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Aloe Vera Gel Protects Ovarian Structure in Diabetic Rat

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Abstract: Structure and function of many organs alters in diabetic status. Ovary is important organ of reproductive system and its structure influenced in diabetes. Some antidiabetic and protective plants by correction of hyperglycemia and its disturbances including oxidative stress may decrease the side effect of *diabetes mellitus* on ovary. *Aloe vera* has hypoglycemic effect. Thus, in present study, its protective effect on rat ovary structure was evaluated. One group of mature female rats was kept as control group. Experimental diabetes was induced by streptozotocin (45mg/kg) in two groups of rats. One of these groups was daily treated by *Aloe vera* gel (300 mg/kg) for 4 weeks. After 4 week, rats sacrificed and their ovaries were removed. The 5-6µ sections were made using paraffin embedding techniques and stained by hematoxylin-eosin and PAS. The numbers of primordial, primary, secondary and tertiary follicles were significantly decreased and atretic follicles significantly increased in diabetic rats with comparison to control group. The secondary and tertiary follicles diameters were significantly decreased in diabetic rats. The ovary structure was supported in diabetic rats by *Aloe vera* gel administration. The number and diameter of secondary and tertiary follicles were increased. Also *Aloe vera* gel normalized blood glucose and increased weight of rats and ovary. The results shown that *Aloe vera* could have beneficial and supporting effects on ovarian tissue and folliculogenesis if used as a hypoglycemic agent in diabetes.

Key words: Diabetes Mellitus · Ovary Structure · *Aloe Vera* Gel

INTRODUCTION

Diabetes is a complex and multifarious group of disorders characterized by hyperglycemia [1]. Diabetes is associated with increased risk of disease such as neuropathy and cardiovascular disorders, but it is also linked to reproductive problems such as spontaneous abortions, neonatal morbidity and mortality, congenital malformation and poor embryo development [2, 3]. Diabetes causes alterations in the timing of the estrous cycle, associated with modifications in ovary function, which induces a decrease or even absence of ovulated oocytes and oocyte maturation in female rats [3-5]. Suppressed ovarian folliculogenesis and steroidogenesis [6], enhanced endometrial adiposity and hypo-vascularization [7] as well as utero-ovarian compartmental metabolic shifts from normal oxidative to lipogenic non-oxidative dominance [7], promote female reproductive incompetence [8].

Some chemical drugs such as biguanides and sulfonylureas are currently available to reduce hyperglycemia in *diabetes mellitus* [9]. These drugs have side effect and thus search for new drug/compound is essential [1, 10]. Many herbs and plant products have been shown to have hypoglycemic action. This leads to increasing demand for herbal products plant with antidiabetic activity and lower side effects [11-14]. *Aloe vera* is one of these antidiabetic plants [15]. This plant has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. Clinical evaluations have revealed that the pharmacologically active ingredients are concentrated in both the gel and rind of *Aloe vera* leaves [16]. Polysaccharide containing plants which *Alo barbadensis* is also among are used in various diseases as anti-inflammatory, antiulcer, antineoplastic and in wound healing and against hepatitis [17]. It is known that it is activating macrophages and has also antiviral effect [18]. In some studies it is shown that

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Aloe has an antioxidative effect. Its antigenetoxic and chemopreventive effects are also proven [19, 20]. The level of blood glucose was significantly decreased after oral administration of ethanolic extract of *Aloe vera* gel in STZ-induced diabetes [16].

The aim of present study was evaluation of protect effect of *Aloe vera* gel on ovarian paranchyma of diabetic rats.

MATERIALS AND METHODS

Aleo vera gel was prepared by Barij Esance Co. (Iran). Female Wistar rats (with weight 200-210 gram) were obtained from animal house of Jondishapor University (Ahwaz, Iran). The animals were kept in an experimental room for one week, for acclimatization to experimental conditions with 12 hour light and dark cycle. The animals were fed at laboratory chow and water *ad libitum*. The rats were divided randomly in three groups (5 rats in each group).

Group A: Control group.
Group B: diabetic group were received stereptozotocin (STZ) (Sigma) in 45mg/kg, intravenously.
Group C (AV group): diabetic rats (blood glucose up 250 mg/dl) were treated orally with *Aleo vera* gel (300 mg/kg) for 4 weeks, once a day.

All the rats were synchronized by PMSG (Fariman Co. Iran) and HCG (Intervet Holand) (40IU/rat) one week before the sampling. Body weight was taken before and after experiment. Blood glucose was also measured on 0 and 28th day of experiment. The rats sacrificed after 4 weeks and the right ovary removed and samples were taken.

The ovarian samples were fixed in 10% formalin saline. The 5-6µ sections were made using paraffin embedding techniques and stained by hematoxylin-eosin and PAS.

Addition to ovarian structures the cell follicles layers and their structure were used for follicular classification. The primary oocyte which surrounded by a simple squamous epithelium classified as primordial follicle. When the primary oocyte surrounded by a simple cuboidal epithelium as a primary follicle. Secondary follicles have a stratified epithelium of granulosa cells. In tertiary follicles the antrum was developed [21]. Follicle was considered atretic, if two or more pyknotic granulosa cells were found on the same section. The numbers of primordial, primary and secondary follicles were counted in 40 and tertiary follicle was counted in 10 microscopic magnifications. Histometrial studies were done using digital microscope and Dino-capture soft ware.

Statistical Analysis: SPSS version 16 was used for statistical analysis. Groups variance were analyzed by one-way Analysis of Variation (ANOVA) and Fisher least significant difference test (LSD) was tested for significant differences between groups. P = 0.05 was considered statistically significant.

RESULTS

The result showed that the streptozotocin (45 mg/kg) induced *diabetic mellitus* in rats and blood glucose reached to 410. 40 ± 35.38 mg/dl. Administration of *Aloe vera* (300 mg/kg) reduced the blood glucose significantly so that it reached to 99.80 ±9.9 mg/dl on the end of 4 weeks ($p<0.0001$). While the blood glucose of untreated rats were 413.00 ±72 mg/dl (Fig. 1).

The mean of weight in diabetic rats were decreased significantly from 210±10 to 140.20±3.47gram ($p<0.0001$). The weights of diabetic rats significantly increased ($p<0.0001$) following administration of *Aloe vera* (226± 1.87 grams) (Fig. 2).

The ovaries of diabetic rats were smaller than control and AV groups. The ovary weight was decreased in diabetic rats. This decreasing was significant compared to control group ($p<0.0001$). The ovary weight was increased in AV group (18.43%) insignificantly compared to diabetic group (Fig. 3).

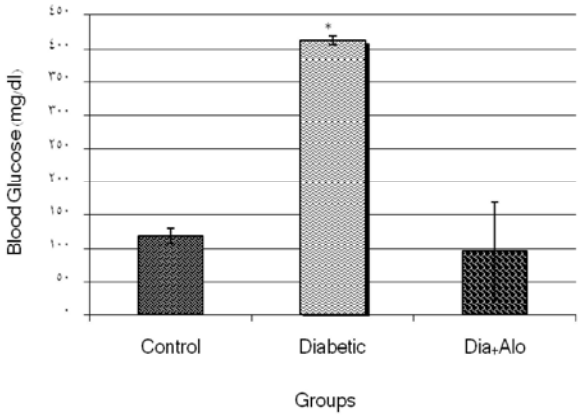

Fig. 1: Mean (±E.S.) of blood glucose after 4week treatment in control, treated and diabetic rats.* represents significant difference between diabetic rats and control or treated groups ($p<0.05$). n=5

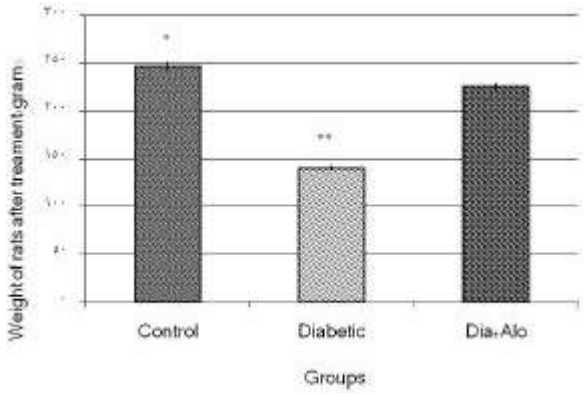

Fig. 2: Mean (±E.S.) of weigth of rats after 4week treatment in control, treated and diabetic rats.* represents control or treated groups($p<0.05$). ** shows significant difference between diabetic rats and other group($p<0.05$). n=5

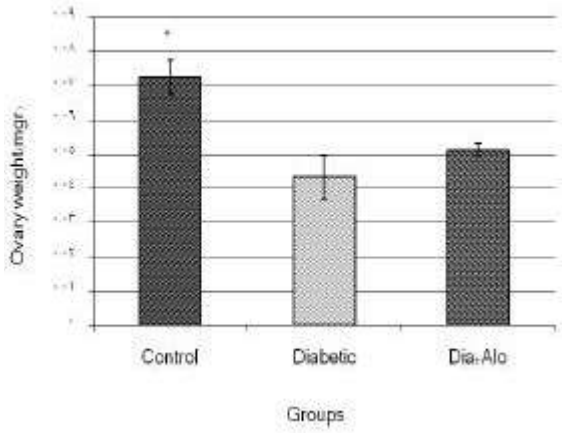

Fig. 3: Mean (±E.S.) weight of ovary after 4week treatment in control, treated and diabetic rats.* represents significant difference between control rats and diabetic or treated groups ($p<0.05$). n=5

Table 1: The mean (± S.E.) number of ovarian follicles in control, diabetic and *Aloe Vera* treated diabetic rats

Groups	Primordial X40	Primary X40	Secondary X40	Tertiary X10	Atresia X40
Group A (control)	17.40±2.25bc	8.80±1.11c	7.40±1.12 bc	3.00±0.44 b	1.60±0.24 b
Group B (diabetic)	9.40±1.54 a	7.20±0.97c	2.60±0.24ac	1.20±0.20ac	5.20±0.91ac
Group C (diabetic+ *Aloe vera*)	11.20±1.77a	14.60±1.03ab	16.80±2.31ab	3.40±0.24 b	1.20±0.20 b

Variable letters show significant difference between groups ($p<0.05$)

Table 2: The mean (± S.E.) diameter (μ) of ovarian follicles in control, diabetic and diabetic rats which treated by *Aloe vera.*

Groups	Primordial	Primary	Secondary	Tertiary	Atresia
Group A (control)	19.45±0.66	32.16±2.47	202±34.22 b	553.72±77.63 bc	367.84±122.20
Group B (diabetic)	17.58±0.49 c	32.49±3.21	84.718±14.90 ac	291.62±46.06 ac	333.43±23.24
Group C (diabetic+ *Aloe vera*)	21.81±1.32 b	37.94±2.79	241.65±18.17 b	881.67±97.32 ab	154.08±24.12

Variable letters show significant difference between groups ($p<0.05$)

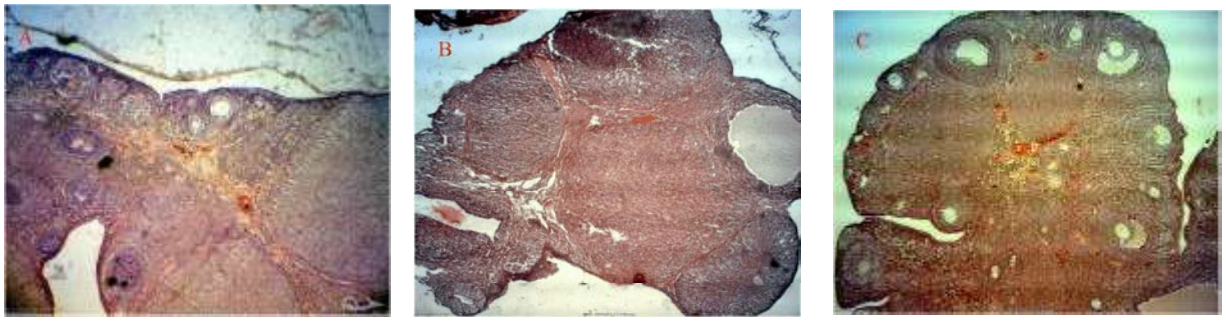

Fig. 4: A. Histological features of the ovaries in control group (X 3.2, H&E). B. Diabetic rats: ovarian structure severely affected. The numbers of growing follicles dramatically were decreased (X 3.2, H&E). C. Diabetic rats which treated by *Aloe vera*. The cortex shows active paranchyma with all type of follicles (X 3.2, H&E)

Histometrical Changes: Histological study showed that the total numbers of growing follicles were decreased in diabetic ovaries, but the total numbers of follicles were increased significantly by administration with *Aloe vera* (Fig. 4). The numbers and diameter of ovarian follicles in different groups were showed in Table 1 and 2.

The mean of primordial follicles number were significantly decreased in diabetic rats ($p=0.004$), but in treated diabetic rats the primordial follicles were more (19.14%), but didn't return to normal status. The diameter of this follicle significantly increased in AV group ($p=0.006$), (Fig. 7 A).

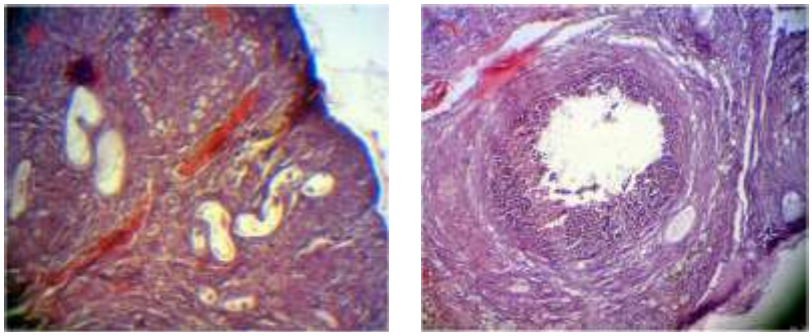

Fig. 5: A & B: Atresia follicles in diabetic ovaries. Disjunction of granulosa cells are noticeable (X 10, H&E)

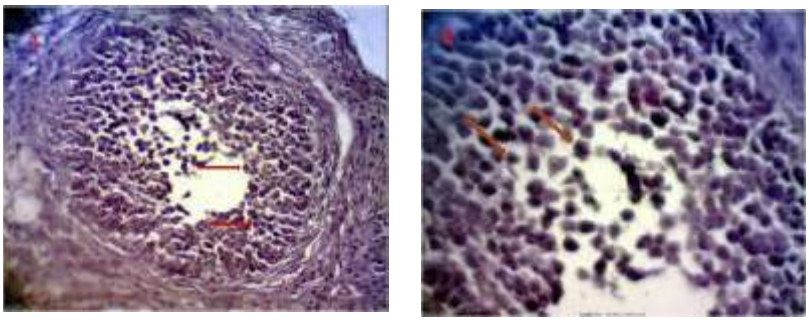

Fig. 6: A: Atresia follicle with picnotic nucleus in granulosa cells layer (*Arrow*), (X 20, H&E). The A photomicrograph as the more Magnification (X 40, H&E)

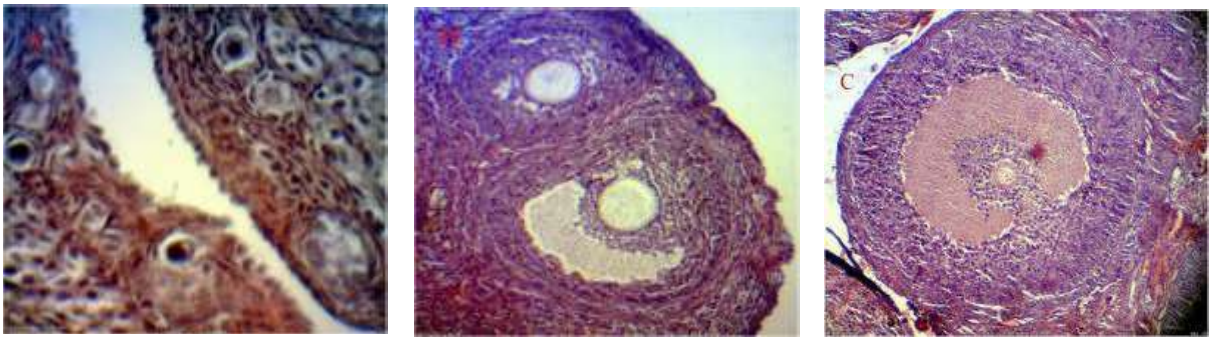

Fig. 7: A. Normal primordial and primary follicles are observed in AV group (X 40, H&E). B: Normal secondary follicles are observed in AV group (X 10, H&E). C: Normal tertiary follicle is observed in AV group (X 10, H&E)

The number of primary follicles decreased in diabetic rats (18.18%), but this decreasing was not significant in comparison to control group. The number of primary follicles significantly increased in AV group (p=0.001). The mean of diameter of primary follicle did not differ between groups (Fig. 7 A).

The number of secondary follicles significantly decreased in diabetic rats (p=0.030), but in *Aloe vera* gel administration diabetic rats increased, however this increasing was significant in comparison with other groups (p<0.0001). The mean of secondary follicles diameter significantly decreased in diabetic rats (p=o.oo5), but in diabetic rats which treated by *Aloe vera* gel significantly increased (p=0.001), (Fig. 7 B).

The tertiary follicles number in diabetic rats were significantly decreased (p=0.043). Administration of *Aloe vera* prevented loses of this follicle, while the number of tertiary follicles significantly increased compared to diabetic rats (p=0.016). The diameter of tertiary follicles were similar to secondary follicles but AV group differed with control group significantly (p=0.011), (Fig. 7 C).

Atretic follicles significantly increased in diabetic rats (p<0.0001) but in AV group it was significantly decreased (p<0.0001), (Fig. 5, 6, 8, 9, 10 and 11).

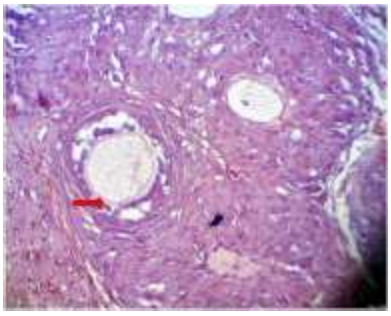

Fig. 8: Differential between zona pellucida in normal secondary follicle (*Bold arrow*) and atresia follicle (*Thin arrow*), (X 20, H&E)

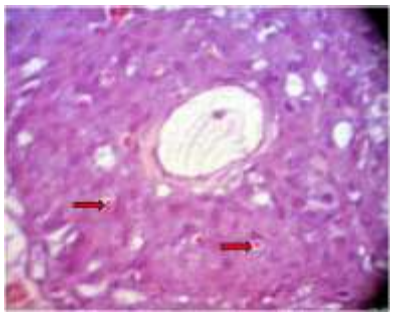

Fig. 9: Atresia follicle with RBC in granulosa cells layer (X 40, H&E)

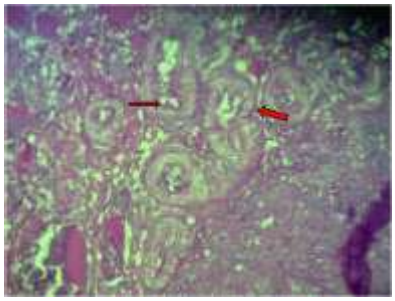

Fig. 10: Atresia follicles with irregular and thicken zona pellucid (*Thin arrow*) and basal lamina of granulosa cells (*Bold arrow*), (X 20, PAS)

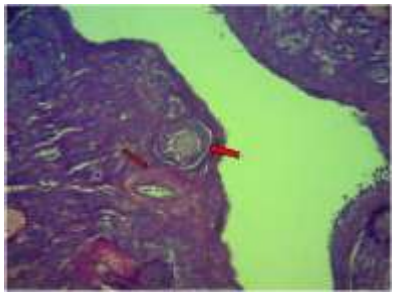

Fig. 11: Atresia follicle comparison with normal follicle (*Bold arrow*). *Thin arrow*: show fold and thicken basal lamina of granulosa cells layer in atresia follicle (X 20, PAS)

DISCUSSION

In recent years, various plant extract have been claimed to be useful for the cure of *diabetes mellitus*, but few of them were tested for their effects on body tissue of diabetic patient. In present study, we investigated the antidiabetic effect of *Aloe vera* gel on ovary structure in STZ-induced diabetic rats. STZ is a compound commonly used for the induction of type I diabetes in experimental rats. STZ caused diabetes by rapid depletion of B cell in pancreas langerhance island, which leads to a reduction of insulin release. In our studies, oral feeding of *Aloe vera* gel reduced blood glucose level by 76.56%. Noor *et al*. [1] and Bolkent *et al*. [14] reported the antidiabetic effect of *Aloe vera* in diabetic mice induced by alloxan (500 mg/kg, twice daily) [14]. Helal *et al.* [21] and Rehman *et al*. [22] reported that the blood glucose is decreased in alloxan diabetic rats which treated by *Aloe vera* for 30 days [21, 22]. The period of these treatments were similar to present study. Rajasekaran *et al.* [16] and Noor *et al.* [1](2008) were observed that oral receiving of *Aloe vera* (300 mg/kg, daily) to diabetic rats reduced the blood glucose levels [1, 16]. These results confirmed our results. AV treatment for 21 days showed potential hypoglycemic activity in oral glucose tolerance test and antidiabetic activity in alloxanized rats [22].

Aloe vera may exert its antidiabetic effect by supporting and maintenance the death of β cells or it may permit recovery of partially destroyed β cells [1]. Also, the hypoglycemic action of the extract of herbal plants may be possible through the insulinomimictic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles [23]. Rajasekaran *et al*., (2006) reported that, the increased levels of plasma insulin indicate that the *A. vera* gel extract stimulates insulin secretion from the remnant β-cells or from degenerated β-cells [24].

Diabetes is characterized by weight loss [5, 23, 25-27] and it was also seen in this study. The body weight decreasing was prevented by administration of *Aloe vera*. This effect of *Aloe vera* was reported in limited studies including [1, 22].

The ovary weights in diabetic rats were decreased significantly, while *Aloe vera* prevented this reduction. Tatewaki *et al.* [28] demonstrated that ovary weight was reduced in diabetic mice [28].

The mean of primordial, secondary and tertiary follicles number decreased significantly in diabetic rats compared to control group. Atretic follicles increased significantly in diabetic rats. Administration of *Aloe vera* prevented loses of primordial, primary, secondary and tertiary follicles and atretic follicles were decreased significantly.

Also Cox *et al*. [29]demonstrated also the follicular diameter of diabetic gilts tended to be smaller than that of control and the numbers of atresian follicles were higher in diabetic gilts [29]. Ovarian dysfunction in diabetes mellitus may be associated with imbalanced glucose utilization, follicular atrophy and impaired steroidogenesis [6]. Tatewaki *et al.* [28] observed the percentages of the primary follicles decreased in diabetic mice [28]. Ballester *et al*.[5] reported that some degenerative signals of ovarian structures caused to observed small cells, with picnotic nuclei spread in the ovary, scavenger cells in follicles, an increasing the intracellular vacuoles in follicles and corpora lutea in diabetic rat ovary [5].

Deficiency of insulin is one of the determinant factors that influence ovary structure in diabetes and, thus, the *Aleo vera*-induced recovery of serum insulin levels would be one of the mechanisms involved in the improvement ovary structure and function [24]. Oxidative stress, through the production of free radices has been implicated in the progression of long-term diabetes complications including microvascular and macrovascular dysfunction [10]. Anti-oxidants such as phenolic compounds and saponins in the gel extract of *Aloe vera* may be responsible, in part, for its protective effect on ovary structure in diabetic rats.

CONCLUSION

The results of present study showed that *Aloe vara* brings back the blood glucose and body weight to normal in the diabetes rats. After treatment with *Aloe vera*, the numbers of normal follicles were increased and atretic follicle reduced significantly. It is shown that *A. vera* could have a beneficial and supporting effects on ovarian tissue and folliculogenesis if used as a hypoglycemic agent in diabetes.

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