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Effect of *Aloe vera* on the Expression of Nerve Factors, p75 and TrkA Receptors in the Hippocampus of Diabetic Rats

Efecto del *Aloe vera* sobre la Expresión de Factores de Crecimiento Nervioso, Receptores p75 y TrkA en el Hipocampo de Ratas Diabéticas

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SUMMARY: Diabetes mellitus can lead to structural disorders in the brain. One of the most common complications of diabetes, diabetic neuropathy is associated with central nervous system disorders. *Aloe vera* has anti-diabetic, antioxidant, and neuroprotective effects. This study was designed to evaluate the effects of *Aloe vera* gel on the hippocampus changes as well as the expression of nerve growth factor and receptors TrkA and P75 in the hippocampus of streptozotocin (STZ)-induced diabetic rats. 25 male Wistar rats were randomly divided into 5 groups including: control (normal saline), diabetic (normal saline), *Aloe vera* gel (400 mg/kg/day; gavage), diabetic + *Aloe vera* gel (400 mg/kg/day; gavage) and diabetic + insulin NPH (10 IU/kg/day; subcutaneous). Experimental diabetes was induced by streptozotocin injection (60 mg/kg; intraperitoneal). All groups treated for 8 weeks. At the end of treatment course, the rat brains were removed for measuring the expression of nerve growth factor, p75 and TrkA receptors were evaluated in the hippocampus. Diabetes induction after 8 weeks caused NGF and P75 expression levels in the diabetic group than other groups significantly increased ($p<0.05$). The TrkA receptor expression in the diabetic group compared with the control had a significant reduction ($p<0.05$). On the other hand in the diabetic group receiving *Aloe vera* gel expression of NGF and P75 expression levels compared to the diabetic group was significantly reduced ($p<0.05$) and the TrkA receptor expression compared with the diabetic group had a significant increase ($p<0.05$). The results showed that oral administration of *Aloe vera* gel in diabetic rats ameliorates diabetes-induced hyperglycemia. On the other hand, *Aloe vera* gel cause modulation of the expression of NGF neurotrophic factor via increased expression of TrkA receptor-specific and non-specific receptor down-regulation of P75 in the hippocampus of STZ-induced diabetic rats.

KEY WORDS: Diabetic; *Aloe vera***; Nerve growth factor; Hippocampus; Rat.**

INTRODUCTION

Diabetes mellitus is a common metabolic disorder associated with hyperglycemia due to impaired insulin secretion and function, or both, leading to several complications such as nephropathy, retinopathy, and neuropathy (Forbes & Cooper, 2013). The effects of diabetes on the central nervous system are gradual and are known as encephalopathic diabetes (Brands *et al*., 2004). These complications can be the result of a chronic increase in intracellular glucose concentration, leading to structural, functional, and neurodegenerative changes in different areas of the brain, especially the hippocampus (Jafari Anarkooli *et al*., 2014).

As an important center for memory and learning, the hippocampus is sensitive to the rise in sugar and its neurons are vulnerable to type 1 diabetes (Reagan, 2007). In diabetic patients, the volume of the hippocampus is significantly reduced compared to non-diabetic patients, which can be attributed to the destruction of nerve cells in this area that are affected by diabetes (den Heijer *et al*., 2003).

Nowadays, a group of nerve growth factors known as neurotrophins and nerve growth factor (NGF) is the first member of the family (Arévalo & Wu, 2006). NGF is a growth factor with a protein structure. These proteins are

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involved in the growth and metabolism of many cells. This factor is effective in the growth, survival, and activity of nerve cells. NGF is a specific factor in the survival of neurons and the acceleration of their repair. Despite being involved in neuronal development, this factor is effective in repairing nerve damages (Tapp *et al*., 2009).

Decreased levels of NGF or its activity play an important role in the pathogenesis of diabetic neuropathy. However, it is not clear whether this deficiency is due to lower NGF levels or its inability to properly activate the receptor and impair postoperative activity. Although studies on NGF have shown its vital role in animal models, clinical studies have failed to address the effects of this factor. Careful analysis of research on animal models and its comparison with the problems associated with early clinical trials can lead us to a better understanding of NGF as a therapeutic tool in diabetic neuropathy (Pittenger & Vinik, 2003).

Neurotrophins are linked to neurons by two types of tyrosine receptor kinases (Trk) (the tyrosine receptor kinases are divided into three types, including TrkA, TrkB, TrkC), and the P75 receptor. The TrkA receptor has a higher and more specific coupling potency for neurotrophins than the P75. The P75 receptor is extensively expressed by Schwann cells, and its expression increases following nerve damage. Increased expression of this receptor can impair the function of the tyrosine receptor kinase, which in turn results in the binding of the P75 receptor to the NGF precursor form, resulting in neurological damage. Studies have shown that an imbalance between NGF and its precursor form can impair the function of the target neuron population because the P75 receptor neutralizes the antiapoptotic pathway by binding to the precursor components. The TrkA receiver is a receiver with a high binding affinity that is responsible for transmitting and directing the signaling pathway and is also a very important mediator for many biological reactions related to neurotrophins, and a low-affinity P75 receptor. Initially, it was thought that the p75 is only contributing in NGF-related receptors' formation, but it was found to be part of NGF's linkage to TrkA. NGF activity is specifically associated with the activation of p75 and TrkA, resulting in the activation of sensory organs and autonomic nerve fibers (Pittenger & Vinik).

In diabetic neurological tissues, the P75 receptor increases several fold, and the NGF precursor has a greater affinity for binding to the receptor and prevents it from differentiating and binding to the TrkA, which this factor initiates the path of apoptosis in nerve cells (Mysona *et al*., 2014). The binding of neurotrophins to specific receptors causes each to have its own specific activity (Heumann, 1994). All of these factors bind to the non-selective p75 receptor but selectively respond to Trk receptors from the family of tropomyosin kinase receptors. NGF acts through the TrkA receptor and BNDF and NT-4 neurotrophins through the TrkB receptor, and NT-3 acts through the TrkC receptor (Lee *et al*., 2001).

Insulin is involved in preventing or reversing the neurological effects of diabetes, such as learning disabilities, synaptic repairs, as well as the speed of neural conduction (Biessels *et al*., 1998). Also, insulin and the insulin-like growth factor have been shown to prevent apoptosis of oligodendrocytes (Brownlee *et al*., 1998). On the other hand, oxidative stress has been suggested as one of the main causes of chronic degenerative changes in diabetes, and treatment with antioxidants has reduced the incidence of some complications of diabetes (Greene *et al*., 1999).

There has been so much interest in sugar reducing agents, especially herbal medicines, in the treatment of diabetes Mellitus. *Aloe vera* belongs to the family Liliaceae that has 360 species. The primary active medical substance in gel and bark of *Aloe vera* (aloin, emodin, aloe-emodin, barbaloin and mono- and polysaccharides such as estriol, organic acids, and vitamin C) can decrease the blood sugar in streptozotocin-induced diabetes significantly. Latex contains carbohydrates, fats, proteins, some vitamins, and anthraquinone, which has been shown to have very strong anti-inflammatory properties (Weiss & Fintelman, 2000). *Aloe vera* gel in mice improved the diabetes status while increasing the antioxidant activity of superoxide dismutase, glutathione peroxidase, catalase, and glutathione transferase enzymes in liver and kidney tissue (Kosif *et al*., 2008).

Aloe vera aqueous extract in the presence of a P75 receptor, which in turn increases the function of NGF, can reduce the death of motor neurons due to compression lesions (Heshmati *et al*., 2015).

Since no comprehensive study has been performed on the effects of *Aloe vera* on NGF and its receptors in the hippocampus in experimental diabetes, the effects of *Aloe vera* gel on the expression of NGF, P75, and TrkA receptors in the hippocampus of diabetic rats by streptozotocin was studied.

MATERIAL AND METHOD

Medications and Regents: The medications and regents used in this study included: Human NPH Insulin (Lansolin® N, Exir Pharmaceutical Company, Boroujerd, Iran), *Aloe vera* gel (Barij Essence Pharmaceutical Company, Kashan,

Iran), Ketamine and Xylazin (Alfasan Chemical Co., Woerden-Holland), Sodium citrate, Citric acid (Sigma, England), Streptozotocin (Enzo, Life sciences, Inc, USA).

This study was performed on 25 healthy Wistar male rats with an average weight of 200 ± 10 g and 12 weeks of age from Jundishapur Laboratory Animals center, Ahvaz, Iran. Rats were kept in a 12-hour light and 12-hour dark at 22 ± 2°C temperature with proper ventilation during the study. During the maintenance and testing of the animals, they had free access to water and compressed food (pellets) for rats (Behparvar, Iran Company). This experimental study was conducted in Department of Basic Sciences of Faculty of Veterinary Medicine of Shahid Chamran University (Ahvaz, Iran). The animal care was provided under the supervision of a qualified veterinarian. The study was approved by the Ethical Review Committee, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran (approval ref no. EE/95.12.4/9025601/scu.ac.ir).

After a week of adaptation to environmental conditions, a number of animals became diabetic through intraperitoneal injection of 60 mg/kg of streptozotocin dissolved in a buffer of citrate (0.1 molar and pH = 4) (19) and the same volume to other rats (2 ml/kg) was injected from the citrate buffer. Diabetes was diagnosed 72 hours after streptozotocin administration. For this purpose, their blood sugar was measured using a glucometer via tail vein and Witar rats which had blood sugar levels more than 250 mg/dl was considered diabetic. After confirming diabetes, healthy and diabetic rats were randomly assigned to 5 groups (8 rats per group) as follows:

Control group: The animals in this group were not diabetic and during the study period received normal saline orally with the same volume of commercially concentrated *Aloe vera* gel in other groups.

Aloe vera **group:** Animals of this group were not diabetic and were treated with *Aloe vera* commercial concentrated gel with a daily volume of 8 ml/kg during the study. The gel contained 50 mg of dry matter per milliliter. Therefore, the daily dose of *Aloe vera* used was 400 mg/kg (Gholami & Saberi, 2015).

Diabetic group: The diabetic animals in this group, like the control group, received normal saline.

Diabetic group + ***Aloe vera*****:** Diabetic animals in this group were treated like the *Aloe vera* group.

Diabetic + Insulin Group: To maintain blood sugar levels in the relatively normal range, diabetic animals in this group received human NPH insulin daily at 10 IU/kg/B.W subcutaneously.

All groups were treated for 8 weeks and their blood sugar and weight were measured on the first and last day of treatment.

Sampling: Eight weeks after treatment, anesthesia was performed intraperitoneally using ketamine (100 mg/kg) and xylazine (5 mg/kg), and after euthanasia, their brains were carefully separated and the hippocampal tissue was then carefully removed from the brain and transferred to 2 ml sterile microtubules and immediately placed in a freezer of 70°C to measure the expression of NGF gene and P75 and TrkA receptors.

RNA extraction and cDNA synthesis: RNA extraction was performed using the guanidium acid-isothiocyanate-phenol-chloroform and RNXTM commercial kit (CinnaGen, Iran). After extraction, the concentration of RNA samples was diluted 1:50 with a tris buffer of 0.05 mM and measured with a microspherometer (Eppendorf, Germany) at 260 nm. The purity of RNA samples was determined by calculating the light absorption ratio of RNA samples at 260 nm to their light absorption at 280 nm, and samples with a ratio of 1/8 and above were used to synthesize cDNA. For each sample, 1 mg of total RNA was reverse transcribed by YTA cDNA synthesis kit using random hexamer primers as described by the manufacturer (Yektatajhiz, Iran). Reaction components were used including 1 mg of total RNA as template, YTA RT 200 U/µL (1 mL), YTA RNase Inhibitor 20 U/µL (1 mL), Random Hexamer primer (1 mL), dNTP Mix 10 mM (2 mL) and Reaction Buffer 5x (4 mL). Then, samples were incubated for 10 minutes at 25°C, 90 minutes at 42°C and 5 minutes at 75°C.

The samples were kept in a hot water bath at 42°C for 90 minutes. The samples were then stored in a freezer at -20°C until a PCR reaction was performed.

PCR reaction in real-time. Evaluate the expression of NGF, P75 and TrkA expression in the hippocampal tissue with PCR test in real-time in Lightcycler® Detection System by Roche, USA, using a kit (SYBR Green qPCR MasterMix 2X) (unique equipment, Iran) based on SYBR Green (SYBR Green 1®) DNA dye. In this study, the GAPDH gene (GenBank: NM-001034055) was used as a calibrator and the expression changes of different genes were evaluated according to the constant expression of this gene. Reactions range from 12.5 microliters and include 6.25 microliters (2X) qPCRTM Green master Mix, 0.25 microliters per primer with a concentration of 10 mM (200nM), 3 ml cDNA (100 ng) and 2.75 ml distilled water without nuclease was performed.

The temperature program used was 5 minutes at 94°C, followed by 45 cycles including 15 seconds at 94°C and 45 seconds at 60°C. The reaction was repeated twice for all samples. Two separate samples, one without cDNA and the other with RNA, were used as negative controls in each reaction.

The list of primers used in this study including the sequence, the length of the piece to be propagated by them, and the bonding temperature of each are in Table I.

Table I. List of sequences with their primer characteristics used in this study.

Gene	Sequence	Length	Melting point
P75	F: TATGGTGACCACTGTGATGG R: CAGCTGTTCCACCTCTTGAA	144	52-60
TrkA	F:GGAGTTGAGAAGCCTAACCA R:TGCACAGTTTTCCAGGAGAG	135	52-60
GADPH	F:CTGGAGAAACCTGCCAAGTA R:GAAGAGTGGGAGTTGCTGTT	122	52-60
NGF	F:CTTCACAGAGTTTTGGCCTG R:CATTACGCTATGCACCTCAGA	147	52-60

Comparative evaluation of gene expression. The method used in data analysis was based on the TDDC method. Expression level of target genes was calculated using comparative threshold cycle formula. The expression level of target genes to reference gene in treated samples compared to controls was calculated using the following formula: Expression level of target gene = $2^{-\Delta\Delta CT}$, Where DDCT is calculated by the following equation: $^{\Delta\Delta CT}$ = [(mCTtarget-mCTreference) test sample - (mCTtarget-mCT reference) control sample]. All qPCR analysis was performed according to The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guideline. The results of the expression of NGF, TrkA, and P75 genes were reported based on the formula.

RESULTS

The effect of *Aloe vera* gel on the blood sugar level of rats in different groups studied. Examination of the results showed that the streptozotocin at a dose of 60 mg/kg induced diabetes mellitus in rats. Following daily intake of *Aloe vera* gel at a dose of 400 mg/kg in diabetic rats and in the diabetic group treated with insulin at the end of the eighth week, blood sugar levels decreased significantly compared to the diabetic control group ($p < 0.05$). While blood sugar in diabetic control rats increased at the end of the eighth week. Table II shows the blood sugar levels in different groups and their comparison.

Table II. Mean ± SEM blood sugar in different groups.

Groups	Blood sugar (mg/dl)	
	Initial	Late
Control	115.4 ± 2.1^{b}	109.7 ± 3^{c}
Aloe vera	113.3 ± 2.2^{b}	107.2 ± 3.7^{c}
Diabetic	436.5 ± 23.5^{a}	541.5 ± 20.5^{a}
Diabetic + *Aloe vera*	536.7 ± 26.6^{a}	255.4 ± 32.5^{b}
Diabetic + Insulin	519.9 ± 26.3^{a}	204.7 ± 44.2^{b}

* Different letters indicate significant difference between groups ($p < 0.05$)

The effect of *Aloe vera* on the weight of rats in different groups. As shown in Table III, the mean weight of diabetic rats decreased after 8 weeks from the time of induction of diabetes from 202.6 ± 3.6 g to 172.7 ± 7.9 g. While the weight of the control group during this period increased from 203.9 ± 3 g to 277.7 ± 7.6 g, the difference in weight between the two groups at the end of the eighth week was significant ($p < 0.05$). Daily intake of *Aloe vera* and insulin in both diabetic groups prevented the weight loss of the rats so that the mean weight of untreated diabetic rats at the end of the eighth week was significantly lower than the above two groups ($p < 0.05$). Changes in the weight of diabetic rats receiving *Aloe vera* did not show a significant difference compared to the insulin-treated group. The results showed that the weight of healthy mice receiving *Aloe vera* increased during the study, but their increase was not significantly different from healthy animals.

Table III. Mean ± SEM of body weight in different groups.

Groups	Blood sugar (mg/dl)	
	Initial	Late
Control	203.9 ± 3^{a}	277.7 ± 7.6^{ab}
Aloe vera	200.1 ± 3^{a}	293 ± 11.9^{a}
Diabetic	202.6 ± 3.6^{a}	172 ± 7.9^{c}
Diabetic + *Aloe vera*	205.7 ± 4.2^{a}	248.3 ± 8.6^{b}
Diabetic + Insulin	207.1 ± 3.4^{a}	270.4 ± 15.4^{ab}

* Different letters indicate significant difference between groups ($p < 0.05$).

Evaluation of Nerve Growth Factor (NGF) and P75 and TrkA Receptors in Hippocampal Tissue Using Real-Time PCR

Results of measuring neurotrophic factor (NGF) expression, 8 weeks after the induction of diabetes. Analysis of NGF expression measurement results in different groups showed that the expression rate of this factor increased in diabetic rats and from 1.04 ± 0.038 in the control group to 8.48 ± 0.40 in the diabetic group, which was a significant increase ($p < 0.05$). Following the use of *Aloe vera* in diabetic mice, the rate of NGF in the hippocampal tissue of these mice decreased to 4.34 ± 0.47, which was significant compared to the diabetic group but did not show a significant difference from the diabetic group receiving insulin (Fig. 1).

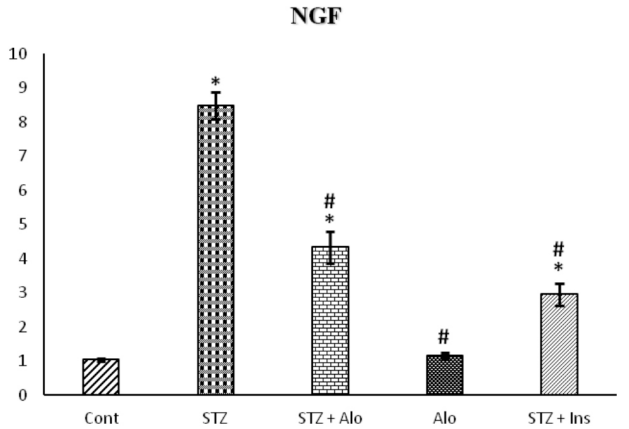

Fig. 1. Comparison values of NGF gene expression in the studied groups. The asterisk sign (*) indicates a significant difference between the groups and the control group and the number sign (#) indicates the difference between the groups and the diabetes control group ($p < 0.05$).

Results of measuring TrkA receptor expression 8 weeks after induction of diabetes. By measuring the expression of TrkA receptor in rats of different groups, it was observed that following the induction of diabetes in rats, the rate of TrkA receptor in the hippocampus decreased from 1.038 ± 0.046 in the control group to 0.867 ± 0.041 in the diabetic group, which was significant ($p < 0.05$). Following the prescribing of *Aloe vera* in diabetic groups, the expression of TrkA receptors increased and the rate of expression in diabetic rats that received *Aloe vera* increased significantly compared to diabetic mice ($p < 0.05$). The expression of the TrkA receptor in diabetic insulin-receiving rats was also higher than in the untreated diabetic group, which was significantly different ($p < 0.05$) (Fig. 2).

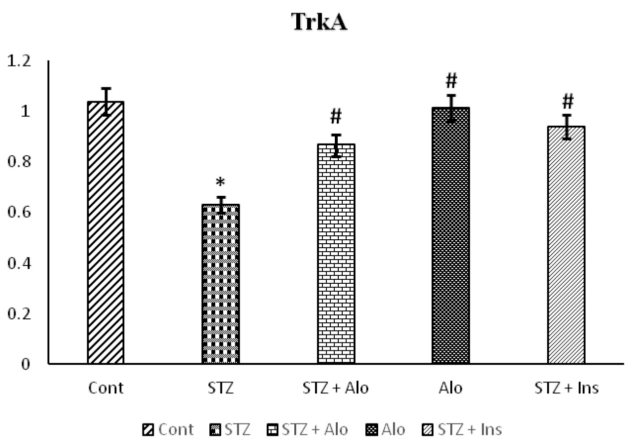

Fig. 2. Comparative values of TrkA expression in the studied groups. The asterisk sign (*) indicates a significant difference between the groups and the control group and the number sign (#) indicates the difference between the groups and the diabetes control group ($p < 0.05$).

Comparing the results of measuring the expression of the P75 receptor expression at the end of the eighth week of treatment. The mean P75 receptor expression in diabetic rats at the end of the eighth week showed a significant difference between different groups ($p < 0.05$). By measuring the expression of the P75 receptor in rats of different groups, it was observed that following the induction of diabetes in rats, the rate of expression of the P75 receptor in the hippocampus increased and its rate ranged from 1.046 ± 0.045 in the control group to 13.942 ± 1.158 in the diabetes group ($p < 0.05$). Following the administration of *Aloe vera*, the expression of the P75 receptor in the diabetic group receiving *Aloe vera* decreased, which showed a significant decrease compared to diabetic mice ($p < 0.05$), but the level of expression of P75 receptor in diabetic mice decreased. Insulin receptors also showed a lower level compared to the untreated diabetic group, so that this difference was significantly different ($p < 0.05$). In the healthy group receiving *Aloe vera*, the rate of change in the expression of the P75 receptor did not differ significantly from the control group (Fig. 3).

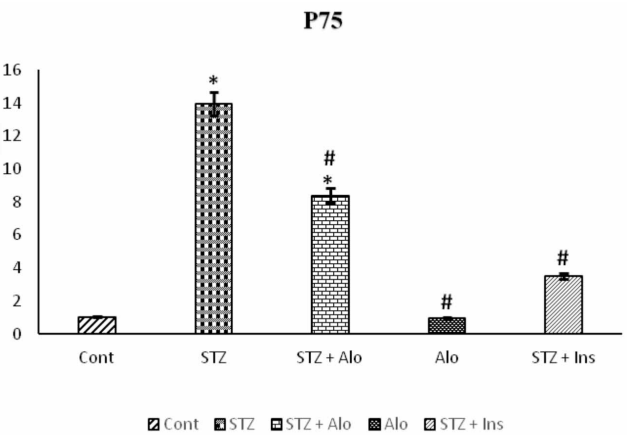

Fig. 3. Comparison of values of P75 receptor expression in the studied groups. The asterisk sign (*) indicates a significant difference between the groups and the control group and the number sign (#) indicates the difference between the groups and the diabetic control group ($p < 0.05$).

DISCUSSION

The results of the present study showed that daily consumption of *Aloe vera* at a dose of 400 mg/kg for 8 weeks in diabetic rats significantly reduced blood sugar and body weight. Also, the induction of diabetes by streptozotocin in rats increased NGF and P75 expression and decreased TrkA.

There are a variety of reports on the antidiabetic effects of *Aloe vera*, including Ghannam *et al*. (1986) on

five non-insulin-dependent diabetic patients and lab rats diagnosed with diabetes by Alloxan. They found that consuming *Aloe vera* reduced serum glucose levels in all patients without altering body weight. In healthy mice, *Aloe vera* reduced blood sugar after 5 days. In diabetic mice, blood sugar dropped after 3 days. They concluded that *Aloe vera* contained hypoglycemic substances whose mechanism of action has not yet been precisely established (Ghannam *et al*.).

Ajabnoor (1990) and Bolkent *et al*. (2005) reported the effects of *Aloe vera* antibodies in the lab rats diagnosed with alloxan.

Rajasekaran *et al*. (2006) examined the antihyperlipidemic effects of *Aloe vera* leaf gel on diabetic rats and observed that blood sugar, liver transaminases, plasma and tissue cholesterol (liver and kidney), triglycerides, free fatty acids, and free fatty acids decreased significantly and plasma insulin increased significantly.

Noor *et al*. (2008) observed that the daily oral intake of *Aloe vera* at a dose of 300 mg in diabetic rats reduced their blood sugar.

Kim *et al*. (2009) investigated the hypoglycemic and hypolipidemic effects of *Aloe vera* gel on non-insulin-dependent diabetes in the liver and reported that oral administration of *Aloe vera* gel prevented the development of diabetic symptoms and suggested that *Aloe vera* gel could type 1 diabetes is helpful.

Lanjhiyana *et al*. (2011) reported that rats treated with diabetic alloxan underwent *Aloe vera* treatment for 21 days, showed hypoglycemic activity in glucose tolerance testing, and demonstrated the plant's antidiabetic properties in diabetic rats. The results of the present study about the reduction of blood sugar by *Aloe vera* are consistent with the results of studies by other authors (Lanjhiyana *et al*.).

Various reasons can be given for the mechanism of action of *Aloe vera* on lowering blood sugar. Clinical evaluations have shown that the pharmacologically active substances are concentrated in the gel and skin of *Aloe vera* leaves. An anthropoid called barbaloin is isolated from the *Aloe vera* plant, which protects the beta cells of the islets of Langerhans from damage caused by free radicals (Lanjhiyana *et al*.).

Esua & Rauwald (2006) showed that three Malloyl glucans, called Vrasil Glucan A, B, and C, were isolated from *Aloe vera* gel, and that Vrasil Glucan B had strong anti-inflammatory and anti-proliferative effects. Veracill Glucan C, on the other hand, shows cell proliferation and anti-inflammatory activity (Esua & Rauwald). *Aloe vera* exerts its anti-hyperglycemic activity in several ways. *Aloe vera* may prevent the pancreatic cell death or, in part, allow the regeneration of damaged cells. It may also induce cell proliferation, like the Momordica charantia plant (Noor *et al*., 2008). Some plant extracts may activate their hypoglycemia through insulin-like activity or by other mechanisms, such as stimulation of glucose reabsorption by environmental factors, inhibition of endogenous glucose production, or activity of gluconeogenesis agents in the liver and muscles (Noor *et al*.).

Rajasekaran *et al*. stated that in general, the hypoglycemic effects of plants depend on the degree of destruction of beta cells. Treatment of the moderate form of STZ-induced diabetes in rats with herbal medicines could be due to the activation of beta cells and their return to normal granulation, indicating their enzymatic effect. The hypoglycemic activity of *Aloe vera* against high blood sugar has been associated with an increase in plasma insulin, which may indicate the enzymatic effect of *Aloe vera* gel. They showed that an increase in insulin levels in diabetic mice treated with *Aloe vera* could be a reason why *Aloe vera* stimulates insulin secretion from remaining cells or from regenerated cells (Rajasekaran *et al*.).

In a recent study of healthy rats that received *Aloe vera* gel, blood sugar was at a normal level, which could indicate that the balance in glucose metabolism was not altered by *Aloe vera* gel. It seems that the effective compounds of *Aloe vera* can only affect blood glucose when the glucose concentration is above a certain level when so-called hyperglycemia has occurred. Also, the healthy rats that received *Aloe vera* were apparently healthy, and their nutrition and behavior were normal, and no symptoms were observed. Therefore, it can be said that the dosage used in this study did not have a harmful effect on eating *Aloe vera*. This result is consistent with the findings of other researchers in the field, such as Noor *et al*. and Rajasekaran *et al*.

The results of the present study on the effect of *Aloe vera* on the body weight of healthy and diabetic rats showed that diabetes has significantly reduced body weight. *Aloe vera* in comparison has prevented weight loss, indicating a positive effect of *Aloe vera* on this process and a kind of therapeutic effect of this plant extract on diabetes.

The results of some researchers, such as Garris (1988) and Ballester *et al*. (2004), describe weight loss due to diabetes.

The effect of *Aloe vera* on body weight has been reported in limited studies, including Noor *et al*. and Lanjhiyana *et al*. Normalizing blood sugar has been shown

to help regain body weight in diabetic rats that have received *Aloe vera*.

In the present study, the weight of a healthy rat that was fed *Aloe vera* did not show a significant decrease after 8 weeks compared to the control group.

Noor *et al*. stated that the weight of diabetic mice that received *Aloe vera* (3 weeks after receiving *Aloe vera*) showed a significant increase compared to the weight of diabetic rats.

According to a study by Pérez *et al*. (2007) on the effect of *Aloe vera* gel on mice with type II diabetes, the gel significantly increased body weight and lowered blood glucose levels and protected animals against the aftereffects of type II diabetes.

Lanjhiyana *et al*. on the effect of *Aloe vera* on body weight stated that in rats, diabetes significantly reduced body weight and consumption of *Aloe vera* at a dose of 300 mg/kg for 21 days prevented this weight loss so that there is a significant difference between the weight of rats that received *Aloe vera* and diabetic rats (Lanjhiyana *et al*.).
Hyperglycemia can have adverse effects on the central nervous system (Jafari Anarkooli *et al*.; El-Akabawy & El-Kholy, 2014). Hyperglycemic oxidative stress plays a central role in nerve damage in the brain under diabetic conditions. Due to its high metabolic rate, very low cell regeneration capacity (low levels of free radical scavenging antioxidants, vitamin C, catalase, superoxide dismutase) and the presence of multiple targets of cellular oxidative stress such as lipids, nucleic acids, and proteins are more sensitive to oxidative stress than other organs (Halliwell, 2006).

The results of this study showed that the induction of diabetes by streptozotocin in rats increased the expression of NGF and P75 and the reduction of TrkA.

According to our research, there is no study of the effect of *Aloe vera* on neurotrophic factors and its specific and non-specific receptors in diabetic mice.

According to the findings of this study, the consumption of *Aloe vera* gel in diabetic mice for 8 weeks can reduce blood sugar as well as increase insulin sensitivity in cells (Kim *et al*.). Clinical studies have also suggested that consuming *Aloe vera* gel in pre-diabetics for 8 weeks can lower blood sugar (Alinejad-Mofrad *et al*., 2015). According to the study's findings, *Aloe vera* can increase the weight of diabetic rats with streptozotocin (Ramachandraiahgari *et al*., 2012). In summary, the proposed mechanisms of the effect of *Aloe vera* in managing diabetes are by increasing glycogen production and reducing fat accumulation in the liver, increasing insulin production and secretion in the pancreas, reducing fat accumulation in muscles, reducing fat accumulation and reducing adipocyte cell size in adipose tissue (Pothuraju *et al*., 2016).

Taking antioxidants such as vitamin B complex and vitamin C can improve structural changes in different areas of the brain in diabetic rats treated with streptozotocin (Eltony, 2016). *Aloe vera* gel contains more than 75 different compounds such as polysaccharides, alkaloids, anthraquinones, anthrones, chromones, coumarins, flavonoids, glycoproteins, water-soluble vitamins (choline, folic acid, thiamine B1, riboflavin B2, pyridoxine: B6, cobalamin: B12, C) and fat-soluble vitamins (E, beta-carotene: precursor of vitamin A) (Akaberi *et al*., 2016). The presence of such compounds has made *Aloe vera* a powerful antioxidant compound. The mechanism of antioxidant and anti-inflammatory effects of *Aloe vera* is by increasing superoxide dismutase and reducing cases such as malondialdehyde, hemorrhage, swelling, and migration of inflammatory cells, as well as reducing the expression of factors such as NF-?B and nitric oxide. Neurotic synthesis (nNOS) has been reported in ischemic/spinal cord injury. The neuroprotective effects of *Aloe vera* gel on sciatic nerve damage/reperfusion were expressed by reducing malondialdehyde, ischemic fiber degeneration, NF-?B, and increasing superoxide dismutase, and according to the results, it was suggested that *Aloe vera* gel have neuroprotective effects through anti-inflammatory and antioxidant mechanisms (Guven *et al*., 2016). Studies on PC12 cells have also shown that *Aloe vera* has neuroprotective effects by protecting the function and structure of mitochondria, as well as inhibiting lipid peroxidation in these cells (Wang *et al*., 2010). The modification of the expression of NGF neurotrophic factor and its specific and non-specific receptors in the brains of diabetic animals by *Aloe vera* gel in the present study may be related to its antioxidant, anti-inflammatory and neuroprotective properties.

In the present study, insulin modulated the expression of NGF neurotrophic factor and its specific and non-specific receptors in diabetes.

Insulin therapy has been suggested to have antioxidant functions in addition to controlling blood sugar. It can reduce oxidative stress as well as reduce cell damage in the hippocampus and cerebral cortex (Wayhs *et al*., 2013).

NGF is a growth factor with a protein structure. These proteins are involved in the growth and metabolism of many cells and are a type of cytokine. This growth factor is effective

in the growth of nerve cells, their survival, and their activity. Nowadays, a group of nerve growth factors known as neurotrophins is the leading cause of nerve growth in this family (Wayhs *et al*.xs). NGF is a specific factor in keeping neurons alive and accelerating their repair. These factors are involved in nerve growth and repair. The role of these factors is shown in neurogenesis, nerve differentiation, axon and dendrite growth (Tapp *et al*.).

In summary, decreased levels or activity of NGF play an important role in the pathogenesis of diabetic neuropathy. However, it is not clear whether this deficiency is due to a lower systemic level or the inability to activate appropriate receptors and disrupt post-receptor activity. However, studies on NGF have shown a vital role in animal models but clinical trials have failed. Careful analysis of research on animal models and comparing it with the problems associated with early clinical trials could provide a better understanding of the NGF in the treatment of diabetic neuropathy (Pittenger & Vinik).

Both receptors connect to the dimer in response to NGF. TrkA homodimers and TrkA and p75 heterodimers develop survival and nerve growth properties, while p75 homodimers trigger apoptosis as soon as they bind to NGF.When either the TrkA dimer receiver or one of the TrkA and p75 dimers are present, they initiate survival and nerve growth characteristics, while if both dimers of p75 receptors are present, the apoptosis process begins (Mukai *et al*., 2003).
Apoptosis is mediated by the binding of NGF to p75, so it occurs in places where TrkA does not exist, due to the simultaneous expression of homolog tyrosine kinase receptors; The p75 receiver is inhibited and eventually, death signals are inhibited (TrkA increases the expression of the homolog TrkA, thereby inhibiting p75 and ultimately inhibiting apoptosis. In the absence of TrkA, NGF also causes apoptosis of oligodendrocytes and demyelination through p75, which has little affinity for NGF (Yune *et al*., 2007).

NGF also produces a brain-derived neurotrophic factor (BDNF) (Acosta *et al*., 2013), which is another global neurotrophin involved in myelin and reduces the regulation of pro-inflammatory signals (Triaca *et al*., 2005).

In the present study, the expression of the NGF gene in the hippocampal tissue of diabetic rats increased compared to the control group, control of *Aloe vera*, diabetic recipient of *Aloe vera*, and diabetic recipient of insulin. TrkA receptors were also reduced in the hippocampus of diabetic mice. However, the expression of this receptor in the control groups of *Aloe vera*, diabetic recipient of *Aloe vera*, and diabetic recipient of insulin did not have a significant decrease compared to the control group. Existing studies have shown that decreased levels of NGF activity play an important role in the pathogenesis of diabetic neuropathy (Pittenger & Vinik).

Linkage of neurotrophins to specific receptors causes each to have its own activity (Lee *et al*., 2001). All of these factors bind to the non-selective p75 receptor but react selectively with Trk tyrosine kinase receptors in the family of tropomyosin receptor kinase families. NGF acts through the TrkA receptor and BNDF and NT-4 neurotrophins through the TrkB receptor, and NT-3 acts via the TrkC receptor .

Mysona *et al*. (2014) showed that in diabetic neurological tissues, the P75 receptor increased severalfold, and the neurotrophic factor precursors (proNGF) were more likely to bind to the receptor and prevents conversion and binding to the TrkA, which triggers the apoptosis pathway in nerve cells.

In the present study, an increase in NGF expression with a P75 receptor and a decrease in the TrkA receptor may be due to an increase in the NGF precursor, which activates pathways that cause diabetic neuropathy and acute encephalopathy. This finding was consistent with the results of Mysona *et al*.

The results of this study indicate an imbalance between NGF and its specific receptor, TrkA, which is likely to increase the prognostic form of NGF, which can impair the function of the target population of neurons because, in the absence of a TrkA receptor, the p75 receptor can fail to form a mature NGF form by forming a ligand to these components.

According to the results of this study, Pittenger & Vinik have shown that an imbalance between NGF and its precursor form can impair the function of nerve cells because the P75 receptor neutralizes the anti-apoptotic pathway by binding to these components. They stated that the TrkA receptor is a receptor with a high affinity that is responsible for transmitting and directing the signaling pathway and is also a very important mediator for many biological reactions related to neurotrophins, while the p75 receptor acts as a low-affinity receptor for neurotrophins. Initially, it was thought that the p75 would only be involved in the formation of NGF-related receptors but it has been shown that this receptor is a component for the binding of NGF to TrkA. NGF activity is specifically associated with the activation of p75 and TrkA, which results in the activation of sensory organs and autonomic nerve fibers (Pittenger & Vinik).

Yune *et al*. have shown that the NGF precursor binds to p75 and the second receptor of sortilin with high affinity, thereby inducing signal pathways that lead to p75-dependent apoptosis.

Although the NGF precursor promotes the survival of neurons by binding to TrkA with low affinity, recent reports suggest that the predisposing NGF precursor, preferably with a high affinity for p75, exceeds TrkA, resulting in neuronal and oligodendrocytes apoptosis even in the presence of TrkA.

Beattie *et al*. (2002) also showed that the binding of NGF precursor to the p75 receptor also activated caspase pathways, leading to the death of oligodendrocytes.

Due to the effect of *Aloe vera* in decreasing the diameter of the hippocampus, as well as improving and reducing the atrophy of the cerebral cortex, it is possible that one of the mechanisms of the positive performance of *Aloe vera* on hippocampus is reduced apoptosis due to diabetes by increasing the expression of NGF and TrkA and decreasing the expression of p75. *Aloe vera* reduces the activity and expression of p75 in diabetic rats treated with *Aloe vera*. It can be concluded that *Aloe vera* is likely to reduce atrophy of the hippocampus by reducing the activity of this receptor in hippocampus, thereby increasing the expression of NGF and TrkA.

CONCLUSION

Generally, the results of the present study showed that experimental diabetes induced by streptozotocin for 8 weeks increased blood sugar and weight loss. However, chronic treatment with *Aloe vera* at 400 mg/kg and insulin at 10 units/kg improved blood glucose levels in diabetic rats. The results of a molecular study in this study showed an increase in NGF and P75 receptor neurotrophic factor expression and a decrease in NGF-related TrkA receptor expression, which was improved by treatment with *Aloe vera* and insulin. Evidence supports this hypothesis that hyperglycemia and neurodegenerative disorders through NGF imbalance and P75 and TrkA receptors have played a key role in the development of structural disorders of the brain that are comparable to those treated with *Aloe vera*. Insulin improves disorders in diabetic rats by reducing hyperglycemia and modulating NGF expression and P75 and TrkA receptors. Further studies are needed to elucidate the underlying mechanisms of these changes.

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MAHABADY, M. K.; TAPEBUR, M. B.; MAZAHERI, Y.; TABANDEH, M. R. & TABATABAEI, S. R. F. Efecto del *Aloe vera* sobre la expresión de factores de crecimiento nervioso, receptores p75 y TrkA en el hipocampo de ratas diabéticas. *Int. J. Morphol., 39(2)*:577-586, 2021.

RESUMEN: La diabetes mellitus puede provocar trastornos estructurales en el cerebro. Es una de las complicaciones más comunes de la diabetes y la neuropatía diabética y está relacionada con trastornos del sistema nervioso central. El *Aloe vera* tiene efectos antidiabéticos, antioxidantes y neuroprotectores. Este estudio fue diseñado para evaluar los efectos del gel de *Aloe vera* en los cambios del hipocampo, así como la expresión del factor de crecimiento nervioso y los receptores TrkA y P75 en el hipocampo de ratas diabéticas inducidas por estreptozotocina (STZ). Se dividieron al azar 25 ratas Wistar macho en 5 grupos de: control (solución salina normal), diabéticos (solución salina normal), gel de *Aloe vera* (400 mg / kg / día; sonda), diabéticos + gel de *Aloe vera* (400 mg / kg / día; sonda) y diabéticos + insulina NPH (10 UI / kg / día; subcutánea). La diabetes experimental fue inducida por inyección de estreptozotocina (60 mg / kg; intraperitoneal). Todos los grupos fueron tratados durante 8 semanas. Al final del tratamiento, se extrajeron los cerebros de las ratas para medir la expresión del factor de crecimiento nervioso y se evaluaron los receptores p75 y TrkA en el hipocampo. La inducción de diabetes después de 8 semanas provocó que los niveles de expresión de NGF y P75 en el grupo de diabéticos aumentaran significativamente en comparación con otros grupos ($p < 0,05$). La expresión del receptor TrkA en el grupo diabético comparado con el control tuvo una reducción significativa ($p < 0,05$). Por otro lado, el grupo de ratas diabéticas que recibieron la expresión en gel de *Aloe vera* de NGF y los niveles de expresión de P75 en comparación con el grupo de ratas diabéticas se redujo significativamente ($p < 0,05$) y la expresión del receptor de TrkA en comparación con el grupo de ratas diabéticas tuvo un aumento significativo ($p < 0,05$). Los resultados mostraron que la administración oral de gel de *Aloe vera* en ratas diabéticas mejora la hiperglucemia inducida por la diabetes. Por otro lado, el gel de *Aloe vera* causa modulación de la expresión del factor neurotrófico NGF a través del aumento de la expresión de receptor TrkA específico y no específico y regulación negativa del receptor de P75 en el hipocampo de ratas diabéticas inducidas por STZ.

PALABRAS CLAVE: Diabético; *Aloe vera*; Factor de crecimiento nervioso; Hipocampo; Rata.

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