EFFECTS OF 4-VINYL-1-CYCLOHEXENE 1,2-EPOXIDE ON THE FERTILITY OF FEMALE DOGS: A HISTOPATHOLOGICAL INVESTIGATIONS

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ABSTRACT

This study aimed to investigate the effects of 4-vinyl-1-cyclohexene 1,2-epoxide on ovarian follicles in dogs. A total of 25 mongrel bitches were used in the study. The animals were randomly allocated to 5 groups. As a control, dose-dependent groups (240 and 320 mg/kg IP), and time-dependent groups; the ovaries were removed on day 5 or 8 following the VCE administration. The ovaries were subjected to routine tissue processing and examined to determine the number of follicles in each ovary. The assessment of the number of primordial follicles showed that compared to the control group, a decrease of 25%, 35%, 34% and 42% had occurred in Groups 2 ($p<0.05$), 3, 4 and 5, respectively ($p<0.01$). The decrease in the number of primary follicles was determined to have occurred at a level of 41% in Group 2, 61% in Group 3, 44% in Group 4, and 56% in Group 5 ($p<0.01$). Secondary follicles were determined to have decreased at a level of 27% in Group 2, 52% in Group 3, 30% in Group 4 and 54% in Group 5 ($p<0.01$), and the decrease in Group 2 was not statistically significant ($p>0.05$). Antral follicles were determined to have decreased at a level of 47% in Group 2 ($p>0.05$), 88% in Group 3, 48% in Group 4, and 76% in Group 5 ($p<0.01$). Our results show that VCE reduced the number of ovarian follicles in bitches, and therefore, could be used for chemical sterilization in dogs.

***Key words**: Vinyl cyclohexene epoxide, dog, ovarian follicle, fertility.*

INTRODUCTION

Various sterilization methods have been developed for dogs aimed at preventing genital diseases, controlling stray dog populations, reducing aggressive sexual behaviour and avoiding unwanted pregnancies as a result of contact with stray animals. Dogs are sterilized by hormonal, chemical or surgical techniques (Kutzler and Wood, 2006). The hormonal method is costly and requires continuity. Although performed with success, surgical methods are associated with several postoperative complications, including obesity, cardiac stress, enuresis, hemorrhage and behavioral disorders (Kalkan and Alaçam, 1999). In view of these considerations, the use of chemical agents has gained importance (Wiebe and Howard, 2009; Goericke-Pesch, 2010; Aydın and Abay, 2013). Female animals are administered with various chemical substances to accelerate the follicular atresia and reproductive senescence, including polycyclic hydrocarbons such as 3-methylcholanthrene (3-MC) and benzo (a) pyrene (BaP) (8, 10). 4-vinyl-1-cyclohexene 1,2-epoxide (VCE) is a metabolite of vinyl cyclohexene (VCH). Both VCH itself and its metabolites have toxic effects on the ovaries, and cause atrophy of ovarian follicles. The objective of this study was to investigate the timedependent effects on the ovarian follicles of a single dose of VCE, which is an aromatic hydrocarbon and to discuss its possible use for the chemical sterilization of dogs.

MATERIAL AND METHODS

Experimental protocol. A total of 25 mongrel bitches, aged 8-20 months, were used in the study. The animals were randomly allocated to 5 groups. The first group (n=5) was maintained for control purposes and was administered with sesame oil by intraperitoneal route. The second (n=5) and third (n=5) groups received a single intraperitoneal VCE (SIGMA) dose of 240 mg/kg, while the fourth (n=5) and fifth (n=5) groups received a single intraperitoneal VCE dose of 320 mg/kg. The ovaries of the bitches were removed by ovariohysterectomy under general anesthesia, on day 5 following the last VCE administration in Groups 1, 2 and 4, and on day 8 following the last VCE administration in Groups 3 and 5.

Histopathology examination. Ovarian samples were fixed in 10% buffered formaldehyde, and subjected to routine tissue processing. The fixed sections were dehydrated by passage through a graded series of alcohol and xylol, and were finally blocked in paraffin. Sevenmicron-thick sections were cut from the blocks. After being deparaffinised and dehydrated, the sections were stained with haematoxylin-eosin (HxE) and examined under a light microscope.

Biochemical measurements. The ovarian samples were transferred into glass tubes, to which 10 mL of 140 mM KCl solution per 1 g of tissue was added. The tissue samples were then homogenized and the resulting homogenate was centrifuged at 2800 g and +4°C for 10 minutes. The supernatant was used for the measurement of the total antioxidant capacity (TAC) and total oxidant status (TOS).

Statistical analysis. The statistical analyses were performed using SPSS version 20.0 software for Windows (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed using Kruskal-Wallis and Mann-Whitney U test.

RESULTS

VCE treatment significantly reduced primordial, primary, secondary, and antral follicle numbers compared with sesame oil-treated animals (Table 1). The assessment of the number of primordial follicles showed that compared to the control group (Fig. 1A).

Time-dependent results. In order to assess the time-dependent effects of VCE, comparisons were made between Groups 2 and 3 (administered with 240 mg/kg of VCE), and between Groups 4 and 5 (administered with 320 mg/kg of VCE). It was ascertained that Groups 2 and 3 significantly differed for all follicle types (Fig. 2A, 2B) ($p<0.05$) and that the time-dependent decrease in the numbers of the primary and antral follicles was particularly significant ($p<0.01$). The comparison of Groups 4 and 5 demonstrated that while the differences between the two groups in the numbers of the primordial and primary follicles (Fig. 2C, 2D) were not significant ($p>0.05$), the differences for the other types of follicles were statistically significant and associated with a higher rate of timedependent decrease ($p<0.01$). Although statistically insignificant, the loss of primordial and primary follicles was greater in Group 5 than Group 4.

Dose-dependent results. To investigate the correlation between the decrease in the number of ovarian follicles caused by VCE and the treatment dose, comparisons were made between Group 2 (240 mg/kg) and Group 4 (320 mg/kg), after surgery on day 5, and between Group 3 (240 mg/kg) and Group 5 (320 mg/kg), after surgery on day 8. No significant difference was determined between Groups 2 (Fig. 2A) and Group 4 (Fig. 2D) for any of the follicle types ($p>0.05$). However, the mean number of primordial follicles in Group 2 was approximately 300 follicles greater than that in Group 4. The comparison of Groups 3 and 5 revealed that the number of primordial follicles was statistically significantly lower in Group 5 ($p<0.05$), and the decrease in the number of antral follicles was statistically significantly greater in Group 3 ($p<0.01$).

Table 1: Follicle numbers among the groups.

Groups	Group 1	Group 2	Group 3	Group 4	Group 5
Primordial	3410±35.5	2589±176.8*	2218±61.2**	2271±84.9**	1996±40.8**
Primer	767±9.5	474± 34.8**	301 ± 27.2**	432±32.1**	342±26.6**
Sekonder	442±7.7	324±53.8	215±6.0**	311±9.7**	206±3.8**
Antral	149±4.8	79±18.0	19±1.5**	78±9.8**	37±2.6**

Results are expressed as mean ± SEM.
*Means in the same row are significantly different (P<0.05).
**Means in the same row are significantly different (P<0.01).

Table 2. Levels of TAC and TOS.

Groups	Group 1	Group 2	Group 3	Group 4	Group 5
AC	5.65±0.57	4,05±0.31	3,82±0.39	3,87±0.38	3,79±0.37
TOS	3,69±0.34	4,39±0.28	4,30±0.29	4,51±0.29	4,44±0.38

Results are expressed as mean ± SEM.
*Means in the same row are significantly different (P<0.05).
**Means in the same row are significantly different (P<0.01).

Biochemical results. The biochemical data obtained in the present study is given in Table 2. Significant differences were determined between the groups in both parameters (p<0.05). As a result, TAC was determined to have significantly decreased in all the treatment groups, compared to the control group (p<0.01). However, no statistically significant dose- or time-dependent difference was detected between the treatment groups (p>0.05). In comparison to the control group, TOS, which is an indicator of oxidative stress, was determined to have significantly increased in all the treatment groups (p<0.05). Nonetheless, no dose- or time-dependent difference was determined between the treatment groups (p>0.05).

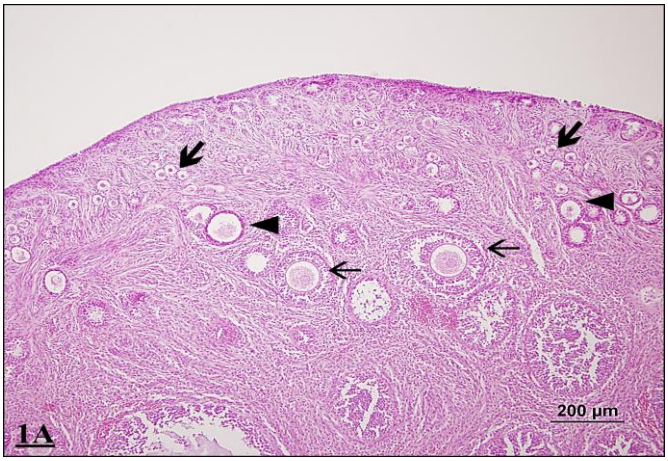

Fig. 1A. Group 1; Thick arrows indicate primordial follicles. Thin arrows show secondary follicles and arrowheads indicate primary follicles (HxE, x100).

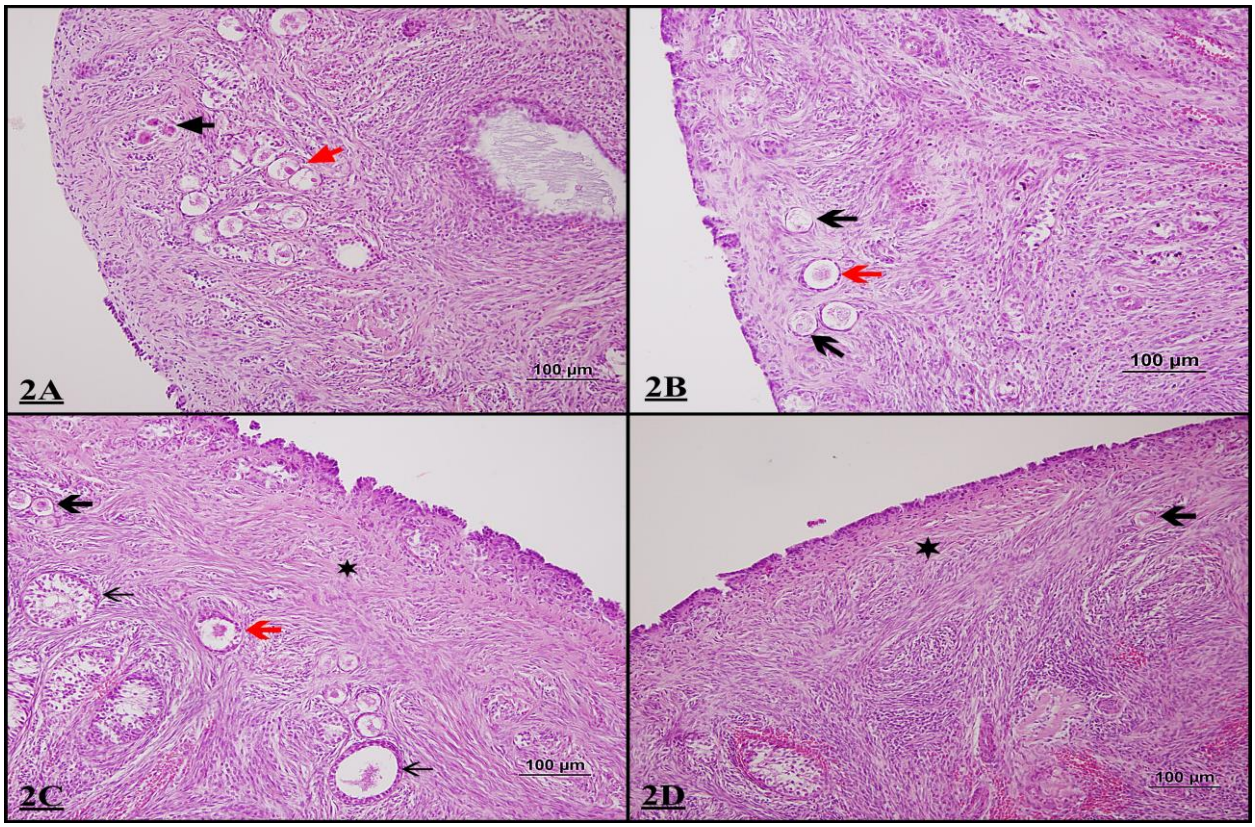

Fig.2A. Group 2; This figure shows necrotic primordial follicle (black arrow) and degenerated primary follicle (red arrow) in the ovaries. (HxE, x200). **2B.** Group 3; Black arrows indicate degenerated primordial follicle and red arrow indicates degenerated primary follicle (HxE, x200). **2C.** Group 4; Thick arrow indicates degenerated primordial follicles. Thin arrows show degenerate secondary follicles and red arrow indicate primary follicle. Fibrosis is seen in the area marked with star (HxE, x200). **2D.** Group 4; Degeneration of primordial follicle (arrow) and fibrosis (star) in the ovaries (HxE, x200).

DISCUSSION

In a study of the dose-dependent effects of VCD, Hoyer and Mayer (2009) administered daily VCD doses of 80 mg/kg, 160 mg/kg and 240 mg/kg to mice for 15 days and determined that the primordial and primary follicles had been entirely destroyed by the end of the study. The present study was terminated 8 days after the final VCE administration, and on the basis of the decrease observed in the number of follicles, it was confirmed that the time-dependent effects of VCE were similar to those of VCD. In the present study, VCE doses have caused significant losses in the primordial and primary follicles, which occurred within 5 to 8 days as a result of the administration of a single dose. Sahambi et al. (2008) investigated the time-dependent effects of VCD and administered a daily dose of 240 mg/kg to mice for a period of 5 days. These mice were sacrificed on days 16, 37, 57 and 100. The researchers reported that the number of primordial and primary follicles had decreased by 96% on day 16, while between days 37 and 100, nearly all of the follicles, excluding the antral follicles, had been depleted. In the present study, in Groups 2 and 3, which received a single VCE dose of 240 mg/kg, the primordial and primary follicles were determined to have decreased by 25% and 41%, respectively, on day 5, and by 35% and 61%, respectively, on day 8. The researchers ascertained that, following the administration of 320 mg/kg of VCD, the number of follicles decreased significantly from day 6 onwards. Similar findings were observed as from day 5 in the present study, and a significant decrease was determined in the

number of follicles. Devine et al. (2001) also investigated the effects of VCD on liver and ovarian glutathione (GSH) levels, and administered a daily VCD dose of 80 mg/kg to rats for a period of 15 days. It was determined that liver glutathione levels had significantly decreased at 2 hrs post-administration, whereas the liver concentrations displayed no significant alteration at 6 and 26 h post-administration. It was also determined that during the 15-day administration period of VCD, the ovarian GSH levels did not alter, but at 26 hrs after administration, the liver and ovarian GSH levels were determined to have increased. In parallel with this data, it was ascertained that throughout the 15-day administration period, while the numbers of primordial and primary follicles decreased significantly, no change occurred in the number of secondary follicles. As a result, no correlation was determined to exist between the GSH level and the follicle loss caused by VCD, and it was indicated that GSH was involved only in the metabolization of VCD in the liver. However, in the present study, it was determined that in the ovaries of the animals administered with VCE, while the antioxidant capacity decreased, the level of oxidative stress increased. This contradicted the findings of the above-mentioned study by Devine et al., and the difference was attributed to a higher dose having been used in the present study, thereby exceeding the threshold tolerable by the ovaries.

CONCLUSION

The results of this study suggest that VCE could be used for chemical sterilization of bitches. Nevertheless, it is considered that the effects of VCE on other organs and tissues should be investigated, and that the present study would contribute to future research in this field.

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