



Full Length Article

The Effect of Aloe Vera Extract on the Sperm Quality in Male Diabetic Rats

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ABSTRACT

Diabetes is a metabolic disorder which can be diagnosed by the hyperglycemia resulting from defects in insulin secretion and performance or both. Diabetes reduces the process of spermatogenesis and makes this phenomenon disordered. The objective of the present study is to investigate the effects of Aloe Vera extract on the production and quality of sperms of male diabetic rats. In the study, 24 diabetic adult male Wistar rats each with a weight of about 200-250 gr were provided. Rats became diabetic by the injection of 50 mg / kg/IPstreptozotocin. Then, rats divided into four groups randomly: the first group: diabetic control group which received daily distilled water orally, the second group: the diabetic group which received daily 400 mg / kg Aloe Vera extract orally. The third group, the healthy control group which daily distilled water orally, and the fourth group: the healthy group received daily 400 mg / kg distilled water orally. After 30 days of treatment, the rats were weighed in and then, the blood glucose was measured by glucometer through tails. Then, they were anesthetized using ether and a blood sample was extracted from the animal for investigating the testosterone levels, then testes and epididymis removed for investigating the quality of sperms, caudal part of epididymis was cut and placed in T6 environment, then the sperms were investigated in terms of morphology, number and motion. The testes, after tissue processing and the preparation of paraffin sections, were stained into sections using Hematoxyline-Eosin. Then, the prepared slides were investigated by OLYMPUS Microscope and OLYSIA software program. The obtained data were analyzed using SPSS and ANOVA test. According to the obtained results, the average body weight, spermatogonia cells, Sertoli cells, Leydig cells, seminiferous tubule diameter and the epithelium diameter of seminiferous tubule, serum levels of blood glucose, sperm motility (fast and slow) and the sperm number in the diabetic control group indicated a significant difference ($p < 0.05$) compared to other groups. In addition, the mean sperm form and the amount of the serum levels of hormone testosterone in diabetic control group did not indicate a significant difference ($p > 0.05$) compared to other groups. The obtained results indicated that Aloe Vera extract, regarding its antioxidant properties, could prevent from the damage to the testicular tissue of rats and improve the spermatogenesis by control of the serum levels of blood glucose.

Key words: Aloe Vera extract, Testes, Rat, Diabetes, Sperm quality

Received 17.03.2014

Revised 15.05.2014

Accepted 22.06.2014

INTRODUCTION

About 15% of the couples suffer from infertility whose 30% of the causes is related to men, 30% to women, 10% to both sexes, and 25% of the cases are related to unidentified reasons. Therefore, as indicated by statistics, men have a significant proportion in relation with infertility (1). Disorder in production and performance of sperms and damage in the spermatogenesis are among the commonest causes of infertility in men (2). Diabetes causes symptoms including damage to the reproductive system of adults such as reduction in pregnancy rates, erectile and ejaculation disorders, decrease in the stimulation of sperms, increase in abnormal sperm forms, and reduction in Sertoli and Leydig cells (3, 4, and 5). Hypothalamic-pituitary-gonadal axis hormones takes the responsibility of normal process of spermatogenesis. Studies indicate that in the absence of insulin, the ability of the cells of the pituitary anterior lobe in using glucose decreases and consequently results in decrease in GnRH (6, 7, and 8). The effects of diabetes on the performance of testicles, due to insufficient production of insulin and consequently the decrease in the effect of this hormone in regulating the performances of Sertoli and Leydig cells (9). Diabetes, due to increase in free radicals, can deteriorate the live tissues. To decrease these radicals, bodies use antioxidants; these antioxidants, with their structural composition, can

decrease the radical oxygen and prevent from their damaging effects (10). Most plants have antioxidant properties. One of them is Aloe Vera. Aloe Vera is from lily family and grow in hot and dry areas and among the 24 species, four species have nutritional value (11, 12). Aloe Vera gel stored in the internal part of its leaves includes 5.99% water and 5.0% solid materials (12). Aloe Vera contains alkaloids, saponins, flavonoids, proteins, lipids, amino acids, vitamins such as vitamin C, B1, B2, B6, A, E, enzymes, organic and inorganic compounds and mineral salts such as sodium, calcium, iron, potassium, chloride, manganese, copper and zinc. The most significant properties of it are lowering blood glucose and cholesterol. Among other properties of Aloe Vera one can mention preventing skin lesions and their healing. Oral administration of this herb contributes to the digestion of food and improves heart health and kidney performance (13). Regarding the antioxidant properties and properties of lowering blood glucose of Aloe Vera and also the damaging effects of diabetes on spermatogenesis, the present study was conducted.

MATERIALS AND METHODS

To do the research, 24 male Wistar rats with the weight of 200-250 gr were purchased from Shiraz University of Medical Sciences and kept in the animal den of Yasuj University of Medical Sciences. To comply with the conditions in laboratory conditions, the rats were kept for 12 hours in darkness and 12 hours in light and also in the temperature $22 \pm 2^\circ \text{C}$ with standard food and sufficient water. Randomly, half of the rats was selected as diabetic group and the other half was selected as the non-diabetic one and then, to make the animals diabetic, streptozotocin manufactured by Sigma Co. in Germany was used and to each one-gram vials of the drug, 10 ml citrate buffer (0.1 M) was added. To each rat 50 mg/kg drug was injected in an intraperitoneally way and using insulin syringes. 24 hours after the injections, to control the amount of blood glucose, by making small incisions on the end of rats' tails, a drop of blood was placed on the glucometer strips and the read number was registered. If the rats' blood glucose was more or equal 250mg/dl, they were considered as diabetic and to ensure the process of making rats diabetic, after 10 days, measuring the amount of blood glucose was conducted in the same method and the read number was registered. In case of rats' not becoming diabetic, after 10 days, again Streptozotocin with the same amount was used and the amount of blood glucose and rats' becoming diabetic were investigated after 24 hours and then 10 days later, with the mentioned method. After that, randomly, diabetic and non-diabetic rats were divided into four 4-animal groups:

The first group: diabetic control group which daily received distilled water orally.

The second group: the diabetic group which daily received 400 mg/kg Aloe Vera extract orally.

The third group: the health group which daily received distilled water orally.

The fourth group: the health group which daily received 400 mg/kg Aloe Vera extract orally.

After 30days, through tails, the amount of rats' blood glucose was measured, then using ether the rats became unconscious, blood samples were obtained from the rats' hearts to investigate the amount of serum testosterone levels and the obtained serums were kept in the refrigerator temperature 0°C , then the animals were dissected and their testes were removed to be put in 10% formalin fixation solution. After fixation process, the animals' testes were put in the tissue processing apparatus and then tissue blocks were obtained from them prepared in 5 micrometer serial sections which were stained by hematoxyline – eosin. The obtained slides were investigated using OLYMPUS Microscope and OLYSIA software program and the diameter of seminiferous tubules, the thickness of germinal epithelium, the number of spermatogonia cells, Leydig cells and Sertoli cells were measured and counted. To investigate the quality of the sperms, caudal part of epididymis was sliced in the buffer T6 and were put in incubation system for 10 minutes, then a drop of buffer containing sperms was taken to be mounted on slide and the percentage of sperm motility (fast and slow) and also the percentage of non-motile sperm were counted. After that, 0.5 ml of buffer containing sperm was diluted by the white measuring pipette with proportion of 1/10 and using neubauer slide, the number of sperms were counted and multiplied by $(\times 10^6/\text{mm}^3)$. The obtained data were analyzed using SPSS software program and One-way ANOVA test.

RESULTS

The present study indicates that the mean sperm motility, counting the sperms and the amount of serum level of testosterone in the diabetic group treated by Aloe Vera extract compared to the diabetic control group indicated an increase which was close to the normal level and the difference was statistically significant ($P < 0.05$). The mean amount of the serum level of blood glucose in the diabetic group treated by Aloe Vera extract compared to the diabetic control group indicated a decrease which was close to the normal level and the difference was statistically significant ($P < 0.05$). The mean body weight in the diabetic control group compared to the other groups decreased which this decrease was statistically

significant ($P < 0.05$). The mean morphology of sperms in the diabetic control group compared to other groups did not show significant difference ($P < 0.05$) (Table. 1).

Regarding the table. 2, it is observed that the number of the spermatogonia cells, Sertoli cells, Leydig cells and the diameter of the seminiferous tubules in the diabetic control group compared to other groups decreased, which the difference was statistically significant ($P < 0.05$). The mean diameter of the germ layer in the seminiferous tubules in the rats of diabetic control groups treated by Aloe Vera extract compared to the diabetic control group increased, which the difference between the two groups was significant ($P < 0.05$).

Table 1: Comparing the $M \pm SEM$ variables in studied groups

Group		Normal		Diabetic	
		Control	Aloe Vera	Control	Aloe Vera
Sperm motility (%)	Fast	^b 13.83 \pm 2.92	^b 7.83 \pm 2.08	^c 3.60 \pm 1.12	^b 7.40 \pm 2.51
	slow	^a 37.33 \pm 2.07	^b 22.83 \pm 5.96	^c 13.60 \pm 2.83	^a 24.40 \pm 6.11
	Non-motile	^a 48.83 \pm 4.66	^b 69.33 \pm 7.58	^c 82.80 \pm 3.54	^a 68.20 \pm 8.42
Morphology of Sperm (%)	Normal	96.83 \pm 0.23	94.67 \pm 1.40	96 \pm 0.31	96.06 \pm 0.31
	Abnormal	3.50 \pm 0.22	5 \pm 1.41	4 \pm 0.32	4.01 \pm 0.31
Sperm count		^a \pm 14.87 176.67	^b 146.83 \pm 4.16	^b 138.20 \pm 4.21	^a 169 \pm 6.10
Body weight (gr)		^a 323.43 \pm 7.64	^a 335 \pm 16.71	^b 195.60 \pm 5.98	^b 217.80 \pm 17.06
Blood glucose (mg%)		^a \pm 12.95 118.85	^a 109.33 \pm 10.03	^b 528.80 \pm 27.41	^c 214.20 \pm 14.37
Testosterone		^a 3.76 \pm 0.43	^a 3.39 \pm 0.51	^b 2.32 \pm 0.44	^a 3.19 \pm 0.63

The dissimilar small letters in English in each row indicate significant differences ($P < 0.05$).

Table 2: Comparing the $M \pm SEM$ variables in studied groups

Group	Normal		Diabetic	
	Control	Aloe Vera	Control	Control
Diameter of Seminiferous(μ)	^a 288.99 \pm 7.53	^b 259.42 \pm 6.36	^c 229.45 \pm 6.22	^b 255.06 \pm 6.23
Diameter of germ layer(μ)	^a 84.76 \pm 7.36	^a 76.91 \pm 5.31	^b 51.80 \pm 2.60	^a 83.71 \pm 3.31
Number of Spermatogonia cell (mm ²)	^a 122.43 \pm 3.06	^b 104.67 \pm 4.31	^c 78.40 \pm 4.20	^c 84.80 \pm 3.51
(mm ²) Number of Leydig cells	^a 8.43 \pm 0.29	^c 6.83 \pm 0.47	^c 7.20 \pm 0.58	^c 7.80 \pm 0.37
Number of Sertoli cells(mm ²)	^a 1.86 \pm 0.26	^b 1.33 \pm 0.21	^b 1.20 \pm 0.20	^{ab} 1.63 \pm 0.20

The dissimilar small letters in English in each row indicate significant differences ($P < 0.05$).

Histological changes:

Investigating the testicular tissue sections in studied groups indicate which diabetes causes changes in the diabetic rats' testes in such a way that causes the decrease in the number of Sertoli cells, spermatogonia cells, Leydig cells, seminiferous tubule diameter and layer diameter of seminiferous germinal epithelium layer. In addition, the increase in the matrix in the interstitial space (edema) was observed in the diabetic control group, but in the diabetic group treated by Aloe Vera extract, the amount of edema decreased (Figure. 1, 2, 3, and 4).



Figure1: a section of the testes tissue of normal rats (OLYMPUS Microscope, Magnification X20, the staining of hematoxyline – eosin)

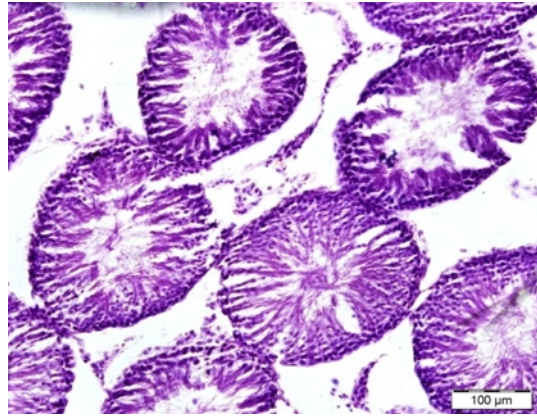


Figure2: a section of the testes tissue of diabetic rats (OLYMPUS Microscope, Magnification X20, the staining of hematoxyline – eosin)

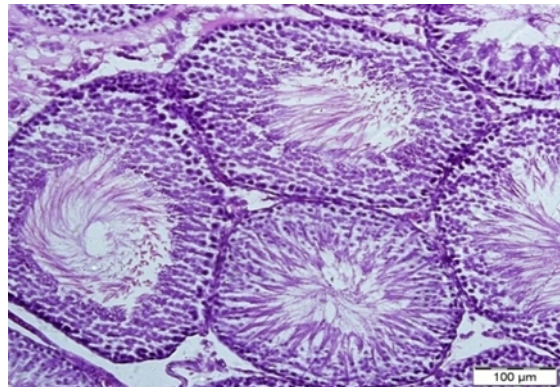


Figure3: a section of the testes tissue of normalrats treated by Aloe Vera extract (OLYMPUS Microscope, Magnification X20, the staining of hematoxyline – eosin)

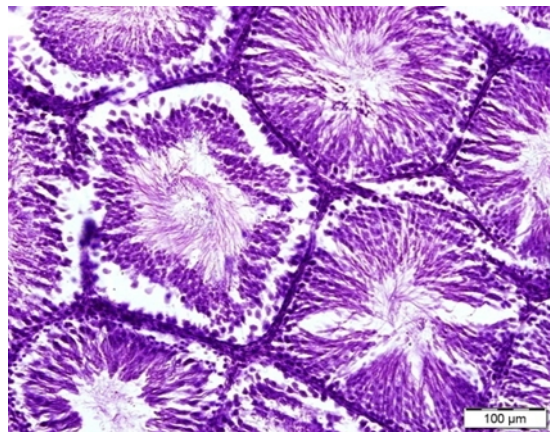


Figure4: a section of the testes tissue of diabetic rats treated by Aloe Vera extract (OLYMPUS Microscope, Magnification X20, the staining of hematoxyline – eosin)

DISCUSSION

A lot of physical and chemical factors cause infertility disorders including diabetes which increases the blood glucose causes the damage to the cell structure of the reproductive system. To decrease the side effects of various factors of new methods and traditional medicine were used. The objective of the present study investigate the effect of Aloe Vera extract on the quality of the sperms of diabetic male rats.

In the present study, it has been indicated that diabetes causes the decrease in the number of spermatogoniaSertoli cells, Leydig cells, the diameter of seminiferous tubules and the diameter of the germ tube. Furthermore, it has no effect on the sperm forms, but decreases the sperm mobility and body weight. It was observed that the mobility and dynamics decreased in the healthy rats treated with Aloe Vera extract. Aloe Vera causes the adjustment of the mentioned disorders in diabetic rats and in some cases, even the amount of studied factors in diabetic and healthy groups treated by Aloe Vera extract

were close to the normal level. Cameron et al. (1985) in a study indicated that spermatogonia cells in diabetic rats who had received Aloe Vera extract increased compared to the control group. These results is consistent with the results of the present study (13). Furthermore, thickening the basement membrane of seminiferous tubules has a significant role in decreasing spermatogenesis. Diabetes increases the thickness of the basement membrane and causes the decrease in the amount of producing sperm. In addition the decrease in the number of Sertoli cells results in the decrease in spermatogonia cells (15, 16). Guneli et al. in a study, indicate that diabetes causes testicular tissue changes through creating cell death (Apoptosis), the increase in the thickness of testicular capsule, the atrophy of seminiferous tubules, the reduction in tubule diameter and the decrease in the somatic Leydig and Sertoli cells (17, 18). Kiani (2011) in a study indicates that the structural changes in the diabetic rats' testicular tissue are not because of the streptozotocin related to the side effects of this composition, but the effects of diabetes on the testicular performance are due to the insufficient insulin production and consequently the decrease in the effect of this hormone in the regulation of Sertoli, Leydig and spermatogonia cells activities (16).

In the present study, the researcher found out that Aloe Vera extract causes the decrease in blood glucose in diabetic rats. The present study is consistent with the study done by MonirJadid al Eslami in which she obtained the results that the amount of 400 mm Aloe Vera extract with 5 mg glibenclamide causes the decrease in diabetic rats. In Kiani's study, the increase in the number and size of fat vacuoles in Sertoli cell cytoplasm in the testicular tissue of diabetic rats indicate that the amount of the activity of making steroid of these cells is due to the creation of decreased diabetes (19). Omotayo et al. (2010), in a study observed that the number of Sertoli cells per unit area in diabetic rats treated by Aloe Vera extract increased compared to the diabetic control group. These results are consistent with the results of the present study (20). Kiani (2011) indicates that in environments with high concentrations of glucose, the synthesis of basement membrane components such as collagen, fibronectin and laminin increase, which causes the thickening of basement membrane and may result in the arterial occlusion (19). Therefore, diabetes, by increasing collagen fibers, can decrease the vascular layers of testicular tissue. Hutson (1982, 1984) indicates that the decrease in the thickness of the wall of seminiferous cells in the testicular tissue of diabetic rats is due to the increase in the amount of collagen fibers in the environment between basement membrane and myeloid cells, which can cause disorder in the process of displacement of materials form the wall of seminiferous cells. Decreasing the thickness of the wall of seminiferous cells can be indicative of abnormal performance of fibroblasts around seminiferous tubules (16). Therefore, the diameter of seminiferous tubules decreases. The results obtained from this study indicate that the diameter of seminiferous tubules in the diabetic group decreased compared to the other groups treated by Aloe Vera extract. These results is consistent with the results of the studies done by Hutson and Kiani. In a study done by Rajasekaran et al. (2004) indicates that Aloe Vera moderates the level of hepatic glycogen by decreasing the activity of glycogen phosphorylase and increasing the activity of glycogen synthetase (19, 22). Accordingly, Vestegard et al. (1999) in a study indicate that phosphorylation of glucose through hexokinase which is in the first stage of glycolysis, is severely damaged in diabetes (20). As a result, the blood glucose decreases (20). Pari et al. (2000, 2002) indicate that probably Aloe Vera does the hypoglycemia operation by the potential of insulin release form the beta cells of the islets of Langerhans or insulin release form the bound form (21, 22).

CONCLUSION

The obtained results indicate that diabetic rats which received Aloe Vera extract, regarding the antioxidant and anti-diabetic properties of this plant, gonadal tissue (germinal cells and somatic cells) show less changes than the diabetic control group. Accordingly, it can be claimed that this plant prevents from the damage to gonadal tissue by adjusting hypoglycemia in long term and moderating body glucose.

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