The effects of hydroalcoholic extract of *Aloe vera* gel on spermatogenesis of adult male rats


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**Abstract**

*Aloe vera* is a perennial plant of the Liliaceae family. Its leaves possess anti-microbial and anti-cancerous properties. The present study investigates the probable effect of the hydroalcoholic extract of *Aloe vera* gel on testosterone, LH and FSH, sperm count and motility in adult male wistar rats. In this experimental study, 24 adult male wistar rats were used that weighed an average of 170–240 grams. Randomly, they were assigned to 4 groups of 6 including: G1: control group, and G2, 3 and 4. The control group received 1 ml of distilled water. The 2nd, 3rd and 4th group received respectively 100, 150 and 200 (mg/kg) of hydroalcoholic extract of *Aloe vera* gel orally for 52 days. In the end, a ventricular blood testing was performed. Its serum was separated; the serum concentration of testosterone, LH and FSH were measured through ELISA, and the status of sperms was examined. Serum concentration of testosterone and LH was significantly increased in the 2nd, 3rd and 4th groups as compared to G1. The serum concentration of FSH hormone showed a significant rise in the 3rd and 4th groups as compared to the control group. The number and motility of sperms indicated a significant increase in all the experimental groups as compared to the control group. The hydroalcoholic extract of *Aloe vera* gel, in dosages used in this study, have fertilizing and stimulating impact on spermatogenesis of adult male wistar rats.

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Introduction
So far, a myriad of study has been conducted on the effects of herbal extracts on male fertility. They have attested to the efficacy of herbal medicine in improving fertility (Shahraki et al., 2014; Oh et al., 2007). Due to their natural quality, medical herbs showed to be better adaptable to human body and are often without any negative side effects. Using them properly might not be useful, but for sure it is harmless too (Afolayan et al., 2002).

Aloe vera is class the Liliopsida, the Asparagales order. It has over 250 types in the world the most prevalent of which is Aloe vera. It is local to tropical areas and grows in bushes. Its winter flowers are tubular and yellow in color. Its leaves are thick, serrated and succulent with thorny edges (Bots et al., 2008). This plant contains many vitamins including antioxidant vitamins like A and C, vitamins of B group like thiamin, niacin, B2 (Riboflavin), B12, and folic acid. Sodium, potassium, calcium, magnesium, manganese, copper, chrome, and iron are found in Aloe vera. Gel of this plant has 20 of 22 essential amino acids for human and seven from eight necessary amino acids which body cannot produce it, including: arginine, aspargin, glutamic acid, aspartic acid, and cerin (Modaresi and khodadadi, 2014).

A myriad of therapeutic properties have been attributed to this plant. The present study seeks to investigate the precise impact of the hydroalcoholic extract of Aloe vera gel on sex hormones and sperm in adult male rats.

Materials and Methods
The adult male wistar rats weighing between 170 to 240 gr were bought from the research center of animal lab in Zahedan University of medical sciences. For 5 days, they were kept in controlled conditions as for the light (12 hours in light and 12 hours in dark), at the temperature of 25-27 oC. They were provided with enough food in the animal room of Zabol University of Medical Sciences, so that they could be adapted to the new environment. The aforementioned conditions were also maintained all throughout the experiment. During the experiment, the animals were kept in rat-exclusive plastic cages of the standard size of 15x25x40. The floors were covered in wood sawdust which was replaced every 7 days. All moral rules concerning animal rights were abided by in this study.

Animals and treatment
After 5 days of adaptation to the new context, the adult male wistar rats were randomly divided into 4 groups of 6. Then they were weighed. The gavage took 52 days for all groups and was done between 10:00 to 12:00 every morning.

Group 1 (control): received 1 ml of distilled water each day.
G2, 3 and 4: respectively received 100, 150 and 200 mg/kg of hydroalcoholic extract of Aloe vera gel every day.

2. Aloe vera gel extract

In order to obtain the hydroalcoholic extract of Aloe vera gel, firstly its leaves were taken from the botanical greenhouse of Sistan and Baluchestan University. They were examined and approved by a botany professor. The gel was then cut into smaller pieces using a knife and 500 grams of it was weighed by a digital scale and poured into a beaker. Subsequently, 750 ml of distilled water along with 750 ml of ethanol (Germany, Merck) which had been measured in a graded cylinder was added to Aloe vera gel. Next, a ceramic magnet was inserted at the bottom of the beaker; the beaker was lidded and put on a magnetic mixer for 24 hours at a room temperature. The obtained mixture transferred to an incubator to be dried at the temperature of 37 oc.

Sperm analysis and hormone assay
At the end of the treatment, and considering all moral regulations, the rats were anaesthetized using ether. After weighing, using a 5 ml syringe, blood was taken from within the skin of the chest; and the obtained blood was transferred to a test tube. For 15 minutes, it was centrifuged at the speed of 2000 rounds per minute. The separated serum was transferred to an eppendorf tube. After jotting down sample
descriptors it was kept in a freezer at the temperature of -180 °C until the measurement time.

Measurement of testosterone, LH and FSH hormones was done using ELISA animal kits (Glory Sience Co, USA).

In order to examine sperm count and motility in each group, animal testicles were immediately cut after the blood test. The weight of the extracted testicles was measured and recorded. In this study, epididymis and the left testis were used to count sperms and examine sperm motility. Once the epididymis of the left testicle was evident, one centimeter of its tail was cut out of its surrounding tissues and cut into smaller pieces. They were, then put in 5 ml of Hanks solution at the temperature of 37 °C.

In order to count the number of sperms, one drop of the solution was poured on a Neobar lam, and the number of sperms in its 4 large chambers was counted. The average number of sperms in every chamber was estimated (Pant et al., 2004). In order to examine sperm motility, after placing the cut of epididymis tail in hanks solution and homogenizing it, a 50 µl sampler was used to pick one drop of the solution and place it on the lam. Next, using an optical microscope (x40), sperm motility was examined and the results were reported in percentage.

Results
The obtained results in this study were analyzed using SPSS. The estimated parameters in the 4 groups of this study were compared for significance using one-way ANOVA and Tukey-test. The significance level of all parameters was set at p-value<.05. The results were reported in Mean±SEM. The degree of significance in the different groups was analyzed in 3 levels:

1. P-value<.05 *
2. P-value<.01 **
3. P-value<.001 ***

Testosterone hormone

The serum level of testosterone was found to be 2.16±0.211, 3.24±0.291, 4.34±0.099 and 4.79±0.312 in groups 1, 2, 3 and 4.

![Fig. 1. Comparison of serum level in testosterone hormone (ng/ml) of the 4 groups.](image)

Comparing the control group and the other three in terms of the significance of results revealed that the control group was significantly different from G2 (P-value<.05) as well as G3 and 4 (P-value<.001) (figure 1).

LH hormone

The serum level of LH for group 1 (control), 2, 3 and 4 were found to be respectively .329±0.007, .379±0.006, .425±0.008 and .468±0.013. Comparison of the control group with others in terms of the significance of results shows that G1 was significantly divergent from G2 (P-value<.01), 3 and 4 (P-value<.001) (figure 2).

![Fig. 2. Comparison of serum level in LH hormone (mIU/ml) of the 4 groups.](image)

FSH hormone

The serum level of FSH for group 1 (control), 2, 3 and 4 were respectively .228±0.007, .250±0.004, .263±0.007 and .286±0.006.
Comparison of the control group with the others in terms of the significance of results shows a non-significant divergence of the control group with G2. However, it was found to be significantly different from G3 (P-value<.01) or G4 (P-value<.001) (figure 3).

**Fig. 3.** Serum level of FSH hormone (mIU/ml) of the 4 groups.

**Sperm count**

The number of sperms in G1 (control), 2, 3 and 4 was $1.91 \times 10^6 \pm 0.87712 \times 10^6$, $2.41 \times 10^6 \pm 0.107437 \times 10^6$, $3.49 \times 10^6 \pm 0.066671 \times 10^6$, and $4.21 \times 10^6 \pm 0.172027 \times 10^6$.

As for the significance of findings, comparing the control group with the other three indicated that it was significantly different from G2 (P-value<.05), G3 and 4 (P-value<.001) (figure 4).

**Fig. 4.** Sperm count in the 4 groups.

**Sperm motility**

Sperm motility in percentage was respectively 47.5 ± 1.62, 59.5 ± 0.88, 63.6 ± 1.4 and 70.1 ± 1.92 for groups 1 (control), 2, 3 and 4.

Comparison of the control group with the other three in terms of the significance of differences, revealed that G1 was significantly different from groups 2, 3 and 4 (p-value<.001) (figure 5).

**Discussion**

There was an increase in the plasma testosterone hormone of the experimental groups as compared to the control. This divergence in the significance level of experimental groups relative to the control group indicated the more efficacies of 150 and 200 mg/kg dosages on the level of plasma testosterone hormone. In another study conducted earlier on the aquatic extract of *Aloe vera*, a similar significant rise was observed in the level of testosterone hormone of the treatment group (Estakhr and javdan, 2011). In another study on the alcoholic extract of *Aloe vera* leaves, a reduction was observed in the level of testosterone hormone of the treatment groups as compared to the control (Shariati et al., 2009). Such a reduction was indicative of the presence of a material in *Aloe vera* leaf sheath which weakens the positive effect of its gel on testosterone hormone. Why testosterone hormone in this study was increased could be related to LH hormone. That is because the amount of its testosterone hormone is positively correlated with LH hormone and is in fact under its control (Guyton and Hall, 2011).

There was a rise in the level of plasma LH of the groups treated with *Aloe vera* hydroalcoholic extract as compared to the level of this hormone in the control group. The divergence of significance in treatment groups compared to the control group indicates the higher impact of 150 and 200 mg/kg dosages of *Aloe vera* alcoholic extract than the 100 mg/kg dosage. Measuring FSH hormone in the treatment groups indicates the rise in this hormone as compared to the control. In a recent study conducted using the hydroalcoholic extract of *Aloe vera* leaves for adult rats for 10 days, the amount of LH and FSH hormones was measured. No significant change, as compared to the control group, was however reported (Poorfarid et al., 2012). In another study which used the alcoholic extract of *Aloe vera* on adult rats for 21 days, the amount of FSH hormone showed to have a
significant reduction. No significant change was, however, witnessed in the level of LH hormone (Shariati et al., 2009). Findings of the present study are different from those of other studies which used Aloe vera leaves. In the present research, the hydroalcoholic extract of Aloe vera gel was used. The rise of LH level might be due to the presence of stimulating compounds of FSH cells in Aloe vera gel. These cells are present in the anterior pituitary and produce LH and FSH. Both hormones are produced by one type of cell in the anterior pituitary. FSH hormone affects sertoli cells and therefore, increases spermatogenesis reproduction (Griswold et al., 1998). Moreover, through having mutagenic effects, FSH would raise the activity of sertoli cells (Sharp et al., 1990). On the other hand, production of these two hormones is under the control of GnRH hormone produced in hypothalamus. The rise of LH and FSH hormones can be associated with the effect of the hydroalcoholic extract of Aloe vera gel on the hypothalamus cells producing GnRH. Through increasing GnRH hormone, they would increase the production of LH and FSH (Guyton and Hall, 2011). In a study conducted formerly using the aquatic extract of Aloe vera on rats, a similar rise of sperm motility and count was observed in the treatment groups (Estakhr and Javdan, 2011). The findings of the present study were similar to those of the aforementioned study. The reason for the increase in sperm count of treatment groups using the hydroalcoholic extract of Aloe vera gel could be the rise of plasma level of the two hormones, testosterone and FSH. That is because the spermatogenesis process is regulated by these two hormones. FSH is linked to its particular receptors in the surface of sertoli cells of semen production tubes and would cause the growth of sertoli cells and production of different effective materials which affect spermatogenesis. However, testosterone and dihydrotестosterone produced by leydig cells are also highly influencing the progress of spermatogenesis (Guyton and Hall, 2011).

On the other hand, the analyses conducted on Aloe vera gel attested to the existence of vitamins A, B, C and E (Hamman et al., 2008). The antioxidant effect of these vitamins has been well established in previous study which has showed that vitamins B, C and E are effective in reducing the toxic effects of cadmium on testis tissue as well as the spermatogenesis process (Yang et al., 2006). Moreover, studies conducted on vitamins C, E and other antioxidants such as glutathione and con- enzyme Q 10 were useful in treating male infertility and protecting the DNA of cell nucleus produced by stress, environmental pollution and malnutrition (Jedlinska et al., 2006; Mozdarani and salami, 2006; Murugesan et al., 2005). Moreover, malnutrition and insufficiency of Group B vitamins, due to their role in synthesis, evolution and repairing DNA cells cause damage to the spermatogenesis process (Mozdarani and salami, 2006; Ebisch et al., 2007). Previous study revealed that meals containing folic acid and zinc have managed to increase about 74% of the total sperm count. Moreover, the reduction of zinc in seminal fluid leads to an increase in sperm count and their motility (Ebisch et al., 2007). Zinc and folic acid are both present in Aloe vera gel and can be the reason why sperm count and motility was raised in this study. Vitamins B and C as well as folic acid and zinc cut down on reactive oxygen species (ROS) and, therefore, enhance the quality of semen and reduce the apoptosis of spermatozoa (Pfeifer et al., 2001).

![Fig. 5. Sperm motility in percentage in the 4 groups.](image-url)
To meet their biological needs, sperms produce a certain degree of reactive oxygen species themselves such as H2O2 and consume it (Baker and Aitken, 2004). However, sperms are highly sensitive to ROS that enter seminal fluid through leukocytes. This fact leads to male infertility (Imai et al., 2001). Among other antioxidants present in Aloe vera gel are glutathione peroxidase and superoxide dismutase (Langmead et al., 2004). The protein of the former works as an antioxidant in protecting sperms and their pathways in testis tissue and epididymis (Chu, 1994). Investigations have shown that the reduction of glutathione peroxidase in body leads to infertility (Imai et al., 2001; Williams et al., 1998). Placed on the plasma membrane of sperm, sperm nucleus, epididymis fluid and epididymis area, GPX 3, 4 and 5 protect sperms from the damage of ROS and free radicals and would lead to the final growth and evolution of sperms (Drevet, 2006). Therefore, the extract of Aloe vera gel could be said to have strong antioxidant materials with the help of which it strengthens the antioxidant defensive system. It, then, reduces oxidative stress and increases sperm count and quality.

Conclusion

Findings of the present research help us to conclude that the hydroalcoholic extract of Aloe vera gel in dosages used in this study, has fertilizing and stimulating effects on spermatogenesis in adult male wistar rats. These effects would increase the level of testosterone, LH and FSH hormones, sperm count and motility.

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