

An investigation on the effects of the Aloe Vera extract on the thickness of the retina in male diabetic rats

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Summary

Retina is a part of the central nervous system derived from the neuroectoderm and made up of 5 layers. In this study, the changes in the thickness of the retinal layers as a consequence of diabetes and effect of Aloe Vera gel extract in male rats were assessed. Thirty Sprague Dawley adult male rats (175 ± 25 g) in two age groups (4 and 8 weeks) were divided into 6 groups of 5 as control, diabetic and diabetic receiving 400 mg/kg Aloe Vera extract. Diabetes was induced by IP injection of 50 mg/kg of STZ (streptozotocin). The animals were weighed and their blood sugar was measured by glucometer before STZ administration and 24 h thereafter. Animals were anesthetized with sodium thiopental (40 mg/kg) via IP injection. After removal of eyes on both sides, retina was dissected out precisely and fixed in 4% glutaraldehyde, post fixed in osmium tetroxid 1%, dehydrated and then embedded in TAAB resin. Thin sections (1 µm) were stained with toluidine blue stain and viewed under light microscope. Ten slides were prepared from each animal. The results revealed reduction of the blood glucose levels and body weight in treated rats in comparison to diabetic groups. The thickness of neural retina and its layers were different as well. In the group treated with Aloe Vera the thickness of retina and its layers retained their normal histologic structures.

Key words: Diabetes, Aloe Vera, Retina, Streptozotocin

Introduction

The eyeball basically consists of three layers. Internally is the nervous layer or the retina, which developmentally and functionally is an isolated part of the central nervous system, to which it remains connected by a tract of nerve fibers, the optic nerve (Lesson *et al.*, 1988). The retina in mammals consists of five layers from the outer area to the inside as follows:

- 1) Outer nuclear layer (ONL): Mainly occupied by a nucleus of rod and cone cells.
- 2) Outer plexiform layer (OPL): Axon-dendritic synapses among photoreceptor cells and bipolar, amacrine and horizontal cells are located in this layer.
- 3) Inner nuclear layer (INL): Nucleus of bipolar, amacrine, horizontal and müller cells form this layer.
- 4) Inner plexiform layer (IPL): Synapses of

bipolar cell's axons and ganglion cells dendrites are located in this layer.

5) Ganglion cell layer (GCL): Bodies of ganglion neurons are located in this layer (Banks, 1993; Dellmann and Eurell, 1998; Esfandiari *et al.*, 2008).

Diabetic retinopathy is the most common complication of diabetes mellitus. This condition is a leading causative etiology for the vision loss in human being (King *et al.*, 1994; Vlassara *et al.*, 1994). Induced hyperglycaemia in the earliest stage of diabetes cause tissue damage in different ways. The retina, as part of the central nervous system, uses glucose as an exclusive energy source for dynamic activity such as for capturing images and for primary visual processing (Park *et al.*, 2003). The glucose used for retinal neuronal activity is transported from the blood by two vasculatures: deep and superficial layers of the retina's own blood vessels, and by the

capillaries of the choroid (Essner and Gordon, 1983; Gariano *et al.*, 1994). Therefore, systemically impaired glucose metabolism causes dysfunction in the neural retina soon after the onset of diabetes (Park *et al.*, 2003). Impaired glial reactivity and apoptotic cell death of retinal ganglion cells have also been observed in cases of short-term experimental diabetes and in diabetic human being (Barber *et al.*, 1998; Lieth *et al.*, 1998). Chronic hyperglycaemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (Singh *et al.*, 2010). Park *et al.* (2003) compared the thickness of different layers of retina in the diabetic and normal rats. Martin *et al.* (2004) reported that, by 10 to 12 weeks, however, the thickness of retinas of diabetic mice was significantly less than that in age-matched control mice.

Hindustan *et al.* (2010) reported that the natural herbs for diabetes treatment focus on lowering blood sugar and reducing the damaging effects of the disease. According to Zohary and Hopf (2000), aloe gum extract effectively increases glucose tolerance in both normal and diabetic rats. In this investigation, our aim was to find out the probable changes of the thicknesses of the total retina and all its layers in treated groups by Aloe Vera in comparison with healthy (control) and diabetic groups.

Materials and Methods

Animals

Thirty mature male rats weighing 175 ± 25 g were obtained from Razi Vaccine and Research Institute and kept in a standard condition with 25°C temperature, 12 h light and 12 h dark and fed on standard rat ration, watered with tap water *ad libitum*. The animals were divided into 6 groups of 5 rats each. Group 1 and 2 (healthy control) just received food for 4 and 8 weeks, and group 3 and 4 (diabetic rats) common food for 4 and 8 weeks. Group 5 and 6 (diabetic) in a period of 4 and 8 weeks, food plus 400 mg/kg Aloe Vera extract once daily.

All studies were performed in accordance with the National Institute of Health's Guide for the care and use of

laboratory animals. The body weight was measured at 4 and 8 weeks in all experimental groups.

Experimental induction of diabetes in rats

For inducing diabetes in rats a single dose of streptozotocin (50 mg/kg BW) (SIGMA Aldrich, USA) was injected through IP route. Fasting blood glucose was assessed by glucometer (ACCUCHEK® Active Roche, Germany) before and 24 h after injection for diabetes approval and 10 days after injection for diabetes consolidation. At the end of experiment, the animals were anesthetized for collection their eyes. The retina was collected for tissue processing following dissection of the eyes. Finally, the animals were euthanized by thiopental sodium overdose (40 mg/kg).

Preparation of Aloe Vera gel extract

Aloe Vera powder was prepared from Aloe Vera leaf gel according to the published procedure (Rajasekaran *et al.*, 2005) with slight modifications. Mature, healthy and fresh leaves of Aloe Vera having a length of approximately 75 to 90 cm were washed with fresh water. The leaves were cut transversely into pieces. The solid gel in the center of the leaf was homogenized. The resulting mucilaginous, thick and straw colored homogenate was lyophilized. Then the lyophilized sample was extracted using 95% ethanol and water. The filtrate was collected and evaporated in a rotary evaporator (Anamis Aloe Vera Iran).

Preparation of tissue for microscopy

The separated retina was immersed in glutaraldehyde 4% for 3-4 h. Retinal tissue was rinsed with buffer, post fixed in osmium tetroxid 1% and dehydrated through a graded ethanol series.

The tissue was then placed in a mixture of propylen oxide and TAAB resin (1:1) (TAAB 812, DDSA, MNA, DMP30) and transferred in pure resin. Semithin sections were prepared (Microtome: C. reichert, Austria om U3) and 10 slides were prepared from each animal (Bozzola and Russell, 1999).

The following histomorphometric studies (Dino software) were carried out in all the groups.

- A) Total thickness of the retina
- B) Thickness of PSL, ONL, OPL, INL, IPL and GCL layers

Statistical analysis

The statistical analysis was performed with factorial analyses of variance in SAS (2003), and Tukey's test. Statistical comparison was performed at 4 and 8 weeks separately and $P < 0.05$ was considered significant.

Results

The changes of body weight and fasting blood glucose in different experimental groups at 4 and 8 weeks after diabetic induction are shown in Tables 1 and 2. Fasting blood glucose was significantly ($P < 0.05$) increased at both 4 and 8 weeks

post-diabetic induction. Treatment with Aloe Vera extract caused a remarkable reduction in elevated fasting blood glucose.

Body weight was slightly decreased at 4 and 8 weeks after the onset of diabetes compared to the control and treated groups. However, these differences were not statistically significant.

The thickness of whole retina and its layers in different groups at 4 and 8 weeks post-diabetic induction was measured with micrometry method and is shown in Table 3.

At 4 weeks of age the thickness of whole retina, PSL, ONL and INL layers were increased after treatment with Aloe Vera gel extract. In comparing the thickness of the whole retina and its layers between diabetic and control groups, there were slight decreases in the whole retina, ONL, OPL and INL, which were not significant.

The thickness of the whole retina, PSL, OPL, INL and IPL layers were decreased at

Table 1: Effect of treatment with hydroalcoholic extract of the Aloe Vera on fasting blood glucose (mg/dl) and body weight at 4 weeks (Mean±SD)

Group	Body weight (g)	Fasting blood glucose concentration (mg/dl)
Control	202.4 ± 11.41	113 ± 8.36 ^a
Diabetic	195.2 ± 24.2	414.6 ± 196 ^b
Diabetic + Aloe Vera	171.4 ± 57.13	231.8 ± 84.38

^{a, b} Means±SD within each column at 4 weeks with different letters differ significantly ($P = 0.009$)

Table 2: Effect of treatment with hydroalcoholic extract of the Aloe Vera on fasting blood glucose (mg/dl) and body weight at 8 weeks (Mean±SD)

Group	Body weight (g)	Fasting blood glucose concentration (mg/dl)
Control	194 ± 19.49	114.6 ± 11.21 ^a
Diabetic	180.6 ± 49.2	413 ± 237 ^b
Diabetic + Aloe Vera	168 ± 34.92	229 ± 138

^{a, b} Means±SD within each column at 8 weeks with different letters differ significantly ($P = 0.02$)

Table 3: The thicknesses (µm) of the whole retina and its layers in male rats (Mean±SD). R: Whole neural retina, GCL: Ganglionic cell layer, IPL: Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, and PSL: Photoreceptor segment layer

Layers	Groups					
	4 weeks after the start of experiment	8 weeks after the start of experiment	4 weeks after diabetes induction	8 weeks after diabetes induction	4 weeks after diabetes induction	8 weeks after diabetes induction
	Control (n=5)		Diabetic (n=5)		Diabetic + Aloe Vera (n=5)	
R	129.19 ± 10.6	118.52 ± 3.8	123.44 ± 1.6	117.04 ± 3.7	139.31 ± 1.5	118.7 ± 4.3
GCL	10.75 ± 4.2	10.74 ± 4.2	10.76 ± 1.5	10.76 ± 1.3	10.75 ± 1.2	10.75 ± 2.5
IPL	32.27 ± 11.8	32.27 ± 5.6	32.3 ± 1.7	21.48 ± 4.6	32.25 ± 3.1	21.51 ± 2.6
INL	21.53 ± 6.1	21.47 ± 6.1	10.74 ± 1.3	18.81 ± 3.6	16.09 ± 2.9	21.54 ± 2.1
OPL	10.82 ± 3.4	10.82 ± 2.5	10.75 ± 0.93	5.36 ± 1.2	10.73 ± 0.69	10.77 ± 4.6
ONL	32.26 ± 2.8	21.5 ± 4.2	32.23 ± 1.3	37.6 ± 2	37.57 ± 1.3	32.27 ± 2.7
PSL	21.54 ± 8.5	21.53 ± 3.3	26.85 ± 2.2	21.48 ± 2.7	32.29 ± 2.1	21.49 ± 5.3

n: Number of animals in each group

8 weeks after induction of diabetes, and were not significant. In comparing diabetic and treated groups the thicknesses of whole retina, PSL, OPL, INL and IPL layers were increased at 8 weeks.

The thickness of the whole retina, PSL, OPL and IPL layers were decreased at 4 and 8 weeks in diabetic group, but there were no significant differences between them.

The thickness of OPL, INL and GCL layers at 4 and 8 weeks in treated group were increased. There were no significant differences.

The retinal structure is well organized into layers in normal rats (Fig. 1A), and is better organized in Aloe Vera treated animals than in normal animals (Fig. 1B). In diabetic rats the outer nuclear layer (ONL) is disorganized (Fig. 1C). Treatment with Aloe Vera extract increased the thickness of the whole retina (Fig. 1D).

The retinal structure is well organized into layers in normal rats (Fig. 2A), and is better organized in Aloe Vera treated animals than in normal animals (Fig. 2B). In diabetic rats the outer nuclear layer (ONL) is disorganized (Fig. 2C). Treatment with Aloe Vera extract increased the thickness of the retina (Fig. 2D).

Discussion

Diabetes mellitus, a global public health problem, is now emerging as an epidemic worldover (Kashikar and Kotkar, 2011). Hyperglycaemia subsequent to the diabetes mellitus is caused by inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced (Singh *et al.*, 2010). In diabetes several signs appear, one of which is retinopathy (John, 2011). Diabetic retinopathy is a leading cause of visual impairment and blindness throughout the world (Mensah and Kohner, 2002; Porta and Bandello, 2002). The retina, as part of the central nervous system, uses glucose as an exclusive energy source for dynamic activities such as for capturing images and for primary visual processing (Park *et al.*, 2003). Therefore, systemically impaired glucose metabolism causes dysfunction in the neural retina soon after the onset of diabetes (Daley *et al.*, 1987). It is well

recognized that, chronic hyperglycemia in diabetes is the main cause of damage to blood vessels, neurons and glial cells in the retina (Karachalias *et al.*, 2003). In the present study, the thickness of total retina, ONL, OPL, and INL layers were decreased at 4 weeks after induction of diabetes but they were not significantly decreased compared to control group. A transient increase in the thickness of the retinal layers was recorded in the inner retina at one week after diabetic onset. The main cause for this increase is most likely due to the breakdown of the blood-retinal barrier and the

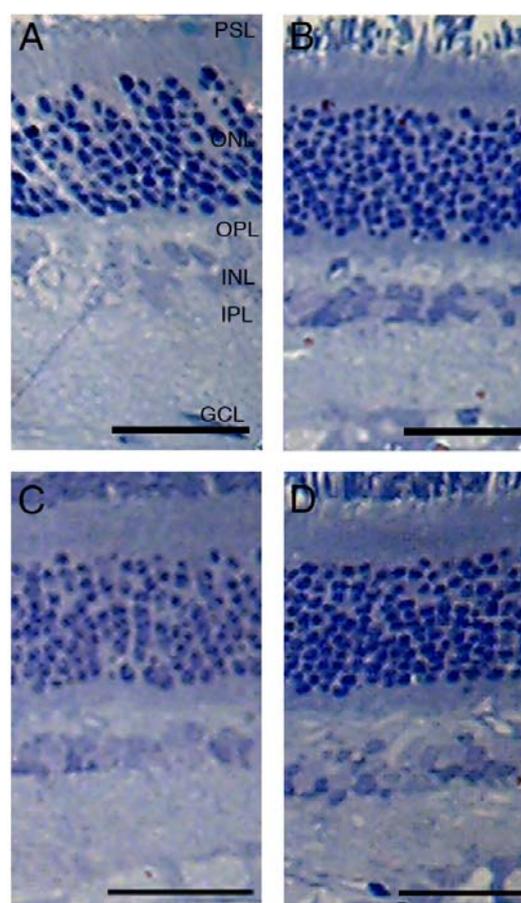


Fig. 1: The thickness of the whole retina and its layers in different experimental groups (4 weeks). **A:** Light microphotographs of retinal sections taken from control rats, **B:** Normal rats treated with Aloe Vera extract, **C:** Diabetic rats, and **D:** Diabetic rats treated with Aloe Vera extract at 4 weeks after diabetic induction. **PSL:** Photoreceptor segment layer, **ONL:** Outer nuclear layer, **OPL:** Outer plexiform layer, **INL:** Inner nuclear layer, **IPL:** Inner plexiform layer, and **GCL:** Ganglionic cell layer (scale bar represents 40 μ m)

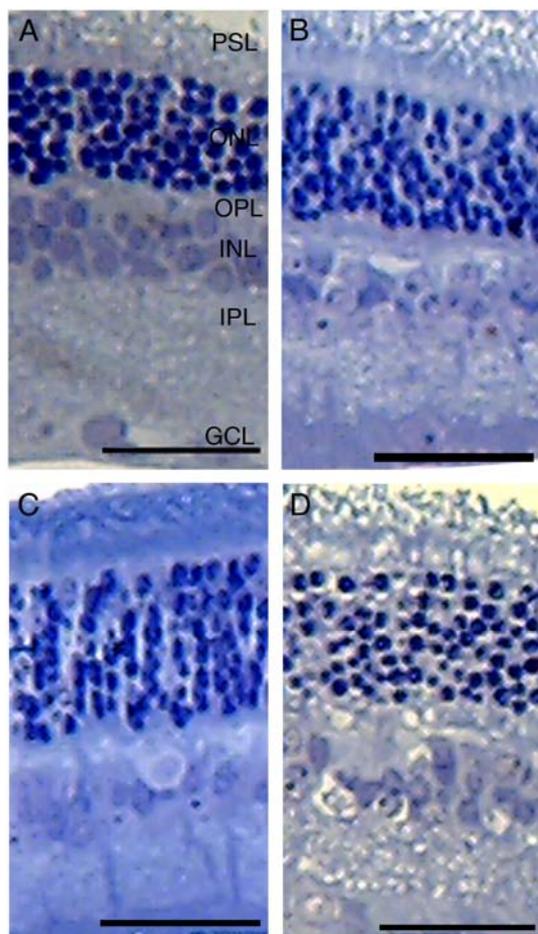


Fig. 2: The thickness of the whole retina and its layers in different experimental groups (8 weeks). A: Light microphotographs of retinal sections taken from control rats, B: Normal rats treated with Aloe Vera extract, C: Diabetic rats, and D: Diabetic rats treated with Aloe Vera extract at 8 weeks after diabetic induction. PSL: Photoreceptor segment layer, ONL: Outer nuclear layer, OPL: Outer plexiform layer, INL: Inner nuclear layer, IPL: Inner plexiform layer, and GCL: Ganglionic cell layer (scale bar represents 40 μ m)

subsequent edematous swelling (Antonetti *et al.*, 1998; Carmo *et al.*, 1998; Barber *et al.*, 2000; Park *et al.*, 2003). Therefore, a gradual decrease in the thickness of the inner retina, to well below that observed in the normal state, seems more likely due to the emergence of the decay of the neural components affected by the altered biochemical environment, including the glucose transport system (Kumagai *et al.*, 1994; Zeng *et al.*, 2000).

In our study the thickness of the total retina, PSL, OPL, INL and IPL were

decreased at 8 weeks after the onset of diabetes but were not significantly decreased compared to age matched control group. According to Martin *et al.* (2004), 10 to 12 weeks, however, the thickness of retinas of diabetic mice was significantly less than that in age-matched control mice.

This differential sensitivity correlates closely with the environment of the vasculature in the retina. Therefore, necrosis in some neurons of the inner retina during diabetes is interpreted to be an inevitable primary effect of impaired glucose metabolism (Joo *et al.*, 1999; Laake, 1999). Herbal treatment for diabetes has been a part of traditional medicine for thousands of years. The natural herbs for diabetes treatment focus on lowering blood sugar and reducing the damaging effects of the disease (Hindustan *et al.*, 2010). Many of them seem to act directly on the pancreas and stimulate insulin level in blood (Singh *et al.*, 2010). According to Sithole (2009) STZ-induced diabetes can be treated by plant extracts which control the blood sugar level as well as improving the lipid profile and ocular complications such as retinopathy. Among these plants, Aloe Vera from the family of liliacea which having about 360 species has been the main focus.

According to the results obtained from this study, after treatment with Aloe Vera extract, the thickness of the total retina, PSL, ONL and INL were increased at 4 weeks, but not significantly. The thickness of the total retina, PSL, OPL, INL and IPL layers were increased at 8 weeks after treatment with Aloe Vera extract but they were not significantly increased. The beneficial effect of Aloe Vera and its bitter nature is through stimulation of synthesis and release of insulin from pancreatic beta cells (Parthasarathy *et al.*, 2010). According to Rajasekaran *et al.* (2006) the blood glucose level in streptozotocin (STZ)-induced diabetic rats was significantly lower after the oral administration of an ethanolic extract of Aloe Vera gel. The antihyperglycaemic activity of Aloe Vera was associated with an increase in plasma insulin. The Aloe Vera gel extract stimulates insulin secretion from the remnant β -cells and/or from regenerated β -cells. Moreover, it maintains glucose homeostasis by controlling the carbohydrate

metabolizing enzymes (Singh *et al.*, 2010). These positive effects are thought to be due to the presence of compounds such as mannans, anthraquinones and lectins (Eshun, 2004). As no significant differences were realized with respect to body weights among various groups, it seems that the Aloe Vera extract has no substantial negative impact on body weight in rat. In conclusion, these findings suggest that the thickness of different layers of diabetic retina could be affected by long term treatment of Aloe Vera gel extract.

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