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# EVALUATION OF SEMEN QUALITY, SEMINAL BIOCHEMICAL TRAITS, AND HISTOLOGICAL FEATURES OF *ALOE VERA* EXTRACT ADMINISTRATED TO RABBIT BUCKS

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**ABSTRACT:** In this study, we aimed to explore the impact of supplementing rabbit bucks' drinking water with *Aloe* vera extract (AVE) on their semen quality, seminal biochemical traits, and testicular structure. We divided twenty-four adult V line rabbit bucks into three equal groups. The second and third groups received daily doses of 2 ml and 4 ml AVE per liter of tap water, respectively, for 12 consecutive weeks, while the first group served as the control (0 ml AVE/ L). The results revealed that the group receiving the lower AVE dose (2 ml AVE/ L) exhibited improved libido (lower reaction time), higher semen volume, and increased litter size in does compared to the control group. These improvements were associated with enhanced semen quality, including higher sperm motility, total sperm output, and reduced abnormal sperm and pus cells in the lower dose group ($P \leq 0.05$). Furthermore, the lower AVE dose group demonstrated significantly increased levels of seminal plasma initial fructose, total protein, albumin, and total antioxidant capacity, while levels of seminal plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA) were significantly decreased compared to both the control and high AVE dose groups. The low AVE dose also positively impacted the histological structure of the testes and spermatogenic activity within the seminiferous tubules of the buck rabbits. In conclusion, administering a low dose of AVE (2 ml/ L) in drinking water to rabbit bucks significantly improved semen quality and positively influenced seminal biochemical traits and testicular histological structure.

**Key words:** Buck rabbits, *Aloe vera*, Semen quality, Antioxidant status, Testis histology.

# INTRODUCTION

*Aloe vera* grows mainly in the dry regions of Africa, Asia, Europe, and America, and are spread almost throughout the world. It is classified as an evergreen perennial plant never-ending creating to 0.8 m by 1 m at a moderate rate. It is considered as herbal preparation with a long history of preventive, therapeutic, and medicinal use. The botanical name of *Aloe vera* is *Aloe Barbadensis* miller. *Aloe vera* is widely used in many proprietary herbal preparations (Guo and Mei, 2016). *Aloe vera* extract gel is a very versatile plant that has many different uses (Shweta *et al*., 2011). According to Hamman (2008), the phytochemical examination of *Aloe vera* gel revealed that it contains polysaccharides, steroids, organic acids, antibacterial agents, amino acids, and minerals, which have skin soothing and cell protective actions. The clear gel inside the leaf is extracted and used as an excellent treatment for various skin wounds and burns, and other skin problems, as it helps speeds up the healing rate, reduces the risk of infection, and improves the quality of skin texture (Hekmatpou *et al*., 2019). It can also be used as a treatment for chronic constipation, low appetite, and recurring digestive problems (Tang *et al*., 2022). Vitamins, enzymes, minerals, carbohydrates, lignin, saponins, salicylic acids, and amino acids are among 75 potentially active constituents in *Aloe vera* (Atherton, 1998).

*Aloe vera* has been shown to have antioxidant activity, which varies depending on the type of gel extract and plant part employed (Hu *et al*., 2003; Hossen *et al*., 2022). With the continuous revelation of the biological activity and physical basis of *Aloe vera*, it has been widely used in the fields of healthy food, cosmetics, and medicine. *Aloe vera* oral medicine can enhance the body's immunity, anti-inflammatory, and detoxification, stimulate blood circulation, and eliminate blood stasis to improve cardiovascular diseases (Shakib *et al*., 2019).

When the entire *Aloe vera* leaf is used, it is difficult to determine whether the biological effects are attributable to the gel or the latex, because excretory chemicals may seep during the manufacture of the gel (Reynolds and Dweck, 1999). Various studies have reported on the properties of *Aloe vera* in lowering blood sugar, hypotensive, protecting the liver, and purifying the blood (Nwajo, 2006). At doses ranging from 100 to 300 mg/kg body weight, ethanolic *Aloe vera* extract improved sexual behavior by increasing mounting and intercourse rates in a rat model; however, a dose of 400 mg/ kg body weight had the opposite effect (Erhabor and Idu, 2017). However, it had a deleterious effect on sperm quality in West African Dwarf (WAD) goat given a 3%-4% AVG extract, resulting in an elevated degree of sperm abnormalities (Oyeyemi *et al*., 2011). In rabbit bucks given AVG, the time gap between two consecutive ejaculations was dramatically increased. It can be used as an antioxidant to enhance the fertility of bucks and semen quality (Bakeer *et al*., 2021).

Other studies have reported that the improvement in testicular weight, germinal epithelium height and seminiferous tubules diameter, and ameliorate reductions in the testicular cell counts can be due to the presence of antioxidant compounds (especially vitamin E) in *Aloe vera* extract. Also, increased activity of antioxidant enzymes and reduced lipid peroxidation which can cause extensive damage to cell membrane lipids could be due to *Aloe vera* extract containing active phenolic and flavonoids compounds (Bala *et al*., 2017; Behmanesh *et al*., 2018).

Therefore, the current study aimed to investigate the impact of *Aloe vera* gel extract administration to V line rabbit bucks on semen quality, seminal plasma constituents,

and histological examination of testes. The goal of this study was to assess the antioxidant effects of various amounts of AVE on the quality of buck rabbit sperm.

## MATERIALS AND METHODS

The present study was conducted out from January to April 2021 at a private breeding rabbit farm in Borg El Arab city, Alexandria governorate. All data and animal care procedures were approved by the Institutional Animal Care and Use Committee in AU-IACUC, Alexandria University, Egypt with the review report number Alex. Agri. 092310218.

**Preparation of *Aloe vera* extract gel**

Mature fresh leaves of *Aloe vera* (*Aloe Barbadensis*) were collected from botanical garden plantations (Itay El Barud, Beheira, Egypt). The length of the leaf reached between 25 and 30 cm, and the width of the leaves was between 4 and 6 cm. After rinsing the leaves were washed with running tap water, the outer shell was removed manually by making a cut with a sharp knife, central solid gel in the center was extracted, and latex of the leaf was removed, and the gel was homogenized and collected in a beaker according to Oyewopo *et al*. (2011). The clear filtrate was kept at 20°C until used (Subbiah *et al*. 2005). This process was repeated every day to be sure that the gel was freshly prepared. *Aloe vera* samples were analyzed at the Marine Science Center GC-MS according Lakshmi and Rajalakshmi (2011). The most important compounds obtained from *Aloe vera* are shown in Table 1.

**Animals, housing management, and treatments**

To investigate the impact of the administration of *Aloe vera* gel extract (AVE) in drinking water (Tap fresh water) to buck rabbits on semen quality, a total of twenty-four V Line adult male rabbits averaged 3.10 kg at 6 months of age were used, the experiment lasted 12 weeks. Bucks were randomly distributed into 3 homogeneous groups (8 bucks each). Rabbits were individually housed in a naturally ventilated building and kept in individual galvanized wire cages. Batteries were equipped with feeders for pellet rations and automatic drinkers.

All treated animal groups were reared under similar management and hygienic conditions with ad libitum supply of fresh water. All groups were fed 200 g diet/ day, the same commercial pellet diet containing 17.80 % crude protein and 2650 kcal/ kg. Rabbits of the 2$^{nd}$ and 3$^{rd}$ groups drunk tap water containing 2- and 4-ml AVE/ L fresh water every day, respectively. The first group was served as a control group that was drinking tap water without AVE.

**Performance and semen quality**

Live body weight of buck rabbits was weighed at the beginning of the experiment (six months of age) and at monthly intervals thereafter till nine months of age. Ten to Twelve hours before weighing rabbits were devoid of feed, but water was allowed at all times.

Semen specimens were artificially collected after 3 months of experimental period with the aid of an artificial vagina using the method described by Herbert and Adejumo (1995). Reaction time, the time between introducing the teaser doe to the buck and ejaculation (RT/ sec), was estimated. The semen volume of each ejaculate was recorded nearest to the 0.1 ml (using a graduated collection tube) after removal of the gel mass. The semen pH was determined by dipping a pH paper into the semen.

Sperm concentration was determined after semen dilution (1:100) with physiological saline by the method of Vizzarri *et al*. (2019). Advance sperm motility (progressive motility) was estimated using phase contrast optics at 40× magnifications and assessed from 0 to

100%. Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration. Total live sperm output and total normal sperm were determined from total sperm output. Assessments of the percentage of live spermatozoa and normal sperm were performed using an eosin–nigrosine blue staining mixture (Tanga *et al*., 2021). Sperm abnormalities and pus cells were determined by making a thin smear on a glass slide and fixing it with buffered formal saline.

A total of 48 receptive V line nulliparous rabbits does were naturally mated by randomly eight treated bucks in each group. Pregnancy was diagnosed abdominally on day 12-14 post mating to calculate pregnancy rate (fertility percentage). Doe cages were prepared by nest boxes pre-expected date of parturition. Kindling rate was computed, and total live (after 12 h of kindling) litter size at birth was recorded.

**Seminal plasma biochemical traits**

Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4 °C and was stored at -20 °C until later analysis. Heparinized tubes were used to collect blood samples which were obtained from the ear vein of each buck every two weeks. Blood samples were centrifuged at 3500 rpm for 20 min to obtain plasma, which was stored at −20 °C.

Testosterone concentration in seminal plasma was measured using an enzyme immunoassay commercial kit (Monobind Inc., Lake Forest, USA). Seminal plasma initial fructose concentration was determined immediately after semen collection according to Mann (1948). Seminal plasma samples were analyzed for total protein (TP) by the Biuret method according to Shaaban *et al*. (2008). Albumin (Alb) concentration was determined by the method of Elzanaty *et al*. (2007). Seminal plasma globulin concentration was obtained by subtracting the values of albumin from the corresponding values of total protein. The enzymes activities of seminal plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by using commercial kits (bio-diagnostic company, EL-Doki, Giza, Egypt). Lipid peroxidation biomarkers such as malondialdehyde (MDA) were assessed in the seminal plasma based on the method developed by Conti *et al*. (1991). Total antioxidant capacity (TAC) was measured according to Erel (2004).

**Histological examination**

After 3 months of the experimental period, four V line rabbit bucks were randomly chosen from each group to be sacrificed to get their testes. After testicular excision, each testis was sliced and fixed with 10% neutral-buffered formalin for at least 24 h, and histological sections with a thickness of 5 microns were cut using a rotary microtome then were processed for hematoxylin and eosin (H&E) staining (Bancroft and Gamble, 2002), and prepared using the routine histological technique by Suvarna *et al*. (2018).

**Statistical analysis**

Data were analyzed as one-way ANOVA design, using the GLM procedure of SAS (Version 9.2; SAS, 2008). All the studied traits were analyzed by using univariate linear model as the response variables and diet (0, 2, and 4 AVE ml/ L) as the factor explanatory variables if the random residual variance follows a normal distribution. When treatment effects were significant, the means were compared using Duncan's Multiple Range Test. Moreover, Polynomial (linear and quadratic) contrasts (adjusted for the equal spacing of treatments) were used to examine dose responses to increasing levels of AVE. Significance was declared when $P \leq 0.05$.

# RESULTS AND DISCUSSION

## Performance and semen characteristics

Data from Table 2 showed no difference in the initial and final body weight of rabbit bucks during the experimental period. While the low dose 2 ml AVE/ L group showed the best body weight compared with other groups and it was also proven to improve libido for bucks measured by the reaction time (sec), which had less time before ejaculation and increased semen volume compared to the control group and high level (4 ml/ L) group in Table 2. Throughout the study's experimental phase, the colour of the sperm ranged from a uniform milky to a creamy white fluid, it was not different between groups during the experimental period.

These agree with the findings of Olugbenga *et al*. (2011) on the color and consistency of semen in bucks. Therefore, it was concluded that the continued administration of *Aloe vera* extract did not have any effect on the semen color of West African Dwarf (WAD) bucks. According to Campos *et al*. (2012), the milky coloration represents the natural state of rabbits, as it indicates good semen quality, and there are also other colors such as citron yellow, which indicates low sperm concentration. Data showed a significant increase ($P \leq 0.05$) in pH with treatment levels as compared to the control group. The high-level, 4ml AVE/ ml group increased by 0.71% (liner effect, $P \leq 0.05$) pH followed by 0.43% of the low-level, 2 ml AVE/ L group compared with the control group.

Also, analysis of litter size at birth data as effected by AVE showed that low level of AVE has significantly increased compared with other levels. The low level 2ml AVE/ L group showed highest by 5.77% (quadratic effect, $P \leq 0.05$) litter size at birth compared with the control group of V line rabbits. However, in terms of fertility as a percentage no significant changes were detected across AVE groups. Because of their bioactive constituents, such as phenols and flavonoids, several plants have high antioxidant qualities that can lessen the detrimental effects of free radicals (Ghotbabadi *et al*., 2012). So far, no research has been conducted on the utilization of ethanolic extract of *Aloe vera* as an antioxidant in buck rabbit sperm. Our findings showed that AVE improved libido sexual. Bakeer *et al*. (2021) demonstrated that there was an increase in successive ejaculations with AVE compared with the control group of the V line rabbit's bucks.

## Semen quality

Based on our findings in Table 3, the use of 2 ml/L of AVE resulted in significant improvement in advance sperm motility and total sperm output compared with control group (0 ml/ L, AVE). The high level of 4 ml AVE/ L group had significantly lowed (linear and quadratic effects, $P \leq 0.05$) in advance sperm motility and total sperm output followed by the 0 ml AVE/ L (control) group and then 2 ml AVE/ L group. The mean percentages of progressive advance sperm motility observed in this study were 67.38, 67.84, and 63.92% for the control, 2 ml AVE/ L, and 4 ml AVE/ L, respectively. Results showed that adding 2 ml/ L of AVE had decreased ($P \leq 0.05$) abnormal sperm and pus cells compared with other groups. The values of live spermatozoa (%), sperm concentration, total live sperm output, and total normal sperm showed non-significant changes compared to the control group (Table 3). The mean percentage of advanced sperm motility, spermatozoa livability (live spermatozoa), and normal sperm were increased from 67.38, 79.79, and 85.44 for control group then to 67.84, 80.22, and 85.17 to for low level of AVE group, respectively.

Semen characteristics of rabbit bucks in this study were like those previously pointed out by previous authors in rabbit bucks (Maurice, 1993; Sodani, 2020; Bakeer *et al*., 2021). Low level of AVE increased ejaculation interval

and advance sperm motility which are indicators of enhanced libido. This may be due to the role of antioxidative phenolic compound in AVE and its effects on lipid peroxidation (Ikeno *et al*., 2002). The phenolic compounds were hypothesized to be enhancers by altering arachidonic acid metabolism, stabilizing the lysosomal membrane, and guarding nuclear structure (Prabhala *et al*., 1990). Phenolic antioxidants in AVE have also been found to act as free radical scavengers (Shahidi *et al*., 1992).

This finding is comparable to that of Erhabor and Idu (2017), who fed rats an ethanolic extract of AVG at doses ranging from 100 to 400 mg/kg Bw. AVG as antioxidants can also improve the quality of rabbit sperm in heat stressed rabbit bucks (Zeweil *et al*., 2013) and the characteristics of frozen rabbit sperm (El-Seadawy *et al*., 2017). Fakhrildin and Sodani (2014) observed that the highest significant increase in sperm concentration, sperm motility rate, progressive motility score A, and normal sperm morphology, as well as sperm viability, were recorded when using a low dose of fresh *Aloe vera* leaf gel extracts (5 µL/ 1 ml semen). Ghaffari *et al*. (2023) showed that male Wistar rats were receiving AVE daily while, the serum shows to significantly improved testosterone concentrations, average sperm count, GE thickness, and ST diameter.

On the contrary, the continuous administration of high level of AVE (4 ml/ L) significantly ($P \leq 0.05$) reduced advance sperm motility by 5.13%, total sperm output by 3.99%, and normal sperm by 5.77% and resulted in increased sperm abnormalities by 9.44% and pus cells by 72.39% compared with the control group, respectively in the rabbits V line buck. Additionally, a high level of AVE supplementation negatively affected advance sperm motility, total sperm output, and normal sperm by reducing their metabolic activity as observed in semen quality data, and a similar finding was made in West African dwarf buck (Bw, 11-15 kg) that received 3-4% of the average fresh AVG (Oyeyemi *et al*., 2011). Also, a negative effect of AVG ethanol extract was observed in mice and rats (Modaresi and Khodadadi, 2014; Erhabor and Idu, 2017), indicating that fresh AVG contains secondary (2ry) metabolites that may negatively affect sperm quality, possibly through their effect on the spermatogenic enzyme LDH, which is involved in glycolysis and ATP production, both of which are required for sperm motility (Odet *et al*., 2008). From our data, the treatment with a high level of AVE can adversely affects sperm motility, sperm output, and normal sperm. Furthermore, sperm capacitation, which is a sequence of enzymatic events that results in the release of acrosomal enzymes that allow fertilization in the female reproductive tract, will be harmed (Oyeyemi *et al*., 2011).

The high level 4ml AVE/ L decreased normal sperm and increased pus cells (liner effect, $P \leq 0.05$) followed by the low level 2 ml AVE/ L when compared with and control group. The mean percentage of normal sperm also decreased from 85.44 to 80.51 and abnormal sperm increased from 11.65 to 12.75% for control group compared with high level of 4 ml AVE/ L tap water every day, respectively. The existence of abnormal sperms in this work is consistent with Mahrous and Ahmed (2021) who report that after administration of AV gel aqueous extract alone (10 or 20%), treated rats showed a small reduction in the proportion of abnormal sperm when compared to the control group. Briefly, a low level of AVE at 2 ml/ L drinking water enhance sperm quality due to its potential spermatogenic activity because it has antioxidant and chemical compounds and may be useful for improving male fertility. Another study also showed that using *Aloe vera* powder at a dose of 60 mg/ kg of body weight leads to an increase in the litter size of rabbits and

enhances fertility rate. This study indicated that consumption of AVE could increase sperm quantity by promoting spermatogenesis and could be used to enhance the fertility rate (Maurice, 1993).

**Seminal plasma characteristics**

Data of seminal plasma showed an improved testosterone level by 1.10% and increased initial fructose, total protein, and albumin by 4.03, 6.064, and 20.41%, respectively in the low level of treated rabbits' group compared with the control in Table 4. The low level 2ml AVE/ L group increased initial fructose, total protein, albumin, and TAC levels (quadratic effect, $P \leq 0.05$) compared with control group. While, the low level 2ml AVE/ L group decreased AST, ALT, and MDA levels (quadratic effect, $P \leq 0.05$) compared with control group. Adding AVE to drinking water of rabbit bucks with different levels studied did not have an effect on seminal plasma globulin level in all groups treated compared with the control group.

In the present study a low level of AVE group recorded a significant improvement in the AST, ALT, and antioxidants activity (MDA and TAC) in comparison with the untreated (control) group. These findings agree with those of Adesokan *et al*. (2009) who indicated that administration of an aqueous extract of *Aloe vera* did not have any negative effect on the liver and kidney functions in rats indicating that the use of AVE is completely safe and has un-toxic effects on the functions of body vital organs. Only few scientific studies on AVE have proven its effect on sex hormones (Telefo *et al*., 2002; Rengin and Gullan, 2009; Poorfarid *et al*., 2022). Despite the wide history and popular acceptance of products containing AVE, only a few articles have discussed the antioxidants in *Aloe vera* tissue and their physiological effects on biological functions (Hęś *et al*., 2019).

Results of the current study can be explained by the fact that the antioxidant activity of *Aloe vera* showed a very noticeable improvement in the rate of sperm motility and normal sperm morphology. This finding is consistent with recent work by Akinola *et al*. (2021) who reported that *Aloe vera* may enhance fertility rate by raising sperm quality and has spermatogenic activity due to chemical compounds present in it and may be useful in producing drugs to improve fertility rate (Sánchez *et al*., 2020).

Bucks treated with AVE at a low concentration (2 ml/ L) had higher seminal plasma total protein and albumin concentrations, which were associated with an improvement in sperm quality. This finding corresponded with many studies that have shown that low seminal plasma protein content is associated with poor sperm quality (White *et al*., 1987; Ashworth *et al*., 1994). Taha *et al*. (2000); Osama and El-Sahn (2006); Elkomy *et al*. (2008) discovered a favorable connection between sperm quality and the level of seminal plasma total proteins. Because these molecules are the amphoteric feature of seminal plasma proteins, a decrease in their protein concentration diminishes their buffering ability and hence semen quality (Dhami *et al*., 1994).

According to Yousef *et al*. (2003), there was a negative relationship between higher ALT and AST levels and lower ejaculate volume, sperm concentration, total sperm output, sperm motility index, and total motile sperm, and a noticeable decrease in the activities of these enzymes coincided with an increase in sperm activities and semen quality. Furthermore, adding AVE to buck's drinking water had a substantial influence on total antioxidant capacity (TAC) and malondialdehyde (MDA). Drinking bucks on fresh water administered with low level (2 ml AVE/ L water) significantly reduced ($P \leq 0.05$) the observed oxidative stress biomarkers (MDA) by 9.02% above the control value, and this effect was dose-dependent. The influence

of drinking AVE administration on TAC, on the other hand, revealed that treating bucks with AVE at a low level significantly enhanced ($P \leq 0.05$) TAC by 10.70% compared to the control group. Treatment with *Aloe* vera extract gel increased TAC levels in seminal plasma, which protects its lipid content and sperm membrane from lipid peroxidation.

Malondialdehyde is an endogenous genotoxic result of enzymatic and oxygen radical-induced lipid peroxidation, according to Muriel *et al*. (2003). Damage to biologic macromolecules (e.g., nucleic acids, membrane lipids, and proteins) and disruption of normal metabolism processes and physiology are caused by oxidative stress (Roberts and Sindhu, 2009; Pohanka *et al*., 2009).

According to the results of semen quality and seminal plasma characteristics, adding a low quantity of AVE (2 ml/ L) to rabbit bucks' drinking water aided spermatogenesis in seminiferous tubules to create complete and motile sperm. Furthermore, it stimulates the secondary sexual gland to secrete the components of seminal plasma, which are required for sperm survival. As a result, it is fair to speculate that the effects of AVE on reaction time, sperm volume, sperm motility, sperm counts, and sperm morphology may be mediated in part by its antioxidant capabilities in animal reproductive organs (Ikeno *et al*., 2002).

**Histological examination**

Figure 1 contain pictures A, B, and C revealed the normal testicular structure of the control group at 9 months of age and groups administrated 2- and 4-ml AVE every day/ L tap water, respectively. Seminiferous tubules lined with spherical and well-stained spermatogonia or germ cells make up testicular tissue. The control group testicular tissue was constituted of a high density of normal shape testicular tubules surrounded by interstitial connective tissues. Active spermatogenesis was seen in seminiferous tubules (ST) bordered with layered germinal epithelium (GE). On the basal lamina were spermatogonia cells with heterochromatin and rounded nuclei. Primary spermatocytes were the biggest spermatogenic cells in the germinal epithelium, containing chromatin in various forms. Sertoli cells exhibited large nuclei and rested on the basal lamina, but Leydig cells in interstitial connective tissues had eosinophilic cytoplasm with large and spherical nuclei (Fig.1A). Rabbit bucks given 2 ml AVE/ L per day, like controls, had normal testicular architecture with ST bordered by GE cells (Fig. 1A and B). In addition to typical testicular architecture with ST bordered by GE cells, there was some improvement in the lining germ cells and spermatogenic lineage cells, as well as supporting Sertoli cells (SC) in layers (Fig. 1B). Leydig cells (LY) which are large polyhedral cells that have spherical nucleus were seen between ST. Cleary observe the increase in the height of GE and increase numbers of sperms were seen inside the lumen of ST. Furthermore, germline cell organization and thickness improved, and connective tissue surrounding seminiferous tubules decreased (Fig. 1B).

However, after 3 months of administration, the unfavorable effects of high levels of AVE gel aqueous extract at a concentration of 4 ml/ L revealed a harmful effect reflected by severe testicular degeneration, necrosis, and vacuolation in ST (Fig. 1C). In addition, in high levels of AVE treated rabbit bucks, there was widespread atrophy and death of all germ cells, as well as significant vacuolation in the epithelium. Furthermore, maturation arrest and the absence of spermatozoa in the lumen were substantial in the majority of seminiferous tubules (Fig. 1C). These histological changes could be explained by redox imbalance disturbances caused by high

levels of AVE, which result in DNA damage, lipid peroxidation, and protein synthesis suppression. In general, the mean thickness of the GE was reduced in 4 ml AVE/ L tap water than in control, according to histological study of rabbit bucks' testicular tissue and low level (2ml/ L) of AVE animals. Also, GE showed derangement and reduced thickness (Fig. 1C).

Most previous studies showed AVE promotes spermatogenesis by altering spermatogenic cells and increasing cell division, as well as to increase testosterone hormone by stimulating Leydig cells (Modaresi *et al*., 2009; Estakhr *et al*., 2010). According to Estakhr and Javdan (2011), AVE boosted testicular weight, testosterone hormone, sperm concentration, and motility while decreasing sperm abnormalities. However, Erfani Majd *et al*. (2021) demonstrated that giving rats *Aloe vera* gel reduced oxidative stress and boosted testosterone levels.

These observations are agreed with the work of Jafaribarmak and Khaksar (2012) who cited that *Aloe vera* extract can enhance the seminiferous tubules diameter, spermatogonia, Leydig and Sertoli cells. The testis's histological observations also showed increase numbers of sperms inside lumen of seminiferous tubules. These results are consistent the with work of Estakhr and Javdan (2011), who demonstrated that in rats when compared to the control group, AVE induces a considerable increase in sperm count and motility, as well as a decrease in sperm abnormalities, and that this extract also causes an increase in testes weight. Shahraki *et al*. (2014) proved that *Aloe vera* has an antioxidative effect, and could promote the spermatogenesis process through its positive effect on testosterone levels as well as histological features of the testis. Histological studies of mice testes by Sodani and Hussain (2014) of groups consuming low level of *Aloe vera* extract orally for 21 days showed enhancement of the entire structure of the spermatogenic layer of the seminiferous tubules consisting of an extensive normal arranged germ cells layer.

## CONCLUSION

It can be concluded that administering *Aloe vera* extract (AVE) in drinking water at a low dose of 2 ml AVE per liter has shown potential benefits for the reproductive performance, semen quality, and seminal biochemical traits of rabbit bucks. This treatment also appears to support the histological structure of the testis cells, enhancing their function and safeguarding them from oxidative damage without causing harm. However, it's worth noting that research on the use of AVE in rabbits is still in its early stages, despite the considerable interest it has generated due to its multiple antioxidant advantages. Consequently, further research is required in this area.

**Table (1):** Total ionic chromatogram (GC-MS) analysis of hexane extract of *Aloe vera* obtained with 70 eV using an Elite-1 fused silica capillary column with Helium gas as the carrier

| No | RT | Compound Name | Molecular formula | Probability | Peak Area % |
|---|---|---|---|---|---|
| 1 | 3.09 | Tetraacetyl-d-xylonic nitrile | C14H17NO9 | 43.05 | 0.43 |
| 2 | 3.09 | Cyclopropane dodecanoic acid, 2-octyl-, methyl ester | C24H46O2 | 11.03 | 0.43 |
| 3 | 3.15 | 2-Oxazolamine, 4,5-dihydro-5-(phenoxy-methyl)-N-[(phenyl amino) carbonyl] | C17H17N3O3 | 5.30 | 0.59 |
| 4 | 3.45 | Silane diol, dimethyl | C2H8O2Si | 9.68 | 3.29 |
| 5 | 3.45 | 2H-Pyran, tetrahydro-2-(2,5-undecadi-ynyloxy) | C16H24O2 | 46.31 | 3.29 |
| 6 | 3.45 | Phenylpropanolamine | C9H13NO | 8.93 | 3.29 |
| 7 | 5.19 | 12,15-Octadecadiynoic acid, methyl ester | C19H30O2 | 46.36 | 2.88 |
| 8 | 6.15 | 2-Myristynoyl pantetheine | C25H44N2O5S | 3.40 | 3.90 |
| 9 | 6.96 | 2,5-Octadecadiynoic acid, methyl ester | C19H30O2 | 32.44 | 1.55 |
| 10 | 7.48 | 1,5:2,4-Dimethanopentalene-3,6-diol, Octa-hydro | C10H14O2 | 17.00 | 0.90 |
| 11 | 8.18 | Geranyl isovalerate | C15H26O2 | 10.21 | 0.97 |
| 12 | 11.89 | 5,7-Dodecadiyn-1,12-diol | C12H18O2 | 15.55 | 0.53 |
| 13 | 17.03 | d-Lyxo-d-manno-nononic-1,4-lactone | C9H16O9 | 14.37 | 1.24 |
| 14 | 17.82 | Hydro-cinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl) methyl] | C20H22O2 | 53.00 | 0.81 |
| 15 | 20.09 | 1-Fluoro-2-methyl-1-(N-methyl-N-phenyl amino)-1,3-butadiene | C12H14FN | 6.22 | 0.50 |
| 16 | 20.16 | [5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-d eca-4,8-dienylidene]- (2-phenyl-aziridin-1-yl)-amine | C28H34N2O | 7.97 | 0.22 |
| 17 | 20.65 | 9,12,15- Octadecatrienoic acid, 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl) oxy] methyl] ethyl ester, (Z, Z, Z) | C27H52O4Si2 | 54.56 | 0.51 |
| 18 | 23.51 | Phenol, 3-methyl- 4- (methyl thio) | C8H10OS | 78.51 | 3.96 |
| 19 | 25.12 | 10-Heptadecen-8-ynoic acid, methyl ester, (E) | C18H30O2 | 14.26 | 0.73 |
| 20 | 26.16 | 1-Monolinoleoylglycerol trimethylsilyl ether | C27H54O4Si2 | 33.31 | 1.88 |
| 21 | 34.41 | 2- [4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde | C23H32O | 25.78 | 0.47 |
| 22 | 34.41 | Vitamin A aldehyde | C20H28O | 4.05 | 0.47 |
| 23 | 36.22 | Gibberellic acid | C19H22O6 | 14.74 | 0.59 |
| 24 | 38.38 | Glycine, methyl ester | C36H69NO6Si3 | 8.15 | 0.67 |
| 25 | 39.53 | Milbemycin B, 6,28-anhydro-15-chloro-25-isopropyl-13-d ehydro-5-O-demethyl-4-methyl- | C33H47ClO7 | 5.07 | 0.36 |
| 26 | 40.39 | psi, psi-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy | C42H64O2 | 6.08 | 0.54 |
| 27 | 46.44 | Cyclopropane, 1-(4-methoxyphenyl)-2-phenyl | C16H16O | 5.36 | 7.93 |
| 28 | 49.26 | Cinnamic acid, 4-hydroxy-3-methoxy | C31H40O15 | 39.61 | 1.68 |
| 29 | 54.15 | 9,10-Secocholesta-5,7,10(19)-triene-3,25,26-triol | C27H44O3 | 3.71 | 1.79 |
| 30 | 54.15 | Androst-4-en-11-ol-3,17-dione, 9-thiocyanato | C20H25NO3S | 8.13 | 1.79 |

**Table (2):** Effect of *Aloe vera* extract in drinking water every day on performance and semen characteristics of male rabbits

| Traits | Treatment, ml/ L | | | SEM | *P*-values | | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | | T | L | Q |
| Initial body weight, kg | 3.11 | 3.07 | 3.10 | 0.35 | 0.635 | 0.468 | 0.185 |
| Final body weight, kg | 3.13 | 3.18 | 3.05 | 0.62 | 0.152 | 0.267 | 0.421 |
| Reaction time, sec | 19.97 | 18.66 | 20.42 | 2.84 | 0.1557 | 0.6266 | 0.0628 |
| Semen volume, mL | 0.91 | 0.94 | 0.86 | 0.13 | 0.1917 | 0.2538 | 0.1553 |
| Color of ejaculate | Milky to creamy white | | | | | | |
| pH | 7.04$^{b}$ | 7.07$^{ab}$ | 7.09$^{a}$ | 0.05 | 0.0056 | 0.0015 | 0.5908 |
| Fertility, % | 74.26 | 72.69 | 71.76 | 19.73 | 0.9312 | 0.7101 | 0.9559 |
| Litter size at birth, kits | 8.67$^{ab}$ | 9.17$^{a}$ | 7.78$^{b}$ | 1.59 | 0.0259 | 0.0825 | 0.0345 |

$^{a, b,..}$ Means in the same row having different superscripts are significantly different at $P \leq 0.05$.

**Table (3):** Effect of *Aloe vera* extract in drinking water every day on semen quality of male rabbits

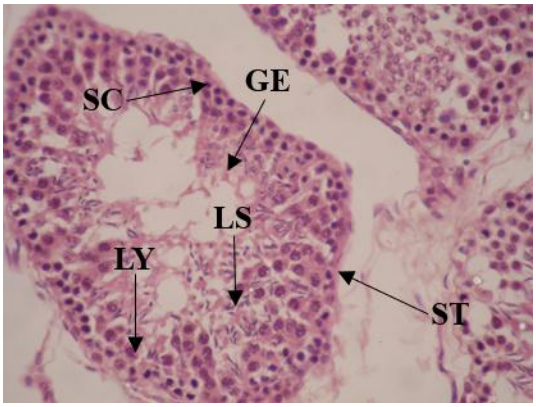
| Traits | Treatment, ml/ L | | | SEM | *P*-values | | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | | T | L | Q |
| Advance sperm motility, % | 67.38$^{a}$ | 67.84$^{a}$ | 63.92$^{b}$ | 3.67 | 0.0012 | 0.0026 | 0.0248 |
| Live spermatozoa, % | 79.79 | 80.22 | 78.11 | 2.99 | 0.0820 | 0.0941 | 0.1391 |
| Sperm concentration, $10^6$/ejac | 379.6 | 384.1 | 375.50 | 11.32 | 0.0715 | 0.2657 | 0.0433 |
| T. sperm output, $10^6$/ejac | 332.6$^{a}$ | 334.0$^{a}$ | 319.3$^{b}$ | 12.53 | 0.0002 | 0.0006 | 0.0128 |
| T. live sperm output, $10^6$/ejac | 298.8 | 303.3 | 294.2 | 12.16 | 0.0802 | 0.2463 | 0.0528 |
| T. normal sperm, $10^6$/ejac | 283.7 | 286.1 | 278.1 | 11.28 | 0.0904 | 0.1312 | 0.1072 |
| Normal sperm, % | 85.44$^{a}$ | 85.17$^{a}$ | 80.51$^{b}$ | 5.55 | 0.0086 | 0.0059 | 0.1442 |
| Abnormal sperm, % | 11.65$^{b}$ | 10.25$^{c}$ | 12.75$^{a}$ | 2.74 | 0.0261 | 0.3048 | 0.0121 |
| Pus cells, % | 3.91$^{b}$ | 4.58$^{b}$ | 6.74$^{a}$ | 3.79 | 0.0005 | 0.0001 | 0.4598 |

$^{a, b,..}$ Means in the same row having different superscripts are significantly different at $P \leq 0.05$.

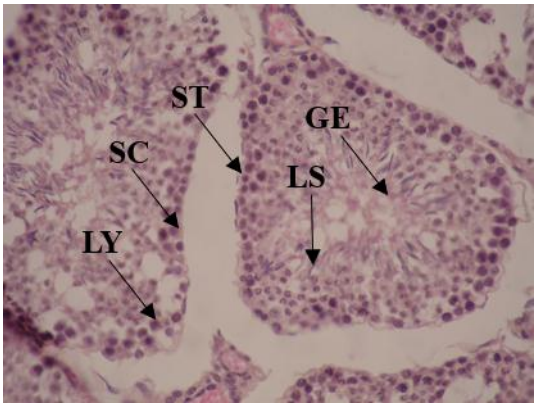
**Table (4):** Effect of *Aloe vera* extract in drinking water every day on semen biochemical traits of male rabbits

| Traits | Treatment, ml/ L | | | SEM | *P*-values | | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | | T | L | Q |
| Testosterone, ng/ml | 4.54 | 4.59 | 4.33 | 0.49 | 0.238 | 0.200 | 0.265 |
| Initial fructose, mg/dl | 73.76$^{b}$ | 76.73$^{a}$ | 73.19$^{b}$ | 3.78 | 0.008 | 0.630 | 0.002 |
| Total protein, mg/dl | 3.31$^{b}$ | 3.53$^{a}$ | 3.24$^{b}$ | 0.30 | 0.008 | 0.463 | 0.002 |
| Albumin, mg/dl | 1.47$^{b}$ | 1.77$^{a}$ | 1.57$^{b}$ | 0.26 | 0.001 | 0.211 | 0.001 |
| Globulin, mg/dl | 1.84 | 1.76 | 1.67 | 0.21 | 0.276 | 0.111 | 0.952 |
| AST, IU/l | 32.92$^{a}$ | 30.53$^{b}$ | 33.75$^{a}$ | 3.86 | 0.031 | 0.501 | 0.011 |
| ALT, IU/l | 43.30$^{a}$ | 36.06$^{b}$ | 41.70$^{a}$ | 4.88 | 0.001 | 0.214 | 0.001 |
| MDA, mmol/l | 1.33$^{a}$ | 1.21$^{b}$ | 1.40$^{a}$ | 0.16 | 0.001 | 0.170 | 0.001 |
| TAC, mmol/l | 1.87$^{b}$ | 2.07$^{a}$ | 1.85$^{b}$ | 0.21 | 0.001 | 0.703 | 0.001 |

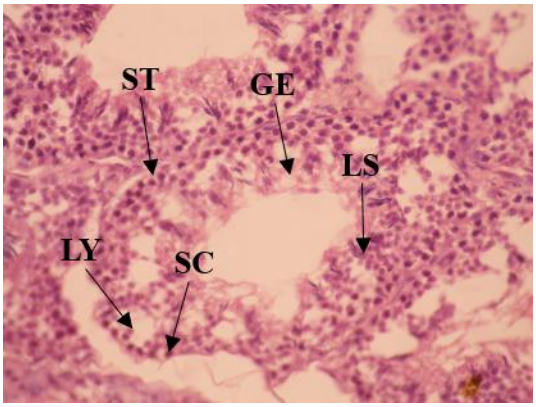
$^{a, b,..}$ Means in the same row having different superscripts are significantly different at $P \leq 0.05$.




**(A)**




**(B)**




**(C)**

**Figure 1:** Histological cross section of V line rabbit testis from: **A)** Control group; **B)** Low level (2 ml/ L tap water) of *Aloe vera* extract (AVE); **C)** High level of AVE (4 ml/ L tap water) show tissue structure, semineferous tubule (ST), germinal epithelium (GE), Sertoli cells (SC), Leydig cells (LY), and Late spermatids (LS). H&E staining 40× magnification.

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## الملخص العربي

### تقييم جودة السائل المنوي والصفات البيوكيمائية للسائل المنوي وقطاعات الهستولوجي لذكور الأرانب المقدم لها مستخلص الصبار

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تهدف الدراسة الحالية إلى دراسة تأثير إضافة مستخلص الصبار (AVE) في ماء الشرب على جودة السائل المنوي، والصفات البيوكيميائية المنوية، والقطاعات الهستولوجية لذكور الأرانب. تم تقسيم أربعة وعشرون من الأرانب البالغة من الخط V إلى ثلاث مجموعات تجريبية متساوية (8 ذكور لكل منها). كانت أرانب المجموعتين الثانية والثالثة تشرب ماء يحتوي على 2 و4 مل من مستخلص الصبار لكل لتر ماء يوميًا لمدة 12 أسبوعاً متتالياً على التوالي. كانت المجموعة الضابطة الأولى بمثابة الكنترول (0 مل AVE لكل لتر). أظهرت النتائج أن الذكور التي كانت في مجموعة الجرعة الأقل (2 مل AVE/ لتر) كانت لديها رغبة جنسية أفضل (وقت رد فعل أقل)، وحجم السائل المنوي أعلى، وحجم المواليد مقارنة بالمجموعة الضابطة. كانت هذه التحسينات موازية لزيادة معنوية لجودة السائل المنوي (الحركة المتقدمة، إجمالي إنتاج الحيوانات المنوية، الحيوانات المنوية غير الطبيعية، وخلايا القيح في مجموعة الجرعة الأقل). بالإضافة إلى ذلك، لوحظ زيادة تركيزات الفركتوز الأولي في البلازما المنوية، البروتين الكلي، الألبومين، وزادت القدرة الكلية لمضادات الأكسدة بشكل ملحوظ في السائل المنوي فى مجموعة الجرعة الأقل (2 مل من AVE) لكل لتر ماء شرب، بينما انخفضت معنوياً قدرة إنزيمات الكبد في البلازما المنوية (AST)، (ALT)، والمالونديالدهيد (MDA) في مجموعة الجرعة الأقل مقارنة بالمجموعة الضابطة والجرعة العالية من AVE. أدى استخدام جرعة منخفضة من AVE إلى تحسين القطاع الهستولوجي لتركيب النسيجي لخصية ذكور الأرنب وزيادة معدلات تركيز الحيوانات المنوية في الأنابيب المنوية. يمكن إستخلاص أن الجرعة المنخفضة من مستخلص الصبار (2 مل AVE لكل لتر ماء شرب) لذكور الأرانب اعطت نتائج إيجابية ومعنوية بتحسين خصائص وصفات جودة السائل المنوي وتأثير إيجابي للصفات البيوكيمائية للسائل المنوي وكذلك التركيب الهستولوجي لخصية ذكور ارانب خط V.

**الكلمات الدالة:** ذكور الأرانب، جودة السائل المنوي، الحالة التأكسدية، هستولوجي الخصية.