

Evaluation of antioxidant potential of *Aloe vera* and pituitary sexual hormones after experimental diabetes in male rat

Behmanesh, M.A.^{1*}, Erfani Majd, N.¹, Shahriari, A.², Najafzadeh, H.³

¹Department of Histology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

²Department of Biochemistry, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

³Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

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Correspondence

Behmanesh, M.A.

Department of Histology,
Faculty of Veterinary Medicine,
Shahid Chamran University,
Ahvaz, Iran

Tel: +98(61) 42429070

Fax: +98(61) 42429538

Email: behmanesh.ma@yahoo.com

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Abstract:

BACKGROUND: Diabetes is a metabolic disease that is associated with hyperglycemia and infertility. Previous studies indicate that *Aloe vera* may positively affect the blood glucose and fertility. **OBJECTIVES:** The present study was carried out to evaluate the effect of *Aloe vera* on serum oxidant/antioxidant activity and reproductive hormones following experimental diabetes. **METHODS:** Sixty adult male Wistar rats were divided to 5 groups. Control group(A) was kept without treatment. Group(E) only received *Aloe vera* gel(400 mg/kg-orally). Experimental diabetes mellitus was induced in 3 groups of rats by streptozotocin(65 mg/kg-Ip). One diabetic group was kept without treatment (B). Another diabetic group received *Aloe vera* gel (400 mg/kg-orally) (C) and another received insulin (10 units)(D). *Aloe vera* gel and insulin was administrated for 30 days, then the rats were anesthetized and the blood collected. The amount of malondialdehyd (MDA), antioxidant activity(AOA), glutathion peroxidase activity(GPX), thiol protein(TSH), testosterone, LH and FSH was determined in serum. **RESULTS:** Level of testosterone was significantly decreased while amount of MDA, TSH, GPX and AOA was significantly increased in non-treated diabetic rats. *Aloe vera* increased antioxidant defense. **CONCLUSIONS:** *Aloe vera* improves antioxidant activity and reduces diabetic complications.

Introduction

Diabetes mellitus is a disease which affects the endocrine system and is considered to be one of the most serious health problems to modern global health (Basmatzou, 2016). Concurrent with the development of diabetes, hyperglycemia can cause structural and functional changes in various organs and tissues(Cai,2000). The existing

evidence suggests that diabetes causes disturbances in the reproductive system and reduces fertility in humans and in animal models (Carlos,2001).

Spermatogenesis is highly affected by the activity of Leydig cells. Diabetes causes changes in Leydig cells activity and thus reduces the level of testosterone in the blood. The reduction of testosterone is one of the main factors affecting the performance of

blood testis barrier (Gautam,2006).

Many mechanisms have been proposed to explain the changes of testicular structure in diabetic conditions. In recent years more attention has been paid to the mechanisms that affect the performance of gonadotropin cells and their secretion. Some reports indicate that the lack of proper activities of hypothalamic–pituitary axis decreases the hormonal levels of gonadotropin cells in blood and this reduction is involved in the structural changes of testis tissue (Ricci, 2009). Testicular function is controlled by pituitary hormones. Follicle-Stimulating Hormone (FSH) regulates the spermatogenesis while Luteinizing Hormone (LH) controls the activity of Leydig cells. In these researches a decreased serum level of FSH and LH following diabetes has also been reported (Hutson,1983).

On the other hand enough evidence exists about the increased oxidative stress in diabetic patients, due to the excessive production of reactive oxygen (ROS) and a reduction in the performance of the antioxidant defense (Jakus, 2004. Maiti, 2004 and Oksanen, 1975). The high concentration of free radicals in diabetes may be due to the increased glucose autoxidation (Ricci,2009). The ROS varieties are caused by cell damage through some mechanisms involving lipid peroxidation, oxidative damage of proteins and DNA through the induction of oxidative stress (Aitken,1989). In normal conditions the removal mechanisms of free radicals inhibit the ROS production and consequently reduce the damages caused by them (Jakus,2004). *Aloe vera* (*Aloe barbadensis*) has long been used for treating a range of certain diseases.

Aloe vera extract has been proved to have anti-diabetic and anti-cancer function. *Aloe*

vera is also used as a substitute to antibiotics (Kianfard, 2011). It is often recommended for diabetic patients due to its hypoglycemic effects (Oksanen, 1975). Anthraquinone are the constituents of *Aloe vera* which have laxative effects and the hypoglycemic properties of *Aloe vera* may be related to these compounds (Okyar, 2001). *Aloe vera* is enriched with the antioxidants that can reduce lipid peroxidation and the eliminated free radicals (Noor, 2008).

Materials and Methods

In order to accomplish the goals of this study, 60 healthy Wistar male rats weighing 200 ± 50 g were purchased from the Experimental Animal Center, in the Medical University of Ahvaz and were transferred to the Histology department of the Faculty of Veterinary Medicine in Shahid Chamran University. The purchased rats were kept in the same environmental conditions (12 h light/ 12 h of darkness), constant temperature and same amount of nutrition intake for two weeks in order for them to adapt to the environment. Following the adjustment phase, the rats were randomly divided into the following groups:

1- Control group (A): received the normal nutrition and were kept in normal conditions (15 male rats).

2- *Aloe vera* Group (B): received 400 mg/kg *Aloe vera* gel orally for 30 days (10 male rats).

3- Non treated Diabetic group: in this group in order to induce diabetes, streptozotocin (STZ) was intraperitoneally injected at dose 65 mg/kg. One week after STZ injection, in order to detect the presence of diabetes, the rats were evaluated in terms of blood glucose levels, through the tail vein

blood and glucometer test. The rats with glucose levels more than 250 Mm/lit were known as diabetic rat group (15 male rats).

4- *Aloe vera*-treated Diabetic group: In this group the diabetic rat received 400 mg/kg *Aloe vera* gel for 30 days by gavage (10 male rats).

5- Insulin-treated group: in this group diabetic rats received 10 IU insulin per day for 30 days (10 male rats).

During the course of the experiment, blood glucose was measured once every two weeks. At the end of the experiment after anesthesia with chloroform, vein section was performed in order to isolate the serum and measure the stress biomarkers and hormones. Malondialdehyde (MDA) was measured as a trailing indicator of lipid peroxidation through TBARS method. Based on this method the concentration of malondialdehyde was obtained based on MDA-TBA complex optical density at 532 nanometer wavelength in comparison with MDA standard curves (Pitton,1987). The measurement of thiolprotein was done based on the resuscitation of Elman reagent (DTNB) through the reduced glutathione and the formation of the yellow complex. The color intensity was measured proportionally to the amount of G-SH at 405 nm (Ellman,1959). Protein carbonyl was measured through the dinitrophenyl hydrazine reaction with this protein, and also by measuring the absorbance of the hydrazone protein complex at 370 nm wavelength (Kosif, 2008). The total antioxidant defense was measured by FRAP assay (Iris, 1996). FSH, LH and the testosterone amount were measured using Elisa method. Data analysis was performed using SPSS version 22 and the one-way analysis of variance (ANOVA) and post-hoc LSD with significance level of ($p \leq 0.05$).

Results

The highest blood sugar levels were observed within three days in diabetic rats and this can be considered as a significant increase compared with other groups. In days 15 and 30, level of blood sugar in diabetic rats showed a significant increase compared with control and *Aloe vera* groups, the diabetic group, the diabetic group which received insulin and the diabetic group that received *Aloe vera*. Comparing the blood sugar level of the diabetic rats which received insulin and the diabetic group who received *Aloe vera*, a significant increase is observed in the control group and the control group which received *Aloe vera* (Table 1).

The most antioxidant activity was observed in the diabetic rats in all three days which signified a significant increase in the control group and the control group who received *Aloe vera* for fifteen days. Furthermore, there is a meaningful decrease in the control group that received *Aloe vera* compared to the diabetic group that received insulin (Table 2). A significant decrease was observed in the protein carbonyl levels at day 15 in the diabetic rats compared to the diabetic group that received *Aloe vera* and the group which received *Aloe vera*. At day 30, a significant increase was observed in the protein carbonyl levels in the control group who received *Aloe vera* compared to the diabetic group and the group which received insulin (Table 2). Malondialdehyde increased in the first day of diabetes induction and compared to the control group this increase can be considered significant. On day 15 and 30, after the induction of diabetes, the amount of this factor in the diabetic

Table 1. The mean and standard deviation of blood sugar of rats in different groups. Letters a, b, c, d, e in each column indicate significant differences at $p \leq 0.05$.

Groups	Blood sugar	The first day of diabetes	15 days after diabetes	30 days after diabetes
Control (A)		118±7.3 ^b	110±8.2 ^{bcd}	112±5.7 ^{bcd}
Diabetics (B)		445±6.3 ^a	407±8.2 ^{acde}	439±3.7 ^{acde}
Diabetes + <i>Aloe vera</i> (C)		-	264±5 ^{abe}	190±2.6 ^{abe}
Diabetic + insulin (D)		-	255±4.7 ^{abe}	184±4.2 ^{abe}
<i>Aloe vera</i> (E)		-	107±6.7 ^{bcd}	108±8.1 ^{bcd}

Table 2. Serum levels (mean ± SD) total antioxidant activity, malondialdehyde, protein carbonyl in diabetic rats treated with *Aloe vera* (n = 10). Letters a, b, c, d, e in each column indicate significant differences at $p \leq 0.05$.

Groups	Biochemical factors								
	Total antioxidant activity			Malondialdehyde			Protein carbonyl		
	The first day of diabetes	15 days after diabetes	30 days after diabetes	The first day of diabetes	15 days after diabetes	30 days after diabetes	The first day of diabetes	15 days after diabetes	30 days after diabetes
Control (a)	488±5	507±8.5 ^b	356.1±8.2	319±9 ^b	426±8 ^b	419±6.8	14.5±1.2	13.56±1.05	13.6±0.4
Diabetics (b)	558±7.5	679±6.6 ^{ac}	429.9±7.4	858±8 ^a	772±7 ^{ad}	765.9±4 ^{ad}	13.4±0.24	11.98±1.07 ^{ce}	12.73±0.69 ^e
Diabetes + <i>Aloe vera</i> (c)	-	627.6±8.1 ^c	395.1±9	-	656.6±8.1	520.2±5.2	-	14.03±0.24 ^b	14.48±0.56
Diabetic + insulin (d)	-	599.2±7.1 ^c	468.6±7	-	499.2±8.6 ^b	439.1±7 ^b	-	13.58±0.88	12.81±0.16 ^E
<i>Aloe vera</i> (e)	-	434.2±6.5 ^{bcd}	432.2±7.3	-	663.6±5.6	533.9±4.3	-	13.97±1.6 ^b	15.22±0.16 ^{Bd}

Table 3. Serum levels (mean ± SD) of markers of oxidative stress in diabetic rats treated with *Aloe vera* (n = 10). Letters a, b, c, d, e in each column indicate significant differences at $p \leq 0.05$.

Groups	Biochemical factors						
	The first day of diabetes	Protein thiols			Glutathione peroxidase		
		15 days after diabetes	30 days after diabetes	The first day of diabetes	15 days after diabetes	30 days after diabetes	
Control (a)	30.11±7.8	37.09±8 ^C	24.03±2.8 ^b	60.56±7.9	52.8±4.9 ^C	41.35±8.8 ^{bc}	
Diabetics (b)	19.07±4.1	47.12±6.1 ^C	46.26±7.1 ^{acc}	162.16±7.5	161.23±8.3 ^C	541.78±8.8 ^{acde}	
Diabetes + <i>Aloe vera</i> (c)	-	21.92±6.3 ^{Abc}	29.01±9.4 ^b	-	651.93±8.5 ^{Abdc}	341.03±5.1 ^{abde}	
Diabetic + insulin (d)	-	35.12±7.8	32.09±3.1	-	170.57±9.1 ^C	122.9±5.2 ^{bc}	
<i>Aloe vera</i> (e)	-	43.09±2.8 ^C	29.88±7.7 ^b	-	81.31±6.1 ^c	63.09±5.5 ^{bc}	

rats shows a significant increase compared with the control group who received insulin and the control rats (Table 2). The thiol proteins levels have a significant decrease in the diabetic and *Aloe vera* recipients compared with the control group, the control group who received *Aloe vera* and the diabetic rats, also on day 30. A significant increase was observed in thiol proteins levels in diabetic rats compared to the control

group, the control group who received *Aloe vera* and the diabetic recipients of *Aloe vera* (Table 2). A significant increase of Glutathione peroxidase was also observed in diabetic rats that received *Aloe vera*. On day 15 and 30, after the diabetes induction a significant increase of this factor was observed in diabetic and diabetic recipients of *Aloe vera* compared to the other groups (Table 2).

The calculated amounts of the FSH, LH

Table 4. Average of FSH, LH and testosterone hormones in rats. Letters a, b, c, d, e in each column indicate significant differences at $p \leq 0.05$.

Hormones Groups	FSH (mIU/ml)			LH (mIU/ml)			Testosterone (ng / dL)		
	The first day of diabetes	15 days after diabetes	30 days after diabetes	The first day of diabetes	15 days after diabetes	30 days after diabetes	The first day of diabetes	15 days after diabetes	30 days after diabetes
Control (a)	406.35±6.3 B	439.91±8.38 b	499.21±6.81 b	8.18±0.62 b	8.56±0.81 B	7.38±0.63 b	9.38±0.47 b	10.2±0.96 b	9.01±0.07 b
Diabetics (b)	251.55±4.63 A	250.34±6.45 ac	268.48±9.98 a	3.3±0.19 ^a	3.41±0.59 acde	3.34±0.18 acde	4.75±0.84 a	4.85±0.39 a	4.58±0.78 ac
Diabetes + <i>Aloe vera</i> (c)	-	307.41±9.2	400.79±6.5	-	7.15±16 ^B	8.34±0.38 b	-	7.66±0.92	7.64±0.23
Diabetic + insulin (d)	-	367.19±9.63	394.81±7.94	-	7.33±0.26 B	7.93±0.58 b	-	7.46±0.85	8.3±0.46
<i>Aloe vera</i> (e)	-	419.75±7.7 ^b	365.47±9.5	-	8.47±0.53 B	7.37±0.18 b	-	8.75±0.77	9.51±0.43 b

and testosterone in serum blood were significantly different in the first 15 and 30 days after the development of diabetes. FSH levels on the first day of diabetes in diabetic rats had a significant reduction compared to the control group. On day 15 after diabetes induction, FSH decreased in diabetic rats and this reduction can be considered meaningful in control group and recipients of *Aloe vera* (ml/ miu). Moreover, on day 30 after diabetes induction FSH levels reduction was statistically significant in diabetic group compared with the control group (Table 3).

In the first day of diabetes induction a significant decrease was observed in LH levels in blood serum of diabetic rats. LH reduction on day 15 and 30 after diabetes induction was statistically significant in diabetic rats compared to the other groups (Table 3).

In the first day of diabetes induction a significant decrease was observed in testosterone levels, of the diabetic rats compared to the control group. Testosterone reduction on day 15 of diabetes induction was also statistically significant in diabetic rats. On day 30 of the diabetes induction, a significant decrease in testosterone level was observed in

the diabetic group (Table 3).

Discussion

The increased blood glucose levels in diabetes leads to structural and functional changes in tissues and organs, including reproductive organs (Cai, 2000). In this study, the mean glucose levels in streptozotocin induced diabetic rats significantly increased in diabetic rats compared to the control group and that is because of the reduction of β -cells in the pancreatic islets after the onset of diabetes. A significant decrease was observed in the amount of blood sugar of the diabetic rats that received insulin and the diabetic rats which received *Aloe vera* with doses of 400 mg/kg for about 15 and 30 days after diabetes induction. Josias and colleagues (Josias, 2008) and Rajasekaran et al (Rajasekaran, 2006) administered *Aloe vera* gel with dosage of 300 mg/kg orally per day for rats for 21 days and reported a significant reduction in the glucose levels of their blood which is consistent with the findings of the present study. Helal et al (Helal, 2003) and Noor et al in 2008 achieved similar results about blood sugar reduction after *Aloe*

vera administration (Helal, 2003; Noor, 2008). Noor et al in 2008 stated that blood sugar reduction after using *Aloe vera* may be due to the prevention of the pancreatic β -cell death by the *Aloe vera*, or by a similar function of insulin, through the glucose uptake by peripheral tissues, and inhibiting glucose production via gluconeogenesis in the liver and muscles (Noor, 2008).

In this study, serum levels of FSH, LH and testosterone in the blood of rats in different groups were compared. In this case a significant decrease was observed in serum levels of FSH, LH and testosterone, in diabetic rats which can be due to the increased oxidative stress after diabetes induction. Furthermore, it was observed that the amount of LH in the serum of diabetic rats that received *Aloe vera* increased compared to other diabetic rats. This can indicate *Aloe vera* potentials to reduce the damaging effects of diabetes and its antioxidant effects. Ricci and colleagues reported the reduction of plasma levels of testosterone following diabetes and stated that it is affected by the reduction in the number of Leydig cells and an increase in oxidative stress after diabetes induction (Ricci, 2009). They also stated that the reduction of testosterone is responsible for the changes in epithelium of tubules in diabetic animals. Stephan and colleagues stated that the Leydig cell reduction and morphological changes that occur in the testis of the diabetic rats was a result of the increased oxidative stress induced by hyperglycemia and the reduction of the antioxidant defense (Stephan, 2007).

Howland and Zebrowski reported that serum testosterone level in diabetic rats was significantly reduced compared to the control group. They also reported that the concentrations of FSH and LH increased in the

pituitary gland of diabetic rats (Howland and Zebrowski, 1976). Ballester et al reported that the reduction of serum levels of FSH, LH, testosterone and insulin subsequent to diabetes (Ballester, 2004). These results are compatible with the findings obtained in this study. In this study biochemical factors such as malondialdehyde, thiolprotein, glutathione peroxidase and total antioxidant activity were examined in order to investigate the role of *Aloe vera* in dealing with the oxidative stress which is caused by diabetes. In this study it was observed that the level of malondialdehyde in the first, 15 and 30 days of induction in diabetic group significantly increased compared to the control group by stimulating the immune system to cope with the oxidative stress (Ballester, 2004). These results were compatible with the results obtained from Stephen et al's study which measured the malondialdehyde levels 60 days after diabetes induction. They also stated that the oxidative stress-induced hyperglycemia after diabetes induction decreases antioxidant defense and increases the malondialdehyde (Stephan, 2007). Mohaseb and colleagues noted the increase in amount of malondialdehyde 8 weeks after diabetes induction (Mohaseb, 2010).

It was also observed that the thiol protein, the glutathione peroxidase and the total antioxidant activity increased significantly in the diabetic rats compared with control group. This fact confirms the stimulation of the immune system to cope with oxidative stress induced by hyperglycemia after diabetes induction. The higher levels of antioxidant activity and other biochemical factors that are involved in the elimination of the reactive oxygen types (ROS) in the diabetic rats which received *Aloe vera* shows *Aloe vera* potentials and its antioxidant com-

pounds, and further, the stimulation of the immune system that could lead to a reduction in the damaging effects of diabetes, following *Aloe vera* administration.

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ارزیابی فعالیت آنتی اکسیدانی آلوئه ور^۱ و هورمون‌های هیپوفیزی جنسی متعاقب دیابت تجربی در موش‌های صحرایی نر

محمد امین بهمنش^{۱*}، نعیم عرفانی مجد^۱، علی شه‌ریاری^۲، حسین نجف زاده ورزی^۳

(۱) بخش بافت شناسی گروه علوم پایه، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران

(۲) بخش بیوشیمی گروه علوم پایه، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران

(۳) بخش فارماکولوژی گروه علوم پایه، دانشکده دامپزشکی دانشگاه شهید چمران اهواز، اهواز، ایران

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چکیده

زمینه مطالعه: دیابت یک بیماری متابولیکی است که اغلب با افزایش قند خون و ناباروری همراه است مطالعات قبلی نشان می‌دهد که آلوئه ور^۱ ممکن است اثرات مثبتی بر قند خون و باروری داشته باشد. **هدف:** مطالعه اثر آلوئه ور^۱ بر فعالیت اکسیدان/ آنتی اکسیدانی سرم و هورمون‌های جنسی هیپوفیزی متعاقب دیابت می‌باشد. **روش کار:** ۶۰ موش نر و بیستار به ۵ گروه تقسیم شد، ۳ گروه از موشها با ۶۵ mg/kg استرپتوزوتوسین داخل صفاقی دیابتی شدند. یک گروه از دیابتی‌ها ژل آلوئه‌ورا ۴۰۰ mg/kg با گاوآز تا ۳۰ روز، گروه دیگر ۱۰ واحد انسولین و ۴۰۰ mg/kg دریافت نمود و گروه دیگر چیزی دریافت ننمود، در پایان هورمونهای جنسی و فاکتورهای استرس سرم ارزیابی گردید. **نتایج:** FSH, LH و تستسترون متعاقب دیابت کاهش یافتند. GPX, AOA, MDA و thiolprotein در گروه دیابتی نسبت به شاهد افزایش معنی دار داشت. موش‌های دیابتی دریافت کننده آلوئه ور^۱ به طور معنی‌داری دفاع آنتی اکسیدانی زیادی نشان دادند. **نتیجه‌گیری نهایی:** آلوئه ور^۱ با افزایش دفاع آنتی اکسیدانی میتواند اثرات مخرب استرس اکسیداتیو ناشی از دیابت را بکاهد.

واژه‌های کلیدی: آلوئه ور^۱، آنتی اکسیدان، دیابت، هورمون‌های هیپوفیزی، موش صحرایی

(* نویسنده مسؤول: تلفن: +۹۸(۲۱)؟ نمابر: +۹۸(۲۱)؟ Email: behmanesh.ma@yahoo.com