

Biochemical and Histological Alterations In Reproductive Tract Tissues of Male Swiss Albino Mice Exposed Commercially Prepared Aloe Vera Gel Product

Ticari Olarak Üretilen Aloe Vera Jel Ürünü Uygulanan Erkek Swiss Albino Farelerin Üreme Sistemi Dokularında Biyokimyasal ve Histolojik Değişiklikler

Research Article

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ABSTRACT

This study was performed to determine the effects of Aloe vera gel (AVG) from the leaves of *Aloe barbadensis* which is commercially available product in the market for human use, on male reproductive tract of Swiss albino mice. Male mice aged 4 weeks separated into four groups randomly received AVG by gavage daily for 28 days. The groups were as follows (n=5): control, $\times 0.5$ dose group received half of recommended AVG dose, $\times 1$ dose group received recommended AVG dose, and $\times 2$ dose group received twice of recommended AVG dose. LH levels of mice in treatment groups decreased compared to control. Testosterone (T) levels decreased in $\times 2$ dose group compared to control. Sperm head count did not change and sperm shape abnormalities in high dose group increased. Histopathological analysis showed that there were atrophic tubules, germ cell debris, picnotic cells and Sertoli cell vacuolization in testes of high dose groups. In prostate glands, atrophic tubules and mononuclear cell infiltration were observed. There were no changes in malondialdehyde and reduced glutathione levels of testes among groups. The findings of this study indicate that oral usage of AVG may cause adverse effects on the male reproductive tract of mice by affecting the secretion of reproductive hormones.

Key words: Aloe Vera Gel, Male Reproductive Tract, Swiss Albino Mice, Toxicity.

ÖZET

Aloe barbadensis'in yapraklarından üretilen ve ticari olarak insanların kullanımı için marketlerde satılan *Aloe vera*'jelin (AVG) erkek Swiss albino farelerin üreme sistemi üzerindeki etkileri açığa çıkartılmaya çalışılmıştır. 4 haftalık erkek fareler rastgele dört gruba ayrılmış ve 28 gün süreyle mide yoluyla AVG verilmiştir. Gruplar kontrol (n=5), önerilen AVG dozun yarısının verildiği $\times 0.5$ grubu, önerilen AVG dozun verildiği $\times 1$ grubu, önerilen AVG dozun iki katının verildiği $\times 2$ grubu şeklindedir. Uygulama gruplarında LH seviyeleri kontrol grubu ile karşılaştırıldığında azalmıştır. Testosteron (T) seviyeleri kontrol grubu ile karşılaştırıldığında $\times 2$ doz grubunda azalmıştır. Sperm başı sayısı değişmez iken sperm anomalileri yüksek doz grubunda artmıştır. Histopatolojik analizlerde yüksek doz gruplarında testisde Sertoli hücre vakuolizasyonu, piknotik hücreler, germ hücre atıkları, atropik tübüller gözlenmiştir. Prostat bezinde atropik tübüller ve mononükleer hücre infiltrasyonu gözlenmiştir. Testisde malondialdehit ve reduktif glutatyon seviyelerinde gruplar arasında fark görülmemiştir. Çalışmanın bulgularından elde edilen sonuç ağızdan alınan AVG'nin hormonların salınımını etkileyerek farelerin üreme sisteminde zararlı etkilere neden olabilir.

Anahtar Sözcükler: Aloe Vera Gel, Erkek Üreme Sistemi, Swiss Albino Fare, Toksisite

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INTRODUCTION

Aloe barbadensis Miller (or *Aloe vera* Linne) is a perennial succulent herb, is mostly referred to as *Aloe vera*. It is one of the over 400 species of *Aloe*, and has been assigned to family Asphodelaceae by the APG II system [1,2]. It is a cactus-like plant that grows in hot, dry climates [3]. *Aloe vera* gel (AVG) is a clear jelly-like substance obtained from the *Aloe vera* leaf pulp. Despite its wide use as a folk remedy over a long period of time, the biochemical details of its action on physiological/pathophysiological functions have not been systematically investigated [4]. Clinical evaluations have revealed that the pharmacologically active ingredients are concentrated in both the gel and rind of *Aloe vera* leaves. Reynold and Dweck's review [5] describes the biologic activities of several aloe species. Moreover, antioxidants present in the *A. vera* extract may be responsible, in part, for the antihyperlipidaemic effect of the gel extract. In addition to the anti-oxidant potential, the hypoglycaemic effect of the gel extract may be implicated as the major reason for the observed antihyperlipidaemic effect of the extract [6].

The whole gel extract of *Aloe vera* has been reported to have various pharmacologic properties, specifically to promote wound, burn, and frost-bite healing, in addition to having antiinflammatory, antifungal, hypoglycemic, and gastroprotective properties. Of those claims, *Aloe vera*'s antiinflammatory and wound healing effects have been the most extensively studied. Besides these effects, AVG is considered to have adverse effects on some organs [3,7,8].

There are a few studies related to effects of AVG on the reproductive system. In a series of studies, Telefo et al [9-11] demonstrated the effect of AVG on the reproductive system in rats. In these studies, besides the effect of AVG on ovarian and uterine weight, an attempt was made to determine ovarian steroidogenesis, estradiol and progesterone levels. The present study was performed to determine the effects of *Aloe vera* extract (AVG), which is a commercially available product on the market for human use, on the male reproductive tract of Swiss albino mice.

MATERIALS AND METHODS

Animals

Male Swiss albino mice at 4 weeks of age were purchased from the Experimental Animals Production Center, GATA (Ankara, Turkey). All animals were housed in polycarbonate cages with stainless steel covers in an air-conditioned room (12 h light/dark cycle with a temperature of $21\pm4^{\circ}\text{C}$ and a relative humidity of $50\pm10\%$). The animals were acclimated to the laboratory for one week prior to the start of the experiments. The animals were provided with standard mouse diet and tap water ad libitum throughout the experimental period. The research protocol for this study was approved by the Gazi University Ethical Council (G.Ü.ET-08.022).

Treatment

Male mice separated into four groups randomly received AVG by gavage daily for 28 days. In this study, the daily dose suggested by the producer company of AVG product as written in the user guide to the product was used. The recommended AVG dose of the product was ($90\text{ mL}^{-1}\text{day}^{-1}$). For this purpose, daily dose of AVG (0,045-0,051 mL) depending on the weight of mice were applied. The groups were as follows (n=5): control, 0.5 dose group received half of recommended AVG dose, 1 dose group received the recommended AVG dose, and 2 dose group received twice the recommended AVG dose. At the end of the study, the animals were weighed and sacrificed under ether anesthesia followed by decapitation, and tissues (testes, epididymis, ventral prostate and seminal vesicle without secretion) were excised immediately. The tissues were weighed in order to calculate the organ/body weight ratios for each animal. The organ weight was considered as absolute organ weight, whereas organ/body weight ratio was considered as relative organ weight.

Histopathological analysis

After weighing, the right testes, epididymis, prostate and seminal vesicle samples were fixed in Bouin's solution for 8 hours. The tissues were embedded in paraffin, cut at $4\text{ }\mu\text{m}$ thickness,

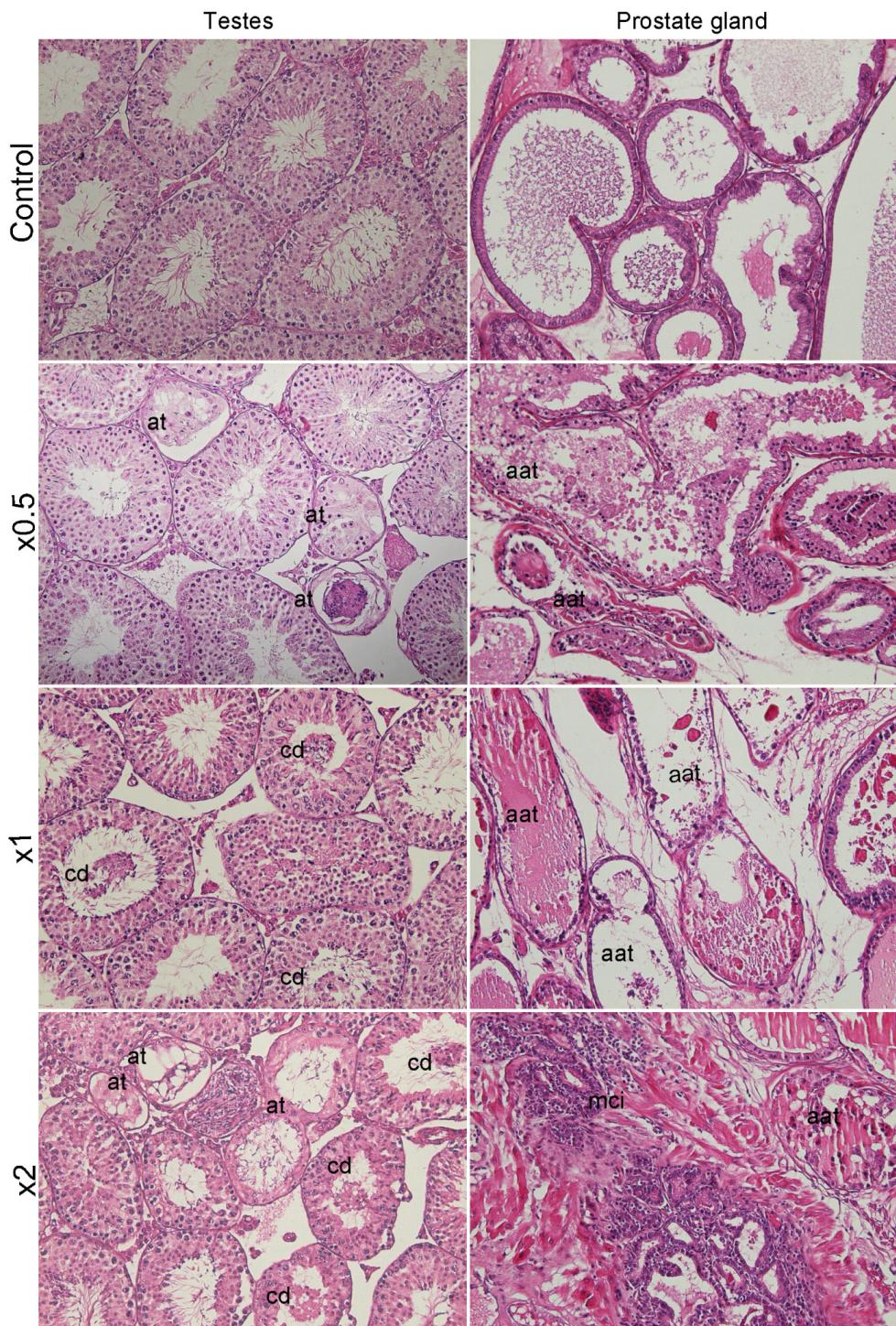


Figure 1. Photomicrographs showing testes and prostate tissues of control and treatment groups. In the testes of treatment groups, atrophic tubules (at) and germinal cell debris (cd) are shown. In the prostate gland of treatment groups, atrophic alveolar tubules (aat) and mononuclear cell infiltration (mci) are shown. (Stained with H&E, $\times 100$).

stained with Harris hematoxylin and eosin, and then examined under light microscope for histopathological evaluation. The left testes were quickly frozen in liquid nitrogen, and all samples were kept at -80°C for further assays.

Sperm counts and morphology of epididymal sperm

The count and morphology of epididymal sperm were analyzed as described previously by Aydogan and Barlas [12]. The weight of the epididymis was recorded so as to calculate sperm counts. The epididymis was put into 1 mL of 0.9% saline and minced with scissors. Aliquot (100 µL) of the mixture was diluted with 800 µL of 0.9% saline, and then sperm were put into stain for an hour after adding 100 µL of Eosin-Y solution to the latter mixture. Sperm head number was counted by using a Neubauer's chamber. The data were expressed as the number of sperm per gram of epididymal tissue. With a view to demonstrating the sperm deformities, a piece of vas deferens was put into 1 mL of 0.9 % saline and conserved at +4°C overnight so as to release the sperm into the liquid. The day after, the samples were rinsed gently, and 100 µL aliquots of the samples were diluted with 900 µL of 0.9 % saline. The sperm samples were transferred to microscope slides and fixed with alcohol. After fixation, the samples were stained with Eosin-Y solution overnight, washed with distilled water, passed through increased alcohol series and mounted with a cover slip. 250 sperms from each sample were evaluated microscopically and classified as head, neck and tail defects. In addition, percentages of abnormal sperms were calculated.

Hormone analysis

At the end of the study, the animals were weighed and sacrificed under ether anesthesia followed by decapitation. The blood was collected from heart and the serum samples were stored at -20°C until hormone analysis. Testosterone (T) (Cayman Chemical, Ann Arbor, MI, USA) and luteinizing hormone (LH) (Endocrine Technologies, Inc, Newark, California) measured by using commercially available EIA kits.

Estimation of lipid peroxidation in testes

Malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) [13]. In brief, the reaction mixture consisted of 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with NaOH, 1.5 mL of 0.8% aqueous solution of thiobarbituric acid and 0.2 mL tissue homogenate (20% in 1.15% KCl). The mixture was made up to 4.0 mL with distilled water and kept in a boiling water bath for 60 min. After cooling with tap water, the mixture was centrifuged at 2500 g for 10 min. The supernatant was taken out and the intensity of pink color was measured at 532 nm on a spectrophotometer. TBARS were quantified using an extinction coefficient of 1.56×10^5 M/cm and expressed as nmol of TBARS per mg protein. Tissue protein was estimated by using Lowry et al method for protein assay [14]. The MDA concentration was calculated using a standard curve prepared from 1,1,3,3-tetraethoxypropane. MDA levels were expressed as nmol MDA/mg protein.

Estimation of reduced glutathione in testes

GSH contents in testes tissue were measured as non-protein sulfhydryl as described by Sedlak and Lindsay [15]. The fresh tissues were immediately homogenized in ice cold 0.02M ethylenediaminetetraacetic acid disodium. Aliquots of tissue homogenate were treated with 50% w/v trichloroacetic acid while shaking, kept for 15 min and centrifuged at 3000 g. Supernatant fractions were mixed with Tris buffer pH 8.9 and 5-5-dithiobis-(2-nitrobenzoic acid) (DTNB). After mixing the contents, absorbance of the mixture was measured at 412 nm within 5 min following addition of DTNB against reagent blank with no homogenate. Reduced glutathione was used as an external standard. GSH levels were expressed as µmol/g wet tissue.

Statistical analysis

Prior to parametric tests, Kolmogorov-Smirnov and Levene tests were used respectively to evaluate data in terms of normality and homogeneity. All values presented in the text are

mean \pm standard error (S.E.). Statistical analyses were performed by using a SPSS 11.0 program for Windows. All values were examined by means of univariate analysis of variance (ANOVA), using a one-way factorial design and by Hochberg's GT2-method to detect differences among groups. A P value <0.05 was considered statistically significant. Besides, histopathological findings were compared by using Fisher's exact test as described by Gad and Weil [16].

RESULTS

Body and organ weights

The absolute organ weights, relative organ weights and final body weights of male mice in control and treatment groups are given in Table 1. There was no significant difference in the final body weights or in the absolute and relative organ weights among groups.

Histopathology

The light microscopic evaluation of the testes and prostate gland in the control and treatment groups is shown in Figure 1. The incidence of exposure-related histopathologic lesions in the reproductive tract of male mice in the control and treatment groups is given in Table 2. The testes, epididymis

and prostate gland of mice in the control group showed regular morphology of the tissues. However, the testes of mice in the treatment groups were very different. The seminiferous tubule atrophy, germinal cell debris, picnotic spermatogenic cells and Sertoli cell vacuolisation were increased in the $\times 2$ dose-treated group compared with the control group. Because of the germinal cell debris, the incidence of unmatured spermatogenic cells in the epididymal lumen were increased in all treatment groups. In the prostate gland, atrophic alveolar tubule and mononuclear cell infiltration were observed in all treated rats. The photomicrographs of epididymis and seminal vesicles of all groups were not shown in the manuscript. The seminal vesicles of mice in the treatment groups were not different in histology compared to the control group.

Sperm counts and morphology of epididymal sperm

The epididymal sperm count and percentages of abnormal sperms in control and treatment groups are given in Table 3. The sperm head count of all treatment groups was significantly increased when compared with the control group. Abnormal sperm percentages, head, neck and tail defects in sperms of mice in the $\times 1$ and $\times 2$ treatment groups

Table 1. Body weight (g), and selected absolute (mg) and relative organ weights (mg/g) of mice in control and treatment groups.

	Control	$\times 0.5$	$\times 1$	$\times 2$
Final body weight	37 ± 2.9	38 ± 3.3	41 ± 3.2	43 ± 1.9
Right testes				
Absolute	98 ± 7.5	108 ± 7.3	106 ± 8.1	106 ± 5.6
Relative	2.7 ± 0.09	3.0 ± 0.45	2.6 ± 0.23	2.5 ± 0.16
Left testes				
Absolute	98 ± 8.5	106 ± 5.2	98 ± 7.3	100 ± 5.2
Relative	2.7 ± 0.06	2.9 ± 0.36	2.2 ± 0.32	2.3 ± 0.15
Right epididymis				
Absolute	50 ± 4.1	44 ± 2.4	52 ± 7.3	47 ± 2.1
Relative	1.4 ± 0.02	1.2 ± 0.09	1.2 ± 0.08	1.1 ± 0.08
Left epididymis				
Absolute	45 ± 2.9	46 ± 2.4	48 ± 6.6	47 ± 3.3
Relative	1.2 ± 0.06	1.3 ± 0.18	1.2 ± 0.14	1.1 ± 0.08
Prostate				
Absolute	40 ± 5.8	48 ± 4.9	44 ± 6.0	48 ± 4.0
Relative	1.1 ± 0.13	1.3 ± 0.18	1.1 ± 0.14	1.1 ± 0.05
Seminal vesicle				
Absolute	225 ± 64.9	226 ± 41.1	266 ± 57.7	210 ± 18.4
Relative	5.9 ± 1.27	5.9 ± 0.86	6.4 ± 0.96	5.0 ± 0.45

n = 5 for each group.

were increased significantly compared to the control group.

Hormone analysis

The serum LH and T levels of mice in control and treatment groups are shown in Figure 2. LH levels of mice in treatment groups decreased compared to the control group. T levels decreased in the $\times 2$ dose group compared to the control group.

Malondialdehyde and reduced glutathione levels in testes

MDA and GSH levels in the testes of all experimental groups of mice are given in Figure 3. The MDA and GSH levels of testes in treatment groups were not different compared to the control group.

DISCUSSION

Aloe vera has been reported to possess immunomodulatory, antiinflammatory, UV

protective, antiprotozoal, and wound- and burn-healing promoting properties. *Aloe vera*'s anti-inflammatory and wound healing effects have been the most extensively studied [3]. Harmful reactions to aloe gel treatment are recorded [17,18]. In fact, limited data are available demonstrating other mechanisms of the extract. This study was conducted with a view to evaluating the effect of *aloe vera* extract on the male reproductive system of mice.

In the present study, the histopathologic evaluation of testes showed that treatment groups had marked defects in morphology. *Aloe vera* extract treatments to male mice caused seminiferous tubule atrophy, germinal cell debris, picnotic cells and sertoli cell vacuolization in the testes tissue. Due to the increased germinal cell debris in the testes tissue of treatment groups, there were an increased number of spermatogenic cells in the lumen of epididymal tissue. The

Table 2. Incidence of exposure-related histopathologic lesions of male mice in the control and treatment groups.

Tissue and lesion	Control	$\times 0.5$	$\times 1$	$\times 2$
Testes				
Seminiferous atrophy	0/5*	3/5	2/5	5/5 ^a
Germinal cell debris	0/5	5/5 ^a	3/5	5/5 ^a
Picnotic cells	0/5	0/5	2/5	5/5 ^{a,b}
Congestion	0/5	0/5	1/5	1/5
Sertoli cell vacuolisation	0/5	1/5	2/5	5/5 ^{a,b}
Epididymis				
Spermatogenic cells in lumen	0/5	4/5 ^a	4/5 ^a	4/5 ^a
Arotrophic tubules	0/5	1/5	3/5	2/5
Prostate				
Atrophy	0/5	5/5 ^a	5/5 ^a	5/5 ^a
Mononuclear cell infiltration	0/5	5/5 ^a	5/5 ^a	5/5 ^a

*Number of mice with the lesion / number of mice examined

^a Significantly different from control group. P<0.05 (Fisher's exact test).

^b Significantly different from $\times 0.5$ group. P<0.05 (Fisher's exact test).

Table 3. Sperm head count and percentages of abnormal sperms of male mice in control and treatment groups.

	Control	$\times 0.5$	$\times 1$	$\times 2$
Sperm head count ($\times 10^4/\text{mg}$ epididymis)	590 ± 90.9	567 ± 57.2	521 ± 110.4	629 ± 62.2
Abnormal Sperm (%)	21.5 ± 2.96	44.2 ± 6.27	52.5 ± 4.37 ^a	66.4 ± 6.90 ^a
Head defect (%)	4.0 ± 0.75	5.2 ± 0.59 ^{c,d}	11.2 ± 1.48 ^{a,b}	12.4 ± 1.47 ^{a,b}
Neck defect (%)	1.5 ± 0.25	4.7 ± 0.97	7.1 ± 1.13 ^a	6.3 ± 0.38 ^a
Tail defect (%)	16.0 ± 3.25	34.3 ± 5.82 ^a	34.2 ± 4.33 ^a	47.7 ± 6.15 ^a

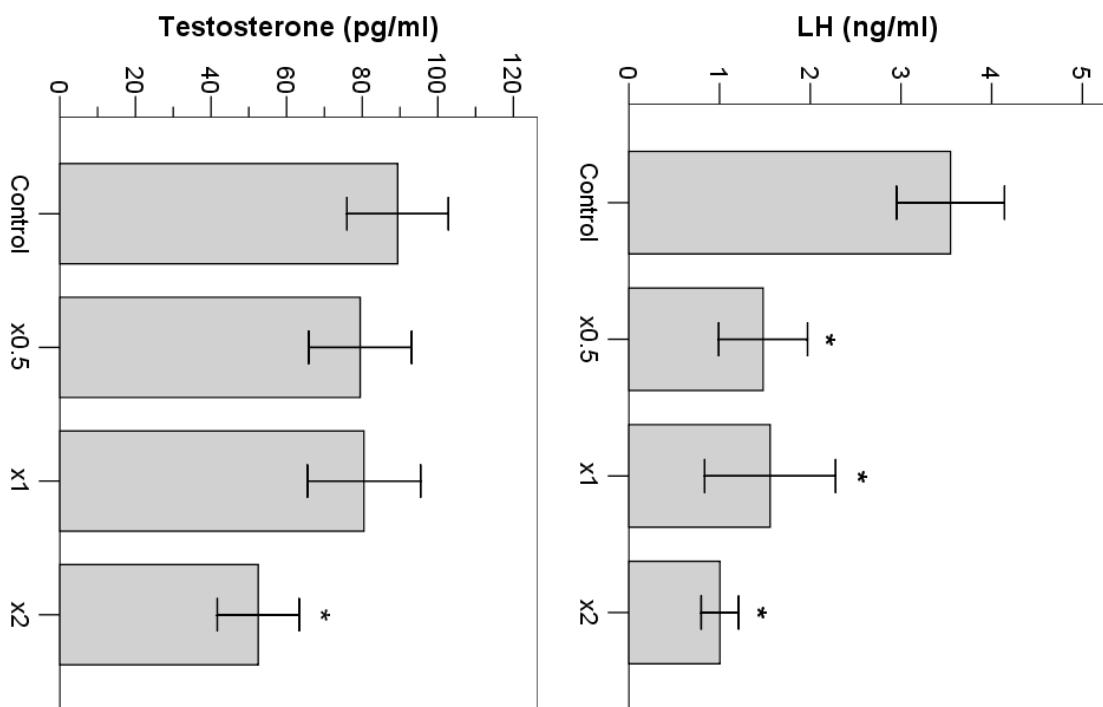
n = 5 for each group.

^a P<0.05 significantly different from control group

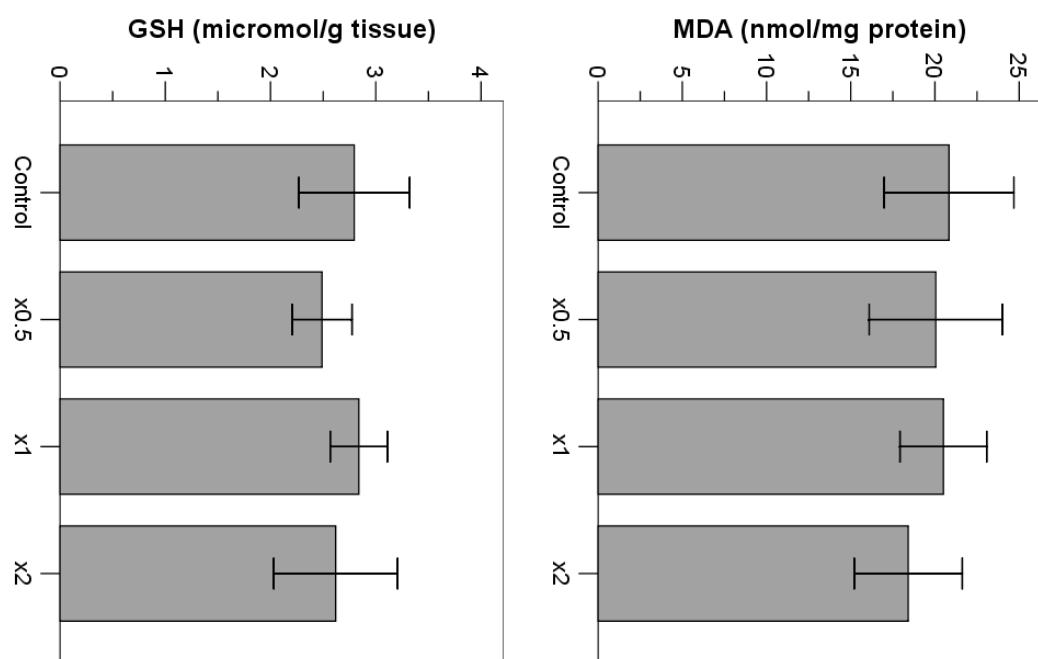
^b P<0.05 significantly different from $\times 0.5$ group

^c P<0.05 significantly different from $\times 1$ group

^d P<0.05 significantly different from $\times 2$ group

**Figure 2.** The serum levels of LH and T of control and treatment groups.

* Significantly different from control group ($P<0.05$).

**Figure 3.** The levels of MDA and GSH in testes of control and treatment groups.

germinal cell debris and the connections between cells were defective. Loss of germ cell attachment, with the appearance of germ cells in the tubular lumen, is a common response to many testicular toxicants. Sertoli cells are responsible for providing developing germ cells with structural and metabolic support. [19]. Disruption of the Sertoli cell tight junctions also disrupts the blood-tubule barrier. Any agent that affects the function and viability of Sertoli cells may also affect the germ cells. In the present study, the increased Sertoli cell vacuolization and germ cell debris in the testes of treated mice indicate that AVG may also adversely affect germ cells by affecting the Sertoli cells. Moreover, the prostate glands of mice in all treated rats have atrophic glands and mononuclear cell infiltration.

Hormonal pathways control the functioning of the entire reproductive process. LH acts exclusively on Leydig cells in the testis and is the primary regulator of T secretion [20]. T is the major androgenic steroid that is synthesized by the Leydig cells and has both intratesticular effects (on spermatogenesis) and peripheral effects (on accessory sex organs). The present results showed that LH levels of mice in treatment groups decreased compared to the control group. Moreover, T levels decreased in the high dose treated group compared to the control group. The reduction in serum T levels of high dose treated mice can be explained exclusively on the basis of the treatment's lowering serum LH levels. In the male mouse, circulating LH is responsible for maintaining normal elevated serum T concentrations. Therefore, in the serum of male mice treated with double the recommended dose of AVG there were likely insufficient LH levels to stimulate Leydig cells to release efficient levels of T to the blood.

It is reported that *Aloe vera* extracts increase ovarian steroidogenesis and significantly increase production of estradiol and progesterone [9,11]. These results were a clear indication of the presence of estrogenic compounds in the plant extracts. *Aloe vera* contains a phytoestrogen named anthraquinones. Several

hydroxyanthraquinones (e.g. emodin, aloe-emodin, chrysophanol) were shown to enhance the estrogen-inducible proliferation of MCF-7 cells at 1-10 μ M in a dose-dependent manner, indicating that they do, indeed, have estrogenic activity [21]. Estrogen inhibits some enzymes in the T synthetic pathway and therefore directly effects testosterone production. The reduced serum levels of LH reflect an inadequate pituitary response to depressed T. Estrogens act primarily by suppressing pituitary gonadotropin secretion, resulting in secondary testicular failure. The primary effect is the reduction in plasma T concentration which is due to reduced LH secretion. This study indicates suppression of T levels associated with a reduction of LH in the circulation. As to the sites of action of E2-17 β , a direct effect on the testes was proposed in addition to the feedback effect on LH release [22]. Therefore, some chemicals in the AVG, such as anthraquinones, can likely exert estrogenic effects in the male reproductive tract of mice by depressing T levels or gonadotropin secretion.

Sperm head count and abnormal sperm percentages are considered to be useful indicators to detect adverse effects on spermatogenesis. During chronic treatment of aloe vera, there was a decrease in red cell count and significant sperm damage [23]. In the present study, the sperm head count of all treatment groups was not different compared with the control group. Abnormal sperm percentages as well as head, neck and tail defects in the sperms of mice in treatment groups were increased significantly compared to the control group. The sperm abnormalities observed in the treatment groups are a serious problem for reproductive function because abnormal sperm could not reach the oviduct after intravaginal ejaculation. The results of the present study suggest that AVG may have deleterious effects on the production of sperm.

In some studies, it is shown that *Aloe* has an antioxidative effect [24]. Therefore it has been showed that *Aloe vera* gel extract has antioxidant and immunomodulator properties. It is known to contain several superoxide dismutase enzymes

in addition to possessing peroxidase [25,26]. The anthraquinones may act as antioxidants and radical scavengers. Reactive oxygen species (ROS) and ROS-mediated reactions are involved in inflammatory response and can contribute to liver necrosis [27]. It is known that increased MDA concentration and decreased GSH concentration indicate an increased generation of ROS which cause lipid peroxidation in the tissues [28,29]. Moreover, ROS attack the membrane of cells in the blood vessels leading to congestion in the tissues. In the present study, MDA and GSH levels of mice testes in treatment groups were not different compared to the control group. The incidence of congestion in the testes tissue of treated mice did not increase compared to the control group. Therefore, the damage in the male reproductive tract of treated mice is independent of oxidative stress.

The toxicity of *Aloe vera* has not been systematically studied. The question arises whether *Aloe vera* is safe. The findings of this study indicate that exposure to AVG may cause adverse effects on the male reproductive tract of mice by affecting the secretion of reproductive hormones. The results of the study also suggest that the mechanism of AVG induced damage to the male reproductive tract is independent of oxidative stress. However, questions still persist as to the mode of action and the origin of the compounds. The precise cellular mechanisms involved will require further study.

Declaration of interest

The authors declare that they have no conflict of interest to disclose.

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