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Original article

Evaluation of intra-testicular injections of calcium chloride and 4-vinylcyclohexene 1,2 monoepoxide for chemical sterilization in guinea pigs

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Abstract

This study was aimed at investigating the use of intra-testicular calcium chloride ($CaCl_2$) and 4-vinylcyclohexene 1,2-monoepoxide (VCM) injections as a side effect-free alternative method for the control of reproduction in guinea pigs. Fifty male guinea pigs were randomly assigned to five groups. In all groups, the chemical agents were injected into both testes in 1% lidocaine hydrochloride. While Groups I, II and III were administered with a single dose (0.25 mL) of sterile physiological saline, 15 mg/100 g $CaCl_2$, and 240 mg/kg VCM, respectively, Group IV and V received a daily dose of 15 mg/100 g $CaCl_2$, and 240 mg/kg VCM for 3 days, respectively. On day 90 post-administration, all animals were weighed and later decapitated under ether anaesthesia. Blood and tissue (testis, liver, hypophysis and adrenal gland) samples were taken. Sperm samples from the cauda epididymis were examined for spermatological parameters. Blood was used for hormone analyses and tissue samples were examined histopathologically (haematoxylin-eosin) and immunohistochemically (Tunel staining). The epididymal sperm count decreased in all treatment groups. Excluding 2 animals, Group V displayed azoospermia. When compared to the control group, Group V displayed the highest prolactin and lowest testosterone levels, and Group III showed the highest testosterone level. Histopathological examination revealed no intoxication finding. Chemical castration with VCM may be a good alternative to surgical castration as it enables mass sterilization without postoperative risks in guinea pig.

Key words: calcium chloride, chemical castration, guinea pig, sperm, 4-vinylocyclohexene 1,2-monoepoxide

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Introduction

Research on the fertility control in animals has been conducted for more than five decades (Mohammed and James 2013). The control of reproduction is primarily aimed at the suppression of unwanted nuisance' male sexual behaviour, reduction of animal population size and suppression of physiological sexual activity. Population management is ensured mainly by means of surgical castration, namely, orchidectomy. However, not only is surgical castration costly and time-consuming, but it also poses the risk of postoperative infection, which needs to be minimized by postoperative care (Jana and Samanta 2011). Although surgical castration does not cause any major problem in livestock, it may result in several complications, including postoperative hormonal imbalance, obesity, cardiac stress, urine incontinence, haemorrhage, ureter ligation, and behavioural changes in pet animals. On the other hand, hormone therapy, which is used as an alternative to surgical castration, has other disadvantages, including high costs, the varying effects of hormonal agents, the need for the frequent repeat of such therapy and the development of serious side effects. Eventually, this has led to an increase in research on chemical castration in the last decade. Chemical castration is based on the arrest of the production of spermatozoids in the testes by the intra-testicular or intra-epididymal administration of a chemo-sterilizing agent, which causes cell and tissue sclerosis (Okwee-Acai et al. 2008). Chemical agents used for chemical castration include cadmium chloride, iron chloride, iron sulphate, danazol, glycerol, lactic acid, formalin, and calcium chloride ($CaCl_2$) (Mohammed and James 2013), but an effective chemosterilizing agent has yet to be established. The first commercial zinc gluconate-based chemical sterilant that fulfilled the criteria of safety and effectiveness by FDA has been approved used for chemical castration of male dogs in 2003 (Oliveira et al. 2012, Fagundes et al. 2014). But, as it caused unacceptable serious inflammatory response in some dogs, it was withdrawn from the market in the United States in 2005. 4-vinylcyclohexene 1,2-monoepoxide (VCM), a metabolite of 4-vinylcyclohexene (VCH) that can also be used for chemical castration, is a by-product generated during the manufacture of insecticides, flame retardants, emollients and rubber wheels, and has ovotoxic effect (Mark-Kappeler et al. 2011). When metabolized in the body, VCH is converted into the more potent VCM and epoxide derivatives such as 4-vinylcyclohexene diepoxide (VCD). In a previous study, while the administration of neither VCH (800 mg/kg, ip) nor VCM (200 mg/kg, ip) caused testicular deformities in mice, it was ascertained that VCD, which is yet another metabolite of VCH, caused damage to the spermatogonia and spermatocytes (Hooser et al. 1995). While VCD administration has been reported to cause ovarian intoxication in female mice and rats as a result of the lysis of germ cells, in the same study, testicular damage was observed in only male mice and not in male rats (Smith et al. 1990). The present study was aimed at determining whether intra-testicular $CaCl_2$ and VCM injections could be used as chemical agents, for the control of reproduction in guinea pigs without any side effects.

Materials and Methods

Experimental animals

In this study, in total, fifty 10 to 12-week-old male guinea pigs, each weighing 500-600 g, were used. Prior to the intra-testicular injections, the animals were acclimatized to the laboratory conditions for a period of 15 days. The animals were kept under appropriate environmental conditions: room temperature 17-19°C, relative humidity 40-60% and light/dark cycles of 12/12 h. The animals were fed with guinea pig feed and tap water was given ad libitum. This study was approved by the Animal Experiments Local Ethics Committee of Dollvet Laboratories (Approval number (2014-20).

Experimental protocol

The body weight of the guinea pigs was measured twice, before the intra-testicular injections and at the end of the trial, using a digital scale accurate to 10^{-4} kg. The maximum effective doses of $CaCl_2$ and VCM for the induction of sterilization were estimated by dividing the 50 animals into 5 groups by random selection (Groups I-V). The animals in the control group (Group I), each received a single bilateral intra-testicular injection of 0.25 ml of sterile physiological saline, containing 1% lidocaine hydrochloride (a local anaesthetic agent, Astra IDL, Bangalore, India). Groups II and III received a single bilateral intra-testicular injection of 0.25 ml of 15 mg/100 g of calcium chloride dihydrate in saline solution ($CaCl_2$, $2H_2O$, Merck, Mumbai, India) and 240 mg/kg of VCM (Aldrich, %98), containing 1% lidocaine hydrochloride, respectively (Jana et al. 2002, Roosa et al. 2015). Group IV and V received a daily bilateral intra-testicular injection of 15 mg/100 g calcium chloride dihydrate in saline solution and 240 mg/kg of VCM in 1% lidocaine hydrochloride for 3 days, respectively.

Intra-testicular injection of $CaCl_2$ and VCM solutions

The different doses of the chemo-sterilizing agents were injected into the dorsocranial aspect of each testis, near the caput epididymis and in parallel to the testes. All injections were performed with caution under ether anaesthesia, using a sterile 12 mm 27-guage needle in order to facilitate intra-testicular administration.

Assays for serum testosterone, estradiol and prolactin levels

Blood samples were collected directly by cardiac puncture into no-additive tubes 90 days post-administration from all animals and serum samples were extracted by centrifugation. The serum testosterone, estradiol and prolactin levels of the guinea pigs were measured by enzyme-linked immunosorbent assay (catalog no. CSB-EQ028156GU; Cusabio Biotech Co, Ltd, catalog no. CSB-EQ027953GU; Cusabio Biotech Co, Ltd, and catalog no. E-EL-GP0358; Elabscience Biotech Co, Ltd, respectively). The interassay and intraassay CV was less than 10%.

Epididymal sperm evaluation

On day 90 post-administration, both the treated and control animals were sacrificed under ether anaesthesia. Immediately thereafter, the testes and epididymis were excised. The cauda epididymis was separated and minced, using a pair of small scissors to release the sperm into 3 ml of warmed physiological saline in a Petri dish. The sperm suspension was then placed in an incubator at 37°C for 10 minutes prior to motility assessment. A 20 µl drop of the sperm was placed on a Makler counting chamber and motile sperm were then counted under a light microscope (X200).

Membrane integrity staining

A SYBR-14/Propidium iodide viability kit (Live/Dead sperm viability kit, catalog no: L-7011, Molecular Probes, Eugene, OR, USA) was used for membrane integrity staining. To diluted sperm 5 µL of SYBR-14 was first added and maintained at 38.5°C for 5 minutes, and then 5 µL of propidium iodide (PI) was added. Five minutes later, 3 µL of each sample was placed onto a glass slide and covered with a coverslip. 100 spermatozoa were counted under an OLYMPUS BX51 fluorescent microscope. Samples, which were stained with PI and observed to display red fluorescence, were considered to be non-viable, whereas those that were stained with SYBR-14 and were determined to display green fluorescence were considered to be viable (Varisli et al. 2009).

Histopathological examination

On day 90 post-administration, all of the animals were sacrificed under ether anaesthesia and tissue samples were taken from both testes, as well as from the liver, adrenal glands and pituitary gland (hypophysis). The tissue samples were fixed in 10% buffered formaldehyde. After being fixed, the tissues were trimmed, subjected to routine tissue processing, and embedded in paraffin. Five-micron-thick serial sections were cut from the paraffin blocks using a Leica RM 2125 RT microtome. The sections were passed through graded alcohol (50%, 75%, 96% 100%) series and xylol, and were stained with haematoxylin-eosin. The testicular tissue samples were examined for necrosis, degeneration, fibrosis, inflammation, hypospermatogenesis, and apoptosis, as well as for Leydig cells. Furthermore, histopathological changes in the other organs and tissues were assessed under a high-resolution microscope (Olympus BX-53, Tokyo, Japan).

Assessment of apoptotic cells

Sections were prepared from the same blocks of testicular tissue onto slides coated with poly-L-lysine. In order to identify the apoptotic changes, the sections were immunohistochemically marked using the TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling) method. The procedure and control stainings were carried out according to the manufacturer's instructions. TUNEL sections were blindly examined by two pathologists under a light microscope (Olympus DP53) interfaced with a camera (Leica, DFC 80). Ten different fields on each slide were examined at high magnification. Staining intensity semi-quantitatively in specific area was scored as negative (-), mild (+), moderate (++), and severe (+++) (Guler et al. 2011, Xu et al. 2004). The extent of staining was scored as – (0%-5%), + (6%-25%), ++ (26%-50%) and +++ (51% and higher) according to the percentage of positively stained cells. Each field was graded according to the score and then the total score was divided by ten. Thereby, the average score was calculated for each slide.

Table 1. Post-treatment body weight change, sperm motility, and epididymal spermatozoa, viable spermatozoa, serum oestrogen, testosterone and prolactin levels of male guinea pigs.

Groups	Body weight change (g)	Sperm count (millons/ml)	Sperm motility (%)	Viable spermatozoa rate (%)	Serum estrogen concentration (pg/ml)	Serum prolactin concentratin (ng/ml)	Serum testosterone concentration (ng/ml)
Control	550.83±20.89^{a}	58.11±6.17^{d}	84.28±1.09^{c}	93.57±0.91^{d}	114.54±0.67^{a}	0.19±0.02^{a}	2.28±0.03^{d}
15 mg $CaCl_2$	637.42±14.59^{b}	12.14±4.90^{b}	18.57±4.52^{b}	17.85±4.81^{b}	138.70±1.97^{b}	0.22±0.05^{a}	0.67±0.16^{b}
240 mg/kg VCM	636.57±18.78^{b}	18.89±4.23^{b}	12.14±2.39^{b}	12.14±2.39^{b}	140.63±0.58^{b}	0.26±0.07^{a}	0.87±0.14^{c}
15 mg $CaCl_2$ for 3 days	628.23±13.27^{b}	9.50±1.37^{c}	15.75±0.19^{b}	10.17±3.57^{b}	136.32±2.77^{b}	0.22±0.02^{a}	0.64±0.02^{b}
240 mg/kg VCM for 3 days	601.28±19.34^{b}	0^{a}a	0^{a}	0^{a}	148.33±0.58^{c}	0.50±0.09^{b}	0.56±0.04^{a}
	p<0.001	p<0.05	p<0.05	p<0.05	p>0.05	p<0.001	p<0.001

Data are mean±SEM, n=50; Groups with different superscripts (a,b,c,d) in the same column are significantly different (p<0.05, p<0.001).

Statistical analysis

Statistical analyses were carried out using the SPSS package program (Ver.14 for Windows). All results were shown as mean ± standard deviation. The level of statistical significance was set as p≤0.05. Firstly, Shapiro Wilk's normallity test was used to analyse data and it was observed that the continous data were not distributed normally. Next, the Kruskal-Wallis test was used to test the mean differences between the groups for estradiol, prolactin and testosterone levels. The Jonckheere-Terpstra rank-based nonparametric test was used to determine any statistically significant trend between ordinal pathological variables.

Results

Side effects

None of the guinea pigs displayed any noticeable complication such as diarrhea, lethargy, emesis, scrotal ulcerations and dermatitis. Scrotal swelling and tenderness are common in the first few days following injection. All the guinea pigs injected with calcium chloride and VCM survived the study in good, healthy condition throughout the experimental period.

Changes in relative body weight

It was determined that the animals included in all of the treatment groups displayed an increase in their body weight, when compared to the control animals (p<0.001). No statistical difference existed between the treatment groups for body weight increase (Table 1).

Effects on epididymal sperm evaluation

The epididymal sperm count was determined to have significantly decreased (p<0.05) in all the $CaCl_2$ and VCM treated guinea pig. When compared to the control group, the lowest spermatozoa count in relation to the treatment dose and agent was determined in Group V, followed by a group ranking of IV<III=II. Except for two animals included in Group V (Fig. 1e-f), all of the animals presented with azoospermia. These two animals could be more resistant to the substance than others. They need higher dose of substance to develop the effect. It may be due to a specific individual response. A decrease in both sperm motility and viable spermatozoa counts (Fig. 1b-c-d) were detected in all of the treatment groups, in comparison to the control group (Fig. 1a) (Table 1).

Effects on serum concentrations of testosterone, estradiol and prolactin

All of the treatment groups statistically differed from the control group for the serum estradiol and testosterone levels. On the other hand, excluding Group V, the treatment groups showed no statistical difference for serum estradiol levels in comparison to the control group. Serum testosterone levels were determined to have decreased in all of the groups, and the highest and lowest testosterone levels according to the control group, were detected in Group III and

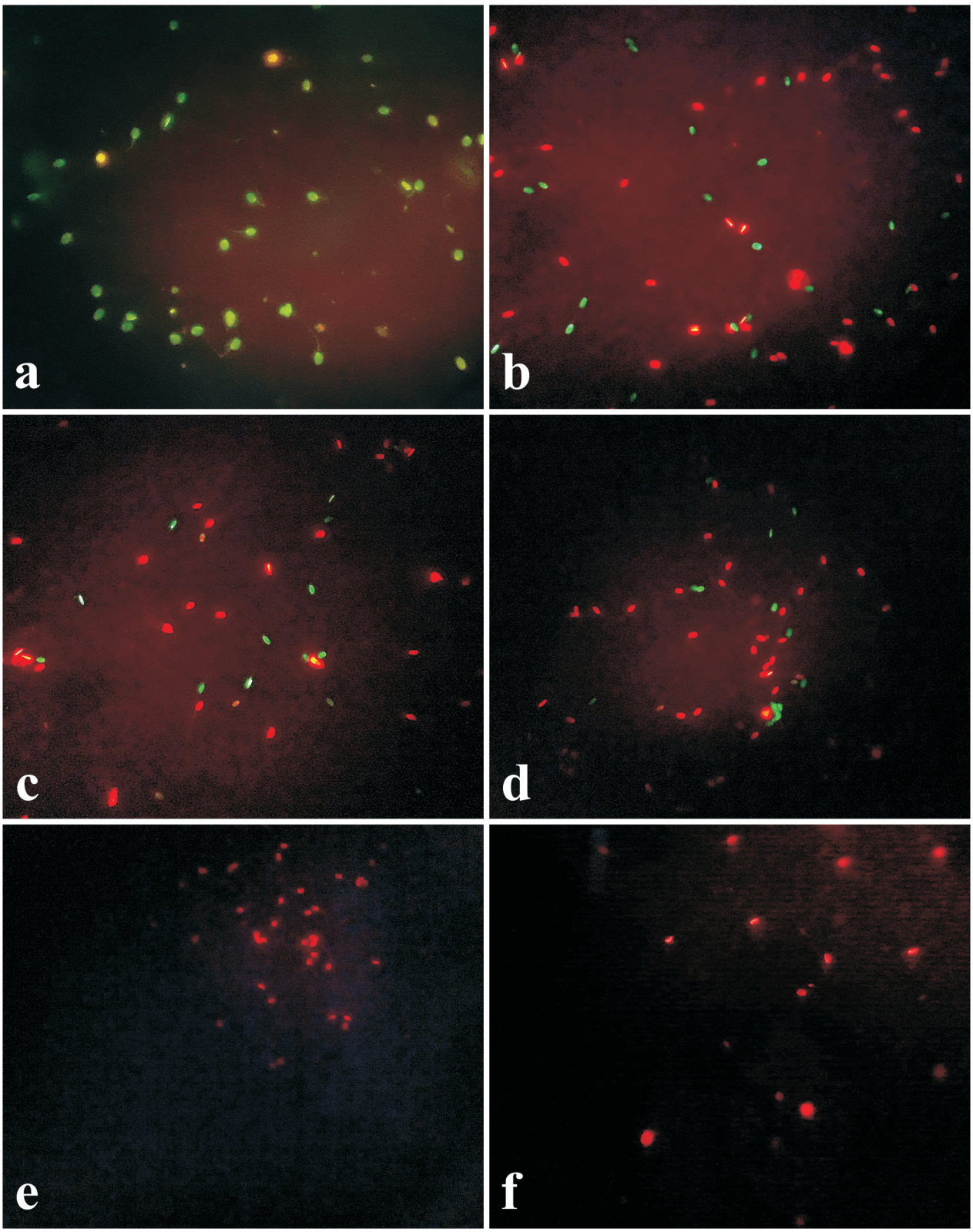

Fig. 1. (a) Fluorescent staining of guinea pig spermatozoa with SYBR/PI method (Control Group, x200 (b) Fluorescent staining of guinea pig spermatozoa with SYBR/PI after intra-testicular injection of 15 mg $CaCl_2$, x200 (c) 240 mg/kg VCM, x200 (d) 15 mg $CaCl_2$ for 3 days, x200 (e, f) 240 mg/kg VCM for 3 days, x100, x200, respectively (green-fluoresced denote live spermatozoa with intact membranes, red-fluoresced indicate dead spermatozoa, yellow- fluoresced represent moribund spermatozoa).

Table 2. The assessment of the presence of Leydig cells and apoptotic cells in testes.

Groups	Control	15 mg $CaCl_2$	240 mg/kg VCM	15 mg $CaCl_2$ for 3 days	240 mg/kg VCM for 3 days	
Number of leydig cells	3.0±0.0[a]	2.71±02[b]	1.40±02[c]	1.57±02[c]	1.14±01[d]	p<0.001
Apoptotic cells	0.9±0.6[a]	1.00±0.0[a]	1.14±0.1[a]	1.25±0.3[a]	1.50±0.5[a]	p>0.05

Data are mean ± SEM, n=50; Groups with different superscripts (a,b,c,d) in the same row are significantly different (p<0.05, p<0.001).

Group V, respectively. When compared to the control group, the highest prolactin level was detected in Group V. The prolactin levels of the other groups were found to be similar (Table 1).

Changes in the histomorphology of the testes

Microscopic examination revealed that, in Group V, the level of testicular atrophy (Fig. 2f) was greater than that observed in the other treatment groups, and extensive fibrosis and distinct necrotic and degenerative foci were also present. While the proportion of normal tissue was greatest in the treatment group administered with 15 mg of $CaCl_2$ (Fig. 2b-c), this group was followed by Groups III and IV. The similar degree of fibrosis and Leydig cell necrosis was observed in Groups II (Fig. 2b) and III (Fig. 2d) was found to be lower than that of Groups IV (Fig. 2e) and V (Fig. 2f). The ranking of the treatment groups for the decrease observed in the number of Leydig cells, in comparison to the control group, was as follows: II=III>IV>V. The level of inflammation and apoptosis was similar in all groups (Table 2). It was ascertained that in the treatment groups the necrotic and degenerative regions of the testicular tissues were characterized by the presence of TUNEL-positive cells (Fig. 3). Furthermore, the interstitial cells presented the nuclear positivity. The pathological examination of the liver, pituitary gland and adrenal glands revealed no sign of intoxication in any of the groups. Nevertheless, a few degenerative hepatocytes and slight hyperaemia were observed.

Discussion

In the present study, the impact of different doses of $CaCl_2$ and VCM, administered for the purpose of chemical sterilization, was investigated and demonstrated in male guinea pigs. Research continues on alternative methods that can replace surgical castration (Jana and Samanta 2006). However, to date, a 100% effective method with no side effect has not been able to be developed for the termination of reproductive ability. The aim of all types of methods used for castration (surgical, hormonal and chemical) is the reversible or irreversible loss of fertility. Each agent or method used for this purpose has different advantages and disadvantages. Different from previous research, following the intra-testicular injections, side effects such as diarrhoea, lethargy, emesis, scrotal ulcerations and dermatitis, were not observed (Jana and Samanta 2006, Kutzler and Wood 2006).

In the present study, all of the treated animals displayed an increase in body weight when compared to the control animals. On the contrary, in a study conducted by Chhabra (1990), following the administration of high doses of VCD (from 6.25 to 200 mg/kg) by either gavage or dermal route for a period of 13 weeks, a decrease was observed in the body weight of both male and female rats. Furthermore, the body weight of male mice exposed to 1500 ppm of VCH by inhalation was also reported to have decreased (Bevan et al. 1996). The differences observed between the results of the present study and previous researches for body weight change can be attributed to different administration routes having been used and effect of a more potent metabolite of VCH having been administered in the present study. Furthermore, it is considered that the body weight increase observed in the present study may have arisen from the increased estradiol level and decreased testosterone level of the treated animals. In agreement with the present study, previous research has shown distinctly increased plasma estradiol levels and decreased testosterone levels in obese males (Coheng 2008).

Motile sperm could not be obtained from the guinea pigs included in Group V, but was able to be obtained from the animals included in the other treatment groups. Damage to the plasma membrane of the sperm cell as a result of VCM for 3 days exposure is considered as a possible cause of sperm immotility. The lowest level of plasma membrane integrity was detected in Group V, whilst the highest level of plasma membrane integrity was determined in Group II-III. Several researches have shown that various substances, which disrupt membrane integrity, adversely affect sperm motility (Bennetts and Aitken 2005).

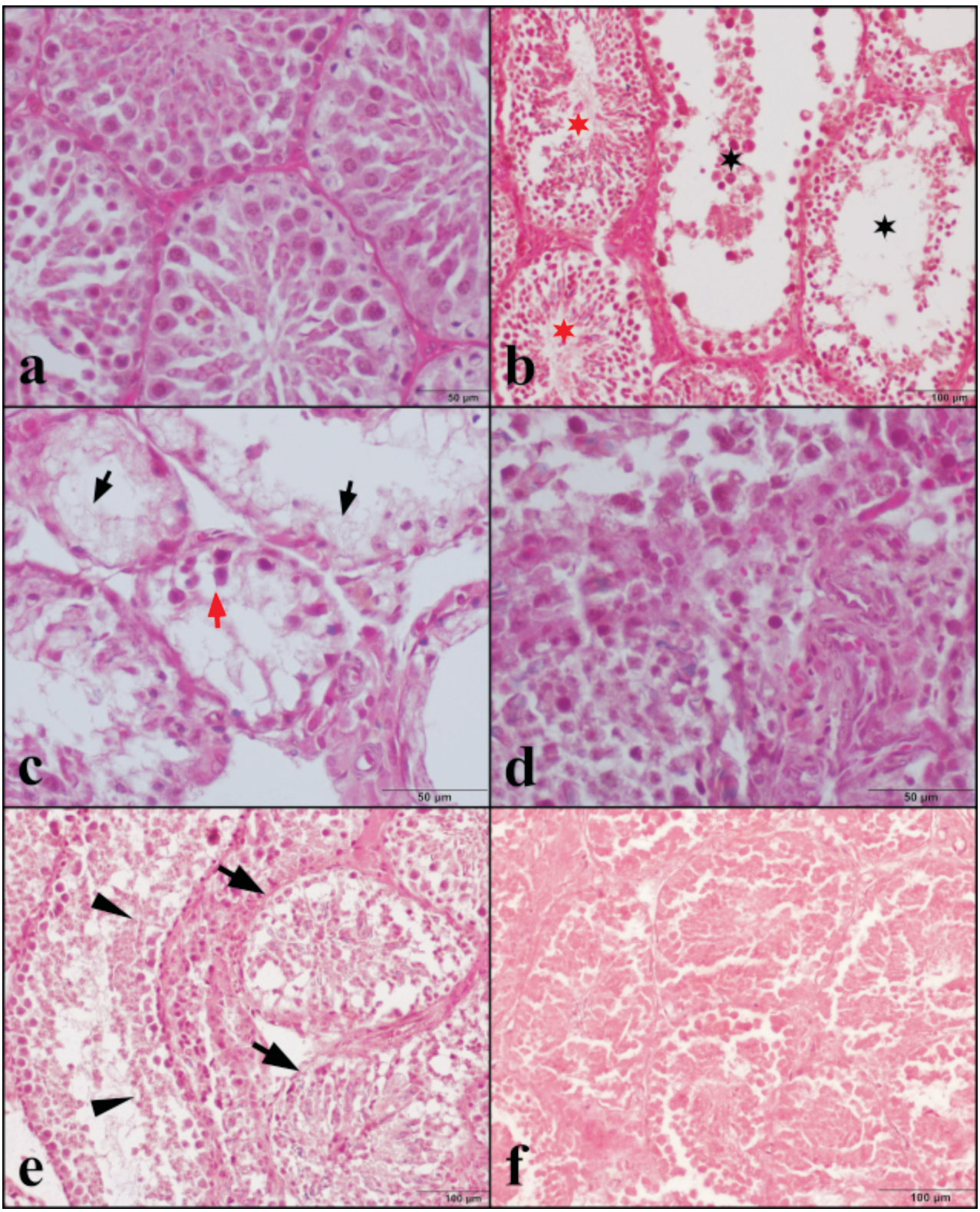

Fig. 2. (a) Control group, normal histological appearance of seminiferous tubules before injection, H&E, x400 (b) Necrotic tubules (black star) and degenerative tubules (red star) after intra-testicular injection of 15 mg/kg $CaCl_2$, H&E, x200 (c) Vacuolar degeneration (arrows) and degenerative cell (red arrow), after intra-testicular injection of 15 mg/kg $CaCl_2$, H&E, x400 (d) Degenerative tubules, after intra-testicular injection of 240 mg/kg VCM, H&E, x400 (e) Degenerative tubules (arrows) necrotic cells (arrowheads), after intra-testicular injection of 15 mg/kg $CaCl_2$ for 3 days, H&E, x200 (f) Complete coagulation necrosis after 240 mg/kg VCM injection for 3 days, H&E, x200.

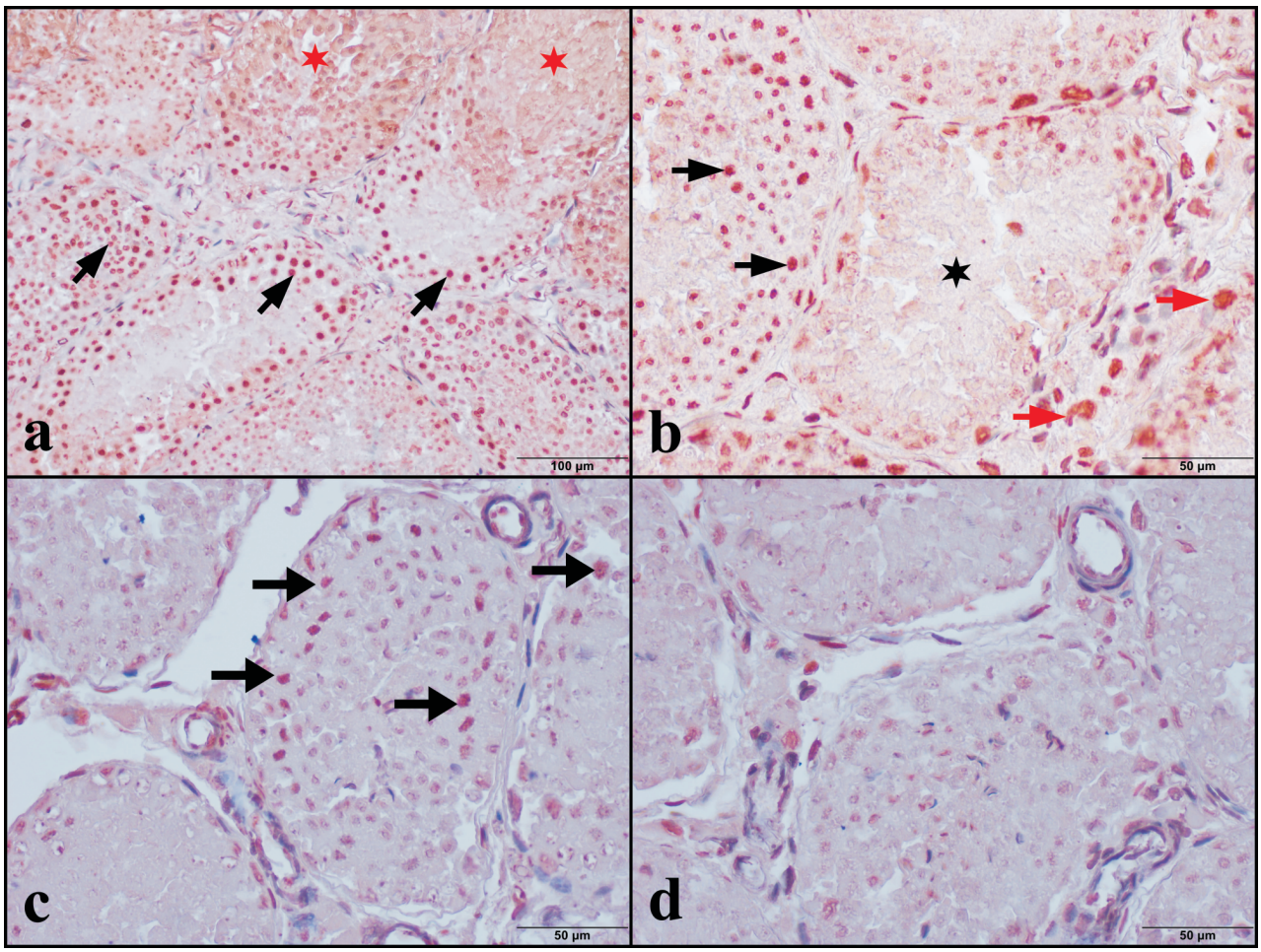

Fig. 3. (a) TUNEL positive apoptotic tubul cells (arrows) ve necrotic tubules (red stars) after intra-testicular injection of 15 mg/kg $CaCl_2$, x200 (b) TUNEL positive apoptotic tubul cells (black arrows), TUNEL positive apoptotic leyding cells (red arrows) necrotic tubul (star) After intra-testicular injection of 240 mg/kg VCM, x400 (c) TUNEL positive apoptotic tubul cells (arrows), After intra-testicular injection of 15 mg/kg $CaCl_2$ for 3 days, x400 (d) Complete coagulation necrosis after 240 mg/kg VCM injection for 3 days, TUNEL stain, x400.

Owing to the sperm reserves in the epididymis, depending on the method of treatment, normally, fertile sperm is found in the testes for a period of up to 60 days post-injection (Kutzler and Wood 2006). In the present study, the different doses of the different chemo-sterilizing agents injected by intra-testicular route were determined to have caused severe oligozoospermia and azoospermia. The maximum response for the spermatological parameters was achieved in Group V. Similar to the results of the present study, Leoci (2014) reported that in dogs administered with 10 and 20 mg $CaCl_2$ asthenozoospermia and oligozoospermia was detected. Furthermore, in several studies carried out in rams, male rats, bucks and male dogs, it has been reported that the intra-epididymal injection of $CaCl_2$ reduces the concentration of spermatozoa (Jana et al. 2002, Okwee-Acai et al. 2008). As sperm production in the testes and sperm maturation in the epididymis are both regulated by testosterone, the significant decrease detected in the epididymal sperm count was attributed to the low level of testosterone (Jana et al. 2002, Okwee-Acai et al. 2008).

Previous research has demonstrated that the intra-testicular injection of $CaCl_2$ causes atrophy of the seminiferous tubulus in male dogs (Ibrahim et al. 2016). Similarly, the present study showed that, in male guinea pigs, the intra-testicular injection of $CaCl_2$ and VCM, led to testicular toxicity and germ cell necrosis depending on the administration dose and period, and thereby, resulted in the atrophy of the testes. The microscopic examination of the tissue samples revealed that the proportion of normal tissue was higher in the testes of the animals included in Group II. In agreement with the present study, Jamanta and Samanta (2011) reported that the administration of a high dose of $CaCl_2$ (20%) to tomcats resulted in the degeneration of the germ cells and the necrosis of the entire testicular tissue. In the present study, microscopic examination revealed marked de-

generation and necrosis associated with significant hypospermatogenesis in the testicular tissue of the animals included in Group V. On the other hand, different from the results of the present study, Hooser et al. (1995) determined that the intraperitoneal administration of VCM to male mice at a dose of 200 mg/kg did not cause testicular deformities. Furthermore, Smith et al. (1990) suggested that the observation of different effects in male mice and rats may be due to differences in epoxide formation in the liver. The level of germ cell degeneration caused by $CaCl_2$ and VCM may be related to the decreased serum concentration of testosterone, which is the prime regulator for the maintenance of the normal physiology and structural morphology of the seminiferous tubules (Tilbrook and Clarke 2001).

In the present study, when compared to the control group, the serum testosterone levels decreased in all of the treatment groups. Similar to the results of the present study, previous researches showed that the injection of a single bilateral intra-testicular dose of $CaCl_2$ caused a decrease in serum testosterone levels in both bulls and bucks, depending on the administration dose and period (Canpolat et al. 2006, Mohammed and James 2013). However, as the chemical castration of bulls with $CaCl_2$ was found to cause only a minimal change in serum testosterone levels and slight histopathological damage to the testes, $CaCl_2$ administration was not considered as a viable alternative to surgical castration (Canpolat et al. 2006). Literature reports indicate that the excessive release of the prolactin hormone from the anterior pituitary gland (hyperprolactinaemia), causes either oligozoospermia or azoospermia in males (Kutzler and Wood 2006). In the present study, azoospermia was detected in Group V, and oligozoospermia was observed in other groups. The highest prolactin level was measured in Group V. In the light of reports that suggest stress to be associated with increased corticosterone levels as well as an increased release of prolactin from the anterior pituitary gland (Riou et al. 2010), it is considered that the guinea pigs included in Group V may have been exposed to a greater level of anaesthesia-induced stress.

It is reported that, in males, the oestrogen hormone derived from testosterone, shows effect on the peripheral tissues, and thereby, plays a role in sexual behaviour and normal male physiology (Wibowo and Wassersug 2013). It was determined that the serum estradiol levels of all of the treatment groups were higher than the levels of the control group and the highest serum estradiol level was measured in Group V. The results of the present study demonstrated that decreased serum testosterone levels caused a varying level of increase in serum estradiol concentrations. Low levels of estradiol may prevent the apoptosis of germ cells (Mishra and Shaha 2005). Particularly in the animals included in Group V, a marked decrease in the number of Leydig cells as well as the presence of necrosis was observed in parallel with the decrease in serum testosterone concentrations. No difference was observed for apoptosis. It is considered that germ cell degeneration may have developed as a result of an increase in programmed cell death (apoptosis) due to androgen deficiency (Fujisawa et al. 1999). However, the cause of cell death may be either necrosis or apoptosis. Due to the alterations it causes in the cell, apoptosis is mostly perceived as part of the necrotic process. In the present study, germ cell degeneration may have resulted from necrosis.

Previous research has shown that the administration of VCD by oral route causes ovarian degeneration in both female mice and rats, and testes degeneration in male mice with very limited effect on the other organs. The differences observed between these species regarding the effects of VCD have been attributed to the differences between the species for epoxide formation in the liver. In view of the main bio-activation site of VCH being the liver, in the present study, the pathological examination of the liver, adrenal glands and pituitary gland revealed slight hyperaemia, vacuolisation and degeneration, yet no sign of intoxication was observed. In agreement with the present study, a previously conducted investigation showed that short-term VCD administration to rodents had only a minimal effect on the liver, spleen, kidneys and adrenal glands (Sahambi et al. 2008). In another study, it was reported that when VCH was orally administered to mice for a period of 90 days, the target tissue could be the kidneys. An increased formation of hyaline drops was determined in the proximal tubules of the kidneys in male rats exposed to VCH. No haematological effect was observed in any of the rats, which were exposed to VCH. On the other hand, these rats presented with renal necrosis caused by tubular cell degeneration.

Conclusions

Intra-testicular VCM administration for 3 days reduced the number of Leydig cells in the testes as a result of necrosis, and thereby, led to the permanent sterilization of the guinea pigs. On the other hand, a single bilateral intra-testicular injections of $CaCl_2$ solution did not cause permanent sterilization. It is considered that chemical castration by means of VCM administration is superior to $CaCl_2$ administration and can be in the future a viable postoperative risk-free alternative to surgical castration for the mass sterilization of in male guinea pigs. Due to the fact that study was conducted in guinea pigs, generated results could not be transferred directly to other animal species. Because, there are

species-dependent differences in the reaction of animals to chemical agents injected into the testis (Kutzler and Wood 2006). Further studies are necessary to evaluate different higher dose in order to use single dose of VCM.

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