RESEARCH ARTICLE

Effect of Intra-Testicular Administration of Clove Oil on Testicular Morphometry in Dogs

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Abstract

The present work was designed to study the effect of intra-testicular administration of clove oil solution on testicular morphometry. Thirty sexually mature adult male dogs were taken and randomly divided into 5 groups comprising six animals in each. The animals were administered intratesticular injection of clove oil (G-I), clove oil along with lignocaine (G-II), clove oil with olive oil (G-III) and eugenol (G-IV) once @ 1.0 mL in each group, while G-V of six animals was kept as untreated control. The testicular morphometry in terms of size (length, width, thickness); testicular circumference and paired volume of intact testes was determined. The testicular size was increased on day 7 as compared to day 0 and then decreased gradually from day 7 to day 45 in clove oil, clove oil along with lignocaine, clove oil with olive oil and eugenol administered groups. The reduction in mean paired volume of testes (PVT) at day 45 was highest in G-II (35.74 %) followed by G-IV (26.57%), G-I (26.22 %), Group-III (25.19 %) and G-V (0.12 %). Mean decrease in scrotal circumference at day 45 was highest in G-II (8.14 %) followed by G-IV (7.64 %), G-I (5.91 %), G-III (3.66%) and G-V (0.29 %). These findings were also supported by semen quality evaluated in selected few animals. The study revealed that intratesticular administration of clove oil along with lignocaine and eugenol separately reduced testicular size which may be attributed due to degenerative changes in the testicles.

Key words: Clove oil, Intra-testicular, Male dogs, Testicular morphometry.

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Introduction

Large number of stray dogs is causing serious problem in many developing countries. In Asia and Africa, dogs are the primary vector of rabies and imparting the biggest threat to humans, that's why dogs have been focused on rabies control programme of World Health Organization. Controlling reproduction in dogs prevents the birth of unwanted puppies which reduces the canine population. In addition to this, many pet owners sterilize their animals to reduce secondary sexual characteristics such as mounting, aggression and urine/territory marking. Additionally, the surgical approach is ineffective when used on a wide scale, particularly for reducing the population of stray dogs. Hence, non-surgical castration may be desirable option in under-developed nations with little resources (Almeida *et al.,* 2010).

Clove oil (oil of *Syzygium aromaticum*) is having medicinal properties like anaesthetic, analgesic, antimicrobial, antiviral, anti-carcinogenic, neuro-protective ability, hypolipidemic and anti-diabetic effects. Eugenol is a naturally-occurring volatile phenolic compound, is a major antioxidant constituent of *Ocimum basilicum* leaves however, eugenol is also principal constituent of several essential oils including *Cinnamomum verum*, *Myristica fragrans*, *Piper betle* and *Syzygium aromaticum*. Eugenol reportedly exhibits antioxidant, antibacterial, anti-inflammatory, antiemetic and antifungal activities at low concentrations while in higher concentration acts as a pro-oxidant causing increased generation of tissue damaging free radicals (Jaganathan and Supriyanto, 2012). It is non-mutagenic, non-carcinogenic and is generally recognized as safe by the Food and Drug Administration of the USA. Abshenas *et al*. (2013) reported that single bilateral injection of eugenol is effective, economical and permanent method of sterilization of male dogs.

This work was planned to study the effect of intra-testicular administration of clove oil and eugenol on testicular morphometry in dogs.

Materials and Methods

The study was performed from September 2021 to October 2022 in thirty healthy adult male dogs irrespective of

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breed, came for castration with intact and developed testicles. Selected dogs were randomly divided into 5 groups, comprising six animals in each. Selected dogs were administered with bilateral intra-testicular pure form of clove oil (HiMedia – GRM340) injection @ 1.0 mL in group-I, clove oil along with 1% lignocaine hydrochloride in group-II, clove oil with olive oil (1:1) in group-III and eugenol (HiMedia – RM6992) in group-IV. Animals in group-V were kept as control without any treatment. The animals were kept isolated at door step of owners and were managed under optimum managemental conditions throughout the study period.

For the assessment of effects of treatment, the testicular biometry was done in all groups on day 0, 7, 15, 30 and 45, and semen quality was studied among selected few animals on day 0 and day 45 of treatment. Measurements of testicular length, width and thickness were taken by vernier calipers. Scrotal circumference was measured using commercially available measuring tape and Paired volume of testis was calculated by the following formula as adapted by Gouletsou *et al.* (2008).

Paired volume of testis (cm^3) = L× W× T× 0.5236

L = Testicular length; W = Testicular width; T = Testicular thickness

The data generated from all the parameters was statistically analyzed using two-way analysis of variance and Duncan's multiple range test. The values were expressed as Mean ± SE and significance was judged at 5 % level.

Results and Discussion

Testicular Biometry

In this study, the mean values of all the parameters of testicular biometry (length, width and thickness) were found to be increased initially after start of treatment and then decreased gradually from day 15 to day 45 in clove oil, clove oil along with lignocaine, clove oil with olive oil and eugenol administered groups, (G-I, G-II, G-III and G-IV), respectively (Table 1, 2 and 3). However, no significant change was recorded in control group. The literature is lacking on testicular morphometry using clove oil, clove oil with 1% lignocaine hydrochloride, clove oil with olive oil and eugenol for contraceptive effect in male dog. However, results of the present study are comparable with the findings of Virendra *et al.* (2021), Thakre *et al.* (2021) and Kulkarni (2022) using zinc gluconate-arginine, calcium chloride (20%) and zinc gluconate-DMSO, respectively.

The mean scrotal circumference increased from day 0 to day 7 post-treatments and then started declining from day 7 to day 15 and day 30 followed by final decrease till day 45, whereas no significant change was observed in animals of control group (Table 4). However, the trend of mean percent reduction in scrotal circumference from day 0 to day 45 was highest in G-II (8.14%) followed by G-IV (7.64%), G-I (5.91%), G-III (3.66%) and G-V (0.29%). Mean values of per cent reduction in scrotal circumference from day 0 to day 45 differed significantly between the treatment groups. Significant difference in the mean values between the treatment and control group may be attributed to the decrease in testicular size in response to injection as compared to the lack of exposure to the injection in control group. No literature could be traced regarding effect of intra-testicular administration of clove oils on scrotal circumference as in present study. However, few published reports, were available with different treatment protocols on same parameters. Our results are comparable with finding of Baba *et al.* (2013), Virendra *et al.* (2021), Thakre *et al.* (2021) and Kulkarni (2022) using different chemicals and methods like cadmium-exposed rabbit bucks administered methanolic extract of *phoenix dactylifera* fruit, zinc gluconate-arginine, 20% calcium chloride and zinc gluconate associated with 0.5% DMSO, respectively.

In the present study, mean paired volume of testis in the treatment groups (G-I, G-II, G-III and G-IV) increased significantly from day 0 to day 7 post-treatment, then started declining from day 7 till day 45. However, in the animals of control group, no significant change was observed in mean

Table 1: Mean testicular length (cm) before and after intra-testicular treatment in dogs

Testicle	Group	Mean testicular length (cm)				
		Day-0	Day-7	Day-15	Day-30	Day-45
Left testicle	I	3.56^{ab}±0.07	3.73^{ABa}±0.07	3.56^{ab}±0.10	3.40^{ab}±0.10	3.21^{b}±0.12
	II	3.68^{ab}±0.13	4.08^{Aa}±0.12	3.76^{ab}±0.14	3.48^{bc}±0.10	3.16^{c}±0.13
	III	3.58^{ab}±0.10	3.87^{ABa}±0.11	3.67^{a}±0.13	3.44^{ab}±0.16	3.15^{b}±0.15
	IV	3.53^{bc}±0.13	4.01^{Aa}±0.16	3.74^{ab}±0.13	3.50^{bc}±0.12	3.28^{c}±0.11
	V	3.44^{a}±0.16	3.43^{Ba}±0.12	3.42^{a}±0.15	3.43^{a}±0.16	3.42^{a}±0.12
Right testicle	I	3.59^{a}±0.11	3.76^{ABa}±0.10	3.60^{a}±0.11	3.48^{a}±0.11	3.31^{a}±0.15
	II	3.65^{b}±0.12	4.12^{Aa}±0.12	3.77^{ab}±0.11	3.44^{bc}±0.10	3.06^{c}±0.09
	III	3.66^{abc}±0.16	3.96^{Aa}±0.13	3.71^{ab}±0.16	3.40^{bc}±0.19	3.14^{c}±0.15
	IV	3.62^{ab}±0.18	4.01^{Aa}±0.16	3.75^{ab}±0.13	3.51^{ab}±0.14	3.28^{b}±0.15
	V	3.38^{a}±0.11	3.37^{Ba}±0.13	3.36^{a}±0.16	3.35^{a}±0.16	3.36^{a}±0.11

Means bearing different superscript within group (lower case) and between groups (upper case) differ significantly ($p<0.05$).

Table 2: Mean testicular width (cm) before and after intra-testicular treatment in dogs

Testicle	Group	Mean testicular width (cm)				
		Day-0	Day-7	Day-15	Day-30	Day-45
Left testicle	I	2.48^{a}±0.07	2.59^{ABa}±0.08	2.51^{a}±0.10	2.46^{a}±0.09	2.36^{a}±0.10
	II	2.53^{ab}±0.08	2.83^{Aa}±0.09	2.57^{ab}±0.06	2.37^{bc}±0.06	2.14^{c}±0.06
	III	2.44^{a}±0.06	2.58^{ABa}±0.06	2.53^{a}±0.07	2.46^{a}±0.06	2.35^{a}±006
	IV	2.49^{abc}±0.07	2.80^{Aa}±0.11	2.57^{ab}±0.08	2.41^{bc}±0.07	2.21^{c}±0.06
	V	2.39^{a}±0.06	2.42^{Ba}±0.07	2.46^{a}±0.07	2.46^{a}±0.07	2.43^{a}±0.06
Right testicle	I	2.44^{a}±0.11	2.53^{ABa}±0.10	2.45^{a}±0.12	2.38^{a}±0.11	2.32^{a}±0.12
	II	2.53^{ab}±0.10	2.85^{Aa}±0.08	2.58^{ab}±0.06	2.37^{bc}±0.06	2.13^{c}±0.10
	III	2.50^{ab}±0.05	2.66^{ABa}±0.06	2.57^{ab}±0.06	2.49^{ab}±0.07	2.37^{b}±0.06
	IV	2.58^{ab}±0.10	2.83^{Aa}±0.12	2.52^{ab}±0.10	2.39^{b}±0.09	2.20^{b}±0.08
	V	2.36^{a}±0.06	2.39^{Ba}±0.08	2.41^{a}±0.06	2.52^{a}±0.08	2.41^{a}±0.08

Means bearing different superscript within group (lower case) and between groups (upper case) differ significantly ($p<0.05$).

Table 3: Mean testicular thickness (cm) before and after intra-testicular treatment in dogs

Testicle	Group	Mean testicular thickness (cm)				
		Day-0	Day-7	Day-15	Day-30	Day-45
Left testicle	I	2.35^{bc}±0.05	2.54^{ABa}±0.04	2.47^{ab}±0.04	2.34^{bc}±0.05	2.20^{c}±0.03
	II	2.29^{bc}±0.10	2.57^{Aa}±0.06	2.35^{ab}±0.05	2.23^{bc}±0.05	2.05^{c}±0.06
	III	2.29^{bc}±0.03	2.49^{ABa}±0.02	2.41^{ab}±0.02	2.27^{c}±0.02	2.10^{d}±0.03
	IV	2.30^{bc}±0.06	2.61^{Aa}±0.05	2.47^{ab}±0.03	2.22^{cd}±0.03	2.09^{d}±0.03
	V	2.20^{a}±0.09	2.21^{Ba}±0.08	2.23^{a}±0.05	2.22^{a}±0.09	2.19^{a}±0.09
Right testicle	I	2.31^{ab}±0.06	2.53^{ABa}±0.07	2.45^{ab}±0.07	2.34^{ab}±0.07	2.18^{b}±0.07
	II	2.33^{ab}±0.11	2.58^{ABa}±0.08	2.38^{ab}±0.09	2.21^{ab}±0.09	2.06^{b}±0.07
	III	2.29^{b}±0.05	2.50^{ABa}±0.05	2.39^{ab}±0.04	2.28^{b}±0.04	2.06^{c}±0.04
	IV	2.30^{b}±0.09	2.62^{Aa}±0.08	2.43^{ab}±0.05	2.21^{bc}±0.05	2.02^{c}±0.05
	V	2.22^{a}±0.08	2.23^{Ba}±0.06	2.25^{a}±0.07	2.24^{a}±0.06	2.21^{a}±0.09

Means bearing different superscript within group (lower case) and between groups (upper case) differ significantly ($p<0.05$).

paired volume of testis at all time intervals. Among treatment groups, the trend of decrease in paired volume of testis from day 0 to day 45 was highest in G-II (35.74%) followed by G-IV (26.57%), G-I (26.22%), G-III (25.19%) and G-V (0.12%). Mean values of percent reduction in PVT from day 0 to day 45 differed significantly between the treatment groups (Table 5). No one reported previously regarding changes in paired testicular volume using clove oil or other chemicals as in this study, however present observations may be correlated with the reports of Immegart and Threlfall (2000), Canpolat *et al.* (2006), Baba *et al.* (2013), Virendra *et al.* (2021), Thakre *et al.* (2021) and Kulkarni (2022) using different chemicals *i.e.,* 70% glycerol solution, 10 mL absolute ethanol and calcium chloride, pinhole castration technique in male dog by using percutaneous spermatic cord ligation, zinc gluconate-arginine, 20% calcium chloride, and 2.0 mL (26.2 mg/mL) of zinc gluconate associated with 0.5% DMSO, respectively.

Some of the available reports using other chemicals revealed significant reduction in testicular volume (47.38%) on day 30 following intra-testicular injection of 20% calcium chloride (Thakre *et al.,* 2021), 30.67% with zinc gluconate associated with 0.5% DMSO (Kulkarni, 2022) and 40.57% using pinhole castration technique in male dog using percutaneous spermatic cord ligation (Baba *et al.,* 2013).

Semen Parameters

Semen was collected by digital manipulation method at day 0 and 45 post injection. All the changes in spermatogenesis were studied in ejaculated semen using routine macroscopic and microscopic tests, *viz.* Color, pH, Live and Dead count, Sperm concentration, Progressive motility etc. Efforts were made to collect semen from the intact dog at day 0 and 45 by digital manipulation method but semen were collected from 12 dogs only and attempts were unsuccessful in 18 dogs because non-cooperation of owners and pets.

The mean sperm concentration, progressive motility and percent live sperm count decreased on day 45 in all the treatment groups I, II, III and IV, however, no difference was recorded in dogs of control group. Color of semen changed from milky white to off white in treatment groups, but no change was found in control group. The literature is scant on semen parameter using eugenol and clove oil in male

Table 4: Mean scrotal circumference (inches) before and after intra-testicular treatment in dogs

Group	Mean scrotal circumference (inches)					Mean reduction in scrotal circumference (%)
	Day- 0	Day- 7	Day- 15	Day- 30	Day-45	Day 0 to 45
I	$5.89^{a}\pm0.18$	$6.27^{ABa}\pm0.16$	$5.99^{ABa}\pm0.20$	$5.75^{a}\pm0.19$	$5.54^{b}\pm0.19$	$5.91^{AB}\pm1.48$
II	$6.37^{a}\pm0.14$	$6.79^{Aa}\pm0.18$	$6.59^{Aa}\pm0.16$	$6.32^{a}\pm0.16$	$5.84^{b}\pm0.08$	$8.14^{A}\pm0.87$
III	$6.09^{ab}\pm0.16$	$6.50^{Aa}\pm0.17$	$6.31^{ABab}\pm0.16$	$6.11^{ab}\pm0.15$	$5.87^{b}\pm0.18$	$3.66^{BC}\pm0.86$
IV	$5.98^{abc}\pm0.23$	$6.55^{Aa}\pm0.21$	$6.28^{ABab}\pm0.18$	$5.81^{bc}\pm0.18$	$5.51^{c}\pm0.11$	$7.64^{A}\pm2.14$
V	$5.71^{a}\pm0.33$	$5.72^{Ba}\pm0.31$	$5.71^{Ba}\pm0.32$	$5.69^{a}\pm0.33$	$5.69^{a}\pm0.33$	$0.29^{C}\pm0.14$

Means bearing different superscript within group (lower case) and between groups (upper case) differ significantly ($p<0.05$).

Table 5: Mean paired volume of testes (cm^3) before and after intra-testicular treatment in dogs

Group	Paired volume of testes (cm^3)					Mean reduction in PVT (%)
	Day-0	Day-7	Day-15	Day-30	Day-45	Day 0 to 45
I	$10.65^{a}\pm0.79$	$12.68^{ABa}\pm0.88$	$11.49^{a}\pm0.96$	$10.27^{a}\pm0.88$	$07.81^{ABb}\pm0.50$	$26.22^{B}\pm1.75$
II	$11.35^{b}\pm1.13$	$15.79^{Aa}\pm1.17$	$12.03^{b}\pm0.81$	$09.55^{bc}\pm0.67$	$07.22^{Bc}\pm0.63$	$35.74^{A}\pm2.25$
III	$10.81^{b}\pm0.68$	$13.61^{Aa}\pm0.81$	$11.94^{ab}\pm0.78$	$09.96^{bc}\pm0.69$	$08.11^{ABc}\pm0.59$	$25.19^{B}\pm0.93$
IV	$11.15^{abc}\pm1.07$	$13.74^{Aa}\pm1.69$	$12.06^{ab}\pm0.62$	$09.82^{bc}\pm0.83$	$08.09^{ABc}\pm0.61$	$26.57^{B}\pm0.78$
V	$09.56^{a}\pm0.84$	$09.61^{Ba}\pm0.84$	$09.59^{a}\pm0.85$	$09.59^{a}\pm0.85$	$09.55^{Aa}\pm0.84$	$00.12^{Ca}\pm0.11$

Means bearing different superscript within group (lower case) and between groups (upper case) differ significantly ($p<0.05$)

dogs as in the present study, hence results could not be compared. Although, results of semen parameters indicating degenerative changes in testes however, less numbers of ejaculates from few animals may not be sufficient to conclude anything as statistical analysis could not be done for the same.

Testicular morphometry parameters showed reduction in testicular size after intra-testicular administration of clove oil along with 1% lignocaine followed by eugenol, clove oil alone and clove oil with olive oil indicating atrophy/ degenerative changes.

Conclusion

The reduction in testicular size following intratesticular administration of clove oil and eugenol in the study may be attributed to degenerative changes in the testicles.

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