lignocaine with zinc solution contraindications. Based on the hypothesis that lignocaine added to the chemical preparation will have a local anesthetic and analgesic effect at the injection site which reduces pain and irritation because the solution contains a higher concentration of zinc gluconate and prevents the need for Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) after the chemo-sterilant is administered. By reducing the likelihood of sclerotic lesions and post-exposure self-mutilation wounds on the scrotal skin, local analgesic effects help prevent subsequent infection and abscess development. The current study evaluated changes in testicular morphometry and ultrasonography after administration of zinc gluconate-DMSO with and without lignocaine intra-testicularly.

MATERIALS AND METHODS

Eighteen healthy mongrel male dogs having fully developed and intact testicles intended for castration were considered for the current investigation. The animals that underwent research did not have any reproductive or anatomical disorders. Separate kennels were given to every dog at the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, M.P., India. All the dogs were given healthy, balanced food in addition to unlimited access to clean drinking water throughout the investigation. The use of animals in experiments was permitted by the aforementioned Institutional Animal Ethical Committee (Approval No.: 14/IAEC/Vety/2020). Three groups *viz.* G-I, G-II, and G-III (control) with six dogs in each group were randomly established from among the dogs.

Animal restraining and sedation

Animals were restrained and sedated using an injection of Xylazine @ 1mg/kg body weight intramuscularly.

Intratesticular injection

All of the dogs received an intramuscular injection of Xylazine hydrochloride (Xylaxin®, Indian Immunologicals, Pvt. Limited) at a dose of 1 mg/kg of body weight to induce drowsiness before the administration of the intratesticular injection. The dogs were not fed for six to twelve hours before the sedative injection. After the sedation, the dogs were physically restrained in a dorsal recumbent position followed by cleaning of the testicles of the dogs with an antiseptic solution.

A single bi-testicular injection of 2 mL zinc gluconate (2.62%) along with 0.5% DMSO was administered to the G-I and G-II animals administered a bi-testicular injection of 2 mL of zinc gluconate (2.62%) associated with 0.5% DMSO along with 1% lignocaine hydrochloride, respectively. The animals in the G-III were kept as controls, receiving no treatment whatsoever. The intratesticular injection was given using a 21-gauge needle with a sterile syringe pointed from the cauda-ventral aspect of each testis, approximately 1.0 cm from the cauda epididymis and towards the dorso-cranial aspect of the testis (Fig. 1).

Testicular morphometry

On days 0, 7, 15, and 30, measurements of the scrotal circumference and testicular morphology were carried out for both groups. The testicular volume was computed using the formula underneath and measurements of testicular length, width, thickness, and scrotal circumference (Fig. 2) were made using Vernier calipers [6].

TesticularPaired Volume (cm^3) = testicular length × testicular width × testicular thickness × 0.5236.

Testicular ultrasonography

Ultrasonographic examination of testes was conducted using a 5-megahertz real-time linear transducer in B-mode (Philips HD 7×E). A sagittal segment of the testes was used to compare the changes in echotexture and consistency of testicular parenchyma following the treatment. The ultrasonographic examination was performed on days 0 and 30 of the study (Fig. 3a, b, c, and d).

Statistical analysis

Using SPSS 24 software, the quantitative data was analyzed using two-way ANOVA for scrotal circumference and testicular morphometry to compare the mean values across the groups and between the days. A significant difference was interpreted as a 95% confidence interval with a p-value of less than 0.05.

RESULTS AND DISCUSSION

A total of 12 dogs (6 each) were injected (Bi-testicular injection) for chemical castration with zinc gluconate in 2 ml (26.2 mg/ml) with 0.5% DMSO in G-I and an additional 1% lignocaine hydrochloride along with a mixed solution of 0.5% DMSO and zinc gluconate in G-II however, in G-III, 6 healthy dogs were kept as control. Testicular morphometry is assisted by measuring the testicular length, width, and thickness then calculating scrotal circumference on days 0, 7, 15, and 30 along with performing testicular sonography before the start of treatment and at the end of the treatment.

Testicular length

On Day 0 (before treatment), the average mean testicular length (cm) was recorded at 3.53 to 3.58 in the left testes and 3.35 to 3.53 in the right testes in all groups. Data analysis revealed that in both the groups receiving intratesticular injection (G-I and II), the mean length of testis increased during the first to the seventh day of treatment then decreased between the seventh to fifteen days followed by a final decrease from day 15 to day 30. However, from a statistical perspective, there was a non-significant change. ($p>0.05$). Whereas, in animals of the control group, no trend of remarkable alteration was exhibited in mean testicular length at all time intervals. In this study, there was a non-significant difference in the mean testicular length between the treatment groups on various days (Table 1).

Testicular morphometry using Vernier calipers and ultrasonographic analysis was also performed to evaluate changes in testicular length after injecting zinc gluconate-arginine intra-testicularly and observed significant diminution in testicular length after an initial increase for the first 7 days following treatment [19]. Testicular morphometry using Vernier calipers to evaluate changes in the mean testicular length after a 20 percent calcium chloride solution treatment and observed an initial increase for the first 7 days followed by a decrease up to the 30th day [14]. Both studies were carried out in stray male dogs, for 30 days duration under similar environmental conditions and the same measurement interval for testicular length as in the present study but with different treatment protocols.

Testicular width

When measured on day 0 (before treatment), the mean testicular width (cm) was recorded as 2.45 to 2.61 in the left testicle and 2.38 to 2.65 in the right testicle. Analysis of data revealed that after intra-testicular injection the mean testicular width slightly increased or was almost comparable to the value on day 7 in treatment groups I and II then from day 7 to day 15, it began to decline till day 30. In male dogs of the control group, no trend of statistically significant variation existed in mean testicular width at all time intervals (Table 2).

In this study, a significant change was recorded in testicular width among dogs of G-I and G-II at all-time intervals. The mean values of G-III (control) do not vary significantly from the values of G-I and G-II at all-day intervals.

The outcomes of the current investigation concurred with the conclusions of various researchers [11,13, 14]. It was examined that testicular width every 15 days for 6 months following treatment with a solution of zinc gluconate-DMSO (ml) injected according to testicular width (mm) [13]. They found no considerable significant changes in testicular size during the study period. Soto and coworker performed testicular width evaluation every 15 days for 2 months (12 evaluations in total) in mixed-breed sexually mature dogs following treatment with a solution of zinc gluconate-DMSO (ml) injected according to testicular width [14]. They found no statistical difference in the testicular width of treated dogs during all 11 evaluations after treatment. Testicular width assessment in mongrel males after being treated with zinc gluconate-based solution daily for the first 7 days and then every 2 weeks until the end of the observation period (150 days) [11]. They

Table 1. Average length of testicles (cm) prior to and following treatment in dogs*.

Testicle	Group	Average length of testicles (cm)			
		Day			
		0	7	15	30
Left testicle	I	3.58±0.46	3.59±0.40	3.41±0.35	3.20±0.28
	II	3.53±0.48	3.55±0.48	3.46±0.53	3.20±0.44
	III	3.55±0.50	3.57±0.46	3.51±0.46	3.53±0.53
Right testicle	I	3.35±0.19	3.40±0.19	3.35±0.19	3.10±0.19
	II	3.53±0.19	3.56±0.19	3.48±0.19	3.25±0.19
	III	3.48±0.19	3.50±0.19	3.46±0.19	3.46±0.19

Table 2. Average width of testicles (cm) prior to and following treatment in dogs*.

Testicle	Group	Average length of testicles (cm)			
		Day			
		0	7	15	30
Left testicle	I	2.45±0.46	2.48±0.43	2.28±0.35	2.10±0.36
	II	2.61±0.50	2.64±0.51	2.55±0.53	2.40±0.58
	III	2.52±0.43	2.55±0.39	2.46±0.43	2.53±0.38
Right testicle	I	2.38^{b}±0.39	2.40^{b}±0.36	2.28^{b}±0.45	2.05^{b}±0.31
	II	2.65^{a}±0.44	2.69^{a}±0.46	2.58^{a}±0.48	2.43^{a}±0.55
	III	2.43^{ab}±0.16	2.95^{ab}±0.25	2.98^{ab}±0.26	2.91^{ab}±0.18

Table 3. Mean thickness of testicles (cm) prior to and following treatment in dogs*.

Testicle	Group	Average length of testicles (cm)			
		Day			
		0	7	15	30
Left testicle	I	2.18±0.31	2.24±0.32	2.21±0.33	2.05±0.36
	II	2.41±0.32	2.44±0.32	2.38±0.25	2.16±0.26
	III	2.30±0.23	2.16±0.21	2.18±0.24	2.15±0.16
Right testicle	I	2.21^{b}±0.19	2.25^{b}±0.15	2.16^{b}±0.18	2.06^{b}±0.16
	II	2.36^{a}±0.29	2.42^{a}±0.45	2.41^{a}±0.36	2.21^{a}±0.33
	III	2.35^{ab}±0.23	2.40^{b}±0.22	2.39^{ab}±0.25	2.20^{ab}±0.25

Table 4. Mean testicular paired volume (cm^3) prior to and following treatment in dogs*.

Group	Testicular paired volume (cm^3)				Mean reduction in TPV (%)
	Day				
	0	7	15	30	Day 30
I	9.96±3.49	9.98±2.41	9.11±3.01	7.27±2.39	30.67^{a}±3.71
II	12.04±4.48	12.09±3.92	11.60±4.37	9.21±3.48	27.00^{a}±6.52
III	12.65±3.75	12.71±3.61	12.74±3.82	12.51±3.83	00.56^{b}±0.81

* Mean values carrying different superscripts (a, b) in a column differ significantly ($p<0.05$).

Table 5. Average scrotal circumference (inches) prior to and following treatment in dogs.

Group	Average scrotal circumference (inches)				Average reduction in scrotal circumference (%)
	Day				
	0	7	15	30	Day 30
I	9.96±3.49	9.98±2.41	9.11±3.01	7.27±2.39	30.67[a]±3.71
II	12.04±4.48	12.09±3.92	11.60±4.37	9.21±3.48	27.00[a]±6.52
III	12.65±3.75	12.71±3.61	12.74±3.82	12.51±3.83	00.56[b]±0.81

Mean values bearing various superscripts (a, b) in a column differ considerably ($p<0.05$).

Table 6. Mean scrotal circumference (inches) in large-sized and medium-sized dogs at different intervals.

Group	Category of dog	Day 0	Day 7	Day 15	Day 30
I	Large size	6.54±0.14	6.58±0.41	6.21±0.31	5.82±0.34
	Medium size	5.60±0.36	5.69±0.38	5.53±0.38	5.47±0.41
II	Large size	6.42±0.10	6.49±0.09	6.39±0.07	6.24±0.12
	Medium size	5.90±0.26	5.96±0.26	5.86±0.26	5.76±0.28

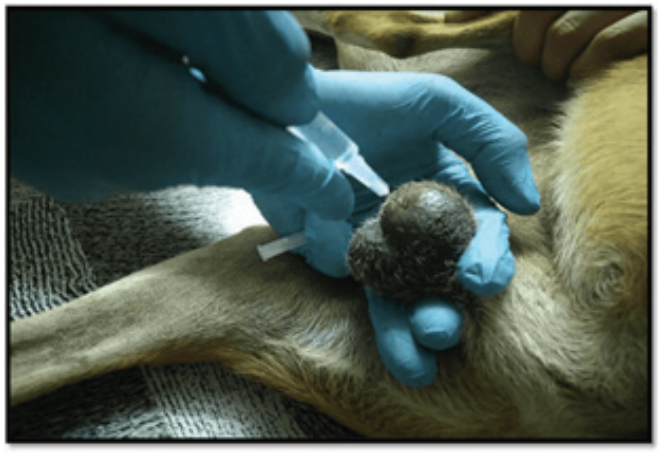

Fig. 1. Intra-testicular administration of chemical caudo-ventrally.

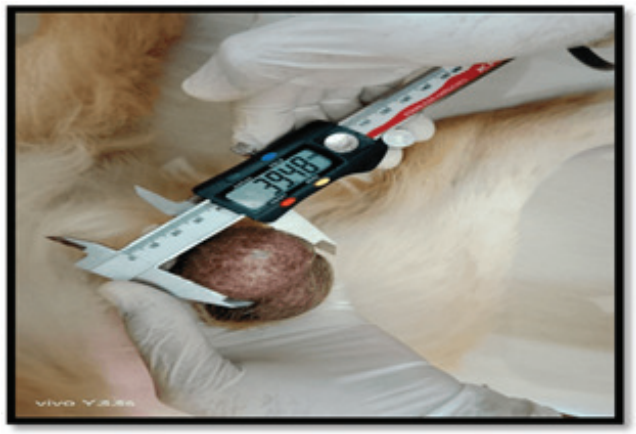

Fig. 2. Measurement of testicular length.

observed an initial increase in testicular width for 2 weeks followed by a decrease up to 50th day post-treatment.

Testicular thickness

On day 0 (before treatment), mean testicular thickness (cm) was recorded as 2.18 to 2.41 cm in the left testicle and 2.21 to 2.36 cm in the right testicle. Data analysis indicated that in both the treatment groups (G-I and II) the mean thickness of the testis progressed from day one of treatment until the seventh day and then started to decline from day seven to day 15 which continued to reduce from day 15 to day 30. No remarkable alterations were observed in male dogs of the control group in mean testicular thickness at all time intervals (Fig. 1, 2) (Table 3).

No study on testicular thickness with the same treatment protocol has been reported previously. However, the current study's findings aligned with the outcomes of other investigations using zinc gluconate-arginine and calcium chloride, respectively [16, 19]. These studies were carried out in stray mongrel males, for 30 days under similar environmental conditions and the same measurement interval for testicular thickness as in the present study but with different treatment protocols.

Testicular paired volume (TPV)

On day 0 (before treatment) mean TPV (cm^3) was recorded as 9.96 to 12.65 cm^3. Data analysis showed that from day 0 to day 7 post-treatment, the mean TPV increased in both treatment groups (G-I and II)

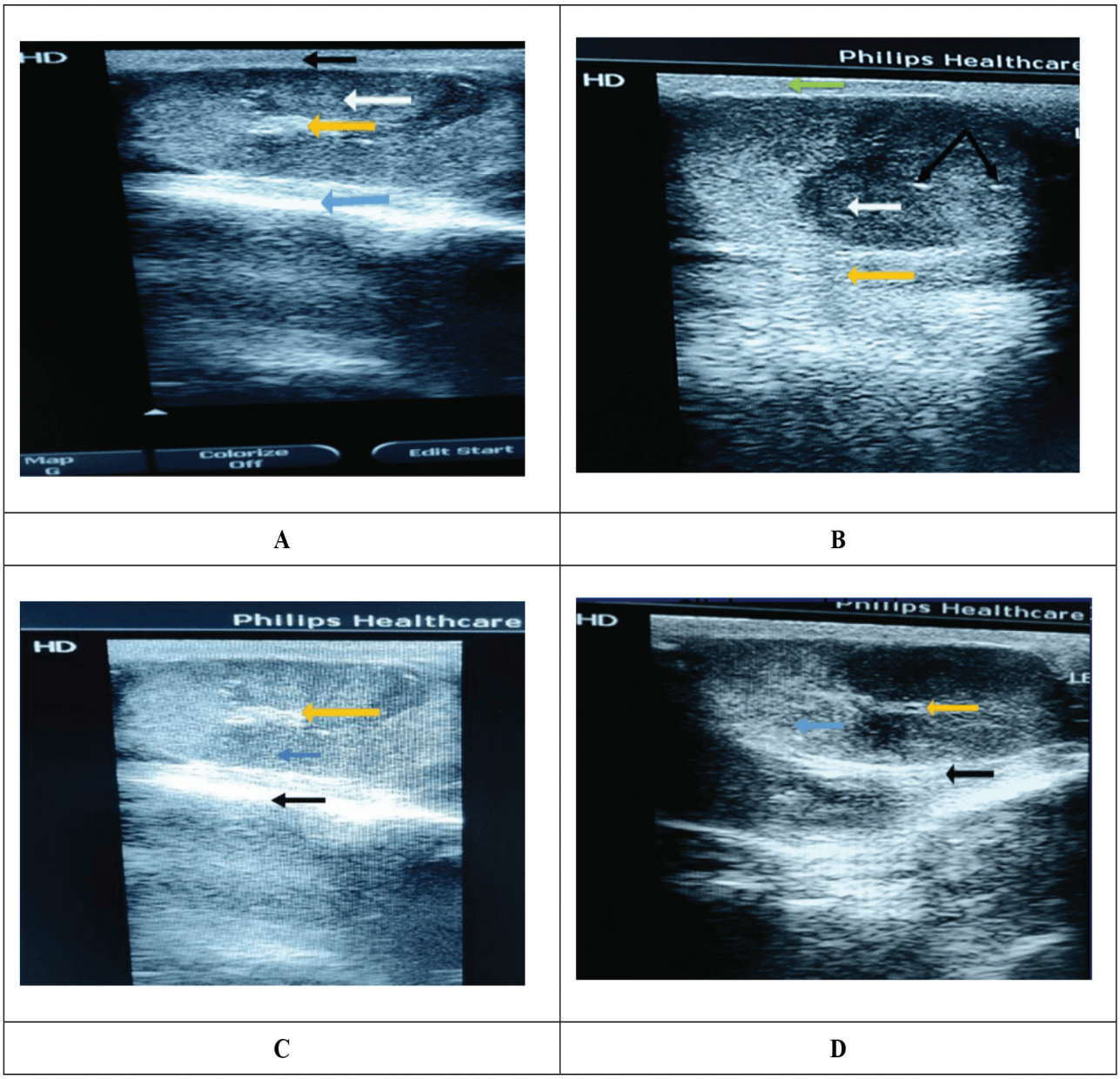

Fig. 3. Ultrasonographic view of the testicle. [A: Testicular sonograph of Group I dog testis (sagittal plane) at day 0 (pre-treatment) showing homogenous echotexture of parenchyma (white arrow), evidence of prominent mediastinum testis (yellow arrow), testicular capsule (blue arrow) and transducer-skin interface (black arrow); B: Testicular sonograph of Group I dog testis (sagittal plane) at day 30 (post-treatment) showing heterogenous echotexture (white arrow), focal hyperechoic spots along the line of infiltration of chemical (black arrows), mild evidence of mediastinal line (yellow arrow) and skin-transducer interface (green arrow): C: Testicular sonograph of Group II dog (sagittal plane) at day 0 (pre-treatment) showing homogenous echotexture of parenchyma (yellow arrow), testicular capsule (black arrow) and hyperechoic mediastinum testis (blue arrow); D: Testicular sonograph of group II dog (sagittal plane) on day 30 (post-treatment) showing mediastinum testis (yellow arrow), heterogenous echotexture (blue arrow) and testicular capsule (black arrow)].

then from day 7 to day 15 it started to decline followed by a reduction till the thirtyth day. However, the difference was found to be statistically non-significant. In animals of the control group, no significant change was recorded in mean TPV throughout the experimental period (Table 4).

In this study, no significant alteration was observed in mean TPV among dogs in treatment groups and

control groups. However, the downward trend in TPV from the first day to the last day of treatment was highest in G-I and then in G-II and G-III. From day 0 to day 30, the mean percentage decrease in TPV did not substantially differ between the groups receiving treatment. Significant differences in the mean values of the percent reduction in TPV among the treatment and control groups might be explained by the fact that the testicles shrank in reaction to the injection, but the control group did not get any injection exposure so no variation was seen in TPV of control group.

An overall decrease in TPV from day 0 to day 30 is by the fibrosis leading to a decrease in testicular size. These observations follow the reports of other similar findings [11, 15, 18].

Virendra and coworkers assessed changes in the paired volume of testes using testicular length and scrotal circumference in response to intra-testicular injection of zinc gluconate-arginine [19]. They observed a significant decrease in testicular volume following treatment. Paired testes volume using testicular length and mean testicular width following treatment with 20 percent calcium chloride solution to evaluate the changes and observed an initial increase for the first 7 days followed by a decrease up to the 30th day [16]. Both studies were carried out in stray mongrel males, for 30 days under similar environmental conditions and the same measurement interval for testicular volume as in the present study but with different treatment protocols. Also, the formula used to calculate TPV in the present study (formula of ellipsoid) was different from the one used in the previous studies.

The evaluation of paired testicular volume for 7 months using ultrasonography in male mongrels treated with double intra-testicular injections of zinc gluconate-DMSO 1-month apart showed a significant decrease in the volume of both testes of the treated groups and postulated that the substitution of healthier testicular tissue by fibrotic and calcified tissue may be the reason behind the reduction in testicular volume and atrophy in the dogs [18]. A reduction in testicular volume was also recorded after intra-testicular administration of glycerol in dogs [8].

Scrotal circumference (SC)

On day 0 (before treatment) mean scrotal circumference (inches) was recorded as 6.07 to 6.24 inches in treatment groups (Table 5). Data analysis of scrotal circumference showed that in all treatment groups (G-I and II) the mean scrotal circumference increased from day 0 to day 7 post-treatment then began to decline from day 7 through day 15 followed by a final reduction till day 30. However, the difference was statistically non-significant. In dogs of the control group, non-significant alteration was recorded in mean scrotal circumference at all time intervals. However, G-I had the largest mean decrease (percentage) in scrotal circumference from day 0 to day 30, followed by G-II and G-III. Between treatment groups, there is no significant difference in the mean values of the percentage reduction in scrotal circumference from day 0 to day 30. The noteworthy trend of significant variations in mean values seen between the treatment and control groups may be attributed to the decrease in testicular size in response to injection as compared to the lack of exposure to the injection in the control group.

In this study, a significant alteration was recorded in the scrotal circumference of dogs of treatment groups at different day intervals. The mean values of group 3 did not vary significantly from the values of G-I and G-II. The overall decrease in scrotal circumference from the first day of treatment to the last day of treatment was recorded which might be due to testicular atrophy, calcification, and fibrosis, which reduce testicular mass.

Similarly, other research assessed changes in scrotal circumference using measuring tape in response to a zinc gluconate-arginine intra-testicular injection and observed a significant decrease in SC following treatment [19]. Assessment of testicular morphometry using vernier calipers to evaluate changes in mean scrotal circumference following treatment with 20 percent calcium chloride solution and observed an initial increase for the first 7 days followed by a decrease up to 30th days [16]. Although, both the studies were carried out in stray mongrel males, for 30 days under similar environmental conditions and the same measurement interval for SC as in the present study but followed different treatment protocols.

A statistically significant difference in the mean values of the scrotal circumference (inches) between the treatment groups from day 0 (pre-treatment) itself may be attributed to the inherently relatively larger size of both the testicles in the large-sized dogs (26-45 kg) as compared to the medium-sized dogs (10-25 kg). Data of large-sized dogs has been described in Table 6 (SC value > 6.30 inches).

Testicular ultrasonography

On day 0, an ultrasound evaluation of the testes in all the treatment groups exhibited normal testicular parenchyma, echotexture, and testicle size, hypoechoic background texture overlaid by a homogenous medium echogenicity along with a well-defined hyperechoic

central line indicating mediastinum. Examination on day 30 revealed significant testicular alterations in the treatment groups in comparison with the control group. Observations included decreased testicular dimensions and increased parenchymal lesions, heterogenous echotexture, mild evidence of mediastinal line, presence of hypoechogenic areas, and heterogenicity in the parenchyma. Hyperechoic changes were seen along the line of infiltration of the chemical indicating progressive degeneration of the parenchyma (Fig. 3).

Previous investigations also showed similar findings consistent with those of other researchers [1, 14, 18]. The injection of zinc gluconate associated with DMSO intra-testicularly in sexually mature dogs led to major lesions in both left and right testicles consisting of heterogenous echotexture, mild evidence of mediastinal line, hypoechogenic areas, and heterogeneity in the parenchyma [14].

During the experimental period, adult dogs who received an intra-testicular injection of zinc gluconate-DMSO had testicular modifications significantly different from those of the control group. These abnormalities included reduced testicular diameters and increased parenchymal lesions. [18]. The intra-testicular injection of zinc gluconate in American black bears resulted in evident alteration in echo density of testicles following zinc administration including scattered hyperechoic areas in some cases and hyperechoic areas throughout the entire parenchyma in remaining ones indicating degenerative changes [1].

CONCLUSION

Thus, it may be stated that bilateral intratesticular administration of 2.62% zinc gluconate mixed with 0.5% dimethyl sulfoxide caused a decrease in testicular size (length and width of testicles paired testicular volume and thickness) and scrotal circumference associated with degenerative changes in the parenchyma of the testis. However, adding 1% lignocaine hydrochloride to the combination of zinc gluconate and DMSO did not reveal any remarkable change during the post-exposure period.

ACKNOWLEDGMENT

The authors express their gratitude to the Madhya Pradesh Council of Science and Technology, Bhopal, for providing financial support for this research project, as well as the administration of the College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, for providing basic infrastructure and necessary facilities.

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Cite this article as: Kulkarni O, Shukla SN, Chouksey S, Kumar J, Arya D. Effects of intratesticular administration of zinc gluconate and dimethyl sulfoxide on testicular morphometry in dogs. Explor Anim Med Res. 2024; 14(1), DOI: 10.52635/eamr/14.1.59-67.