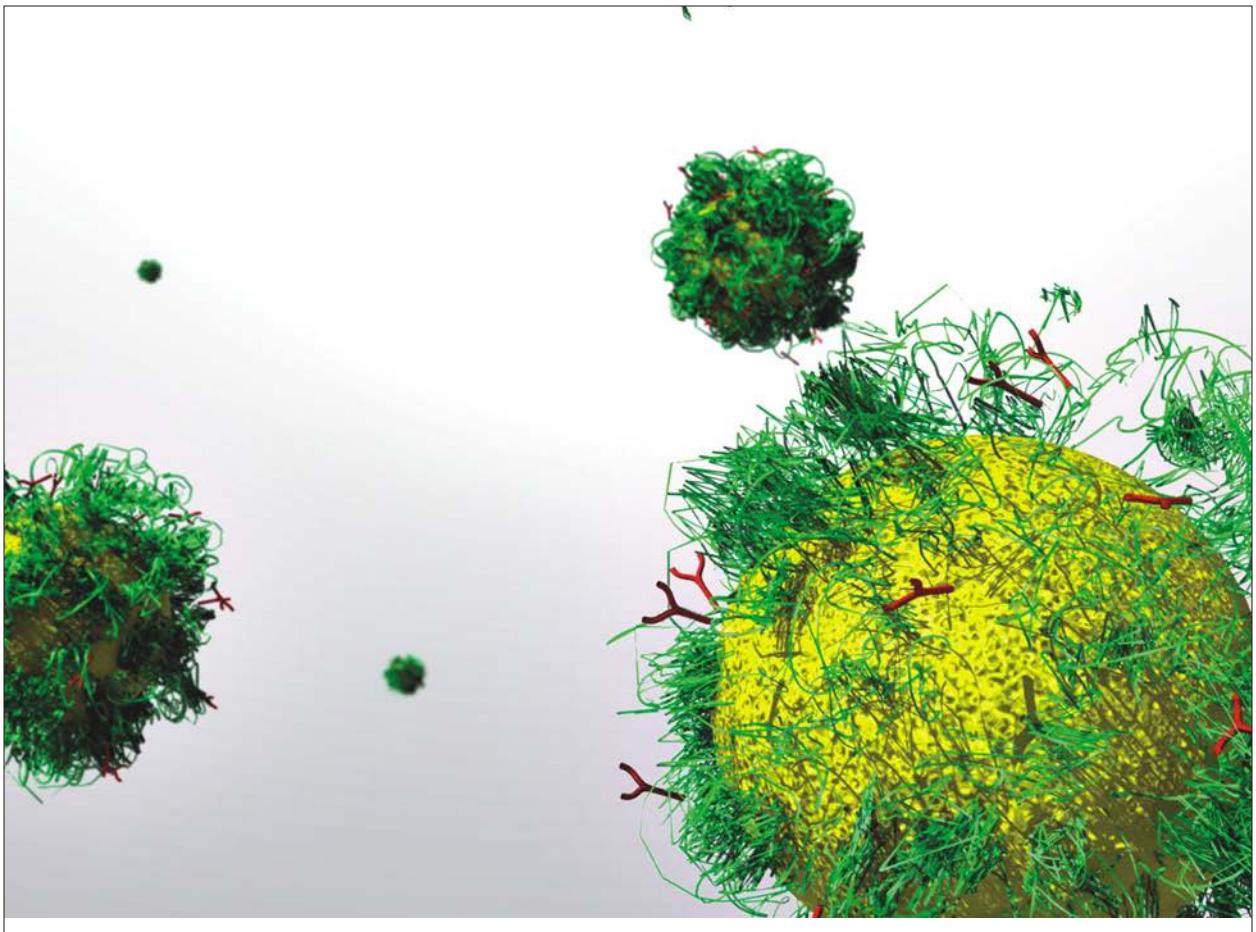

American Journal of Drug Discovery and Development

ISSN 2150-427X

Academic Journals Inc.

www.academicjournals.com

American Journal of Drug Discovery and Development 3 (4): 263-270, 2013
ISSN 2150-427x / DOI: 10.3923/ajdd.2013.263.270

Effect of Aloe Vera on Albumin Glycation Reaction *in vitro*

[1]Seyed Abdolhakim Hosseini, [2]Mehdi Mahmoodi, [3]Mohammad Saeed Hakhamaneshi, [4]Shadi Hosseini, [3]Mohammad Abdi, [5]Sadegh Zarei, [5]Seyed-Mostafa Hosseini-Zijoud and [3]Javad Hosseini

[1]Department of Molecular Medicine and Genetic, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

[2]Department of Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

[3]Department of Biochemistry and Nutrition, Faculty of Medicine, Kurdistan University of Medical Sciences, Kurdistan, Iran

[4]Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[5]Department of Biochemistry, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Corresponding Author: Javad Hosseini, Department of Biochemistry and Nutrition, Faculty of Medicine, Kurdistan University of Medical Sciences, Kurdistan, Iran Tel: 0098 918 784 1007

ABSTRACT

Hyperglycemia in diabetes causes glycosylation of the body proteins that leads to a change in their conformation and function. In this study the influence of the Aloe vera's extracts on the inhibition of the Albumin glycosylation reaction and on the breaking the bond between Albumin and glucose was surveyed *in vitro*. The influence of Aloe vera at 0.1, 0.2, 0.5 and 1 g dL^{-1} concentrations in two states; influence on the inhibition reaction of Albumin glycosylation and influence on the bond breaking between Albumin and glucose was surveyed *in vitro*. The rate of glycosylation was measured by thiobarbituric acid (TBA) and $p<0.05$ was considered as significant Level. Aloe vera's extract can inhibit significantly the Albumin glycosylation reaction in all concentrations except in 1 g dL^{-1} concentration and it has maximum inhibitory effect in 0.1 g dL^{-1} concentration ($p<0.05$) and the rate of this inhibition was time dependent. Additionally, it could break the Albumin-glucose bond and the maximum effect was seen with 0.1 g dL^{-1} concentration ($p<0.05$). The results demonstrate that Aloe vera inhibits the glycosylation of the Albumin and also break the Albumin-glucose bond down, thus it has a hypoglycemic effect.

Key words: Diabetes mellitus, aloe vera, albumin glycosylation

INTRODUCTION

Diabetes is the metabolic disorder of the carbohydrates, lipids and proteins that is caused due to the lack of or decrease in Insulin production or resistance to the function of it (Virdi *et al*., 2003). Severe hyperglycemia in diabetes causes glycosylation of body proteins that in turn leads to secondary complications in the eyes, kidney, nervous system and vessels (Virdi *et al*., 2003). Glycosylation reaction occurs spontaneously when the proteins are exposed to the reducing sugars and its rate is dependent on seveierity of the hyperglycemia and its presence time in the body. Formation of glycosylation proteins causes a change in the conformation and functions and is followed free radical formation through glucose autoxidation that can damage the lipids, proteins and nucleotides and probably causes tissue damage in diabetics (Bonnefont-Rousselot, 2002). One

of the body proteins that bear non enzymatic glycosylation is serum Albumin. Albumin comprises about 60% of the plasma proteins and has important roles like oncotic pressure maintenance and transformation of the endogenous and exogenous biomolecules (Peters, 1985). In fact albumin glycosylation reaction is the conjugation of the aldehyde group of the sugar with free functional amine group in the albumin conformation that is done slowly. The ratio of Albumin glycosylation depends on different parameters like glucose concentrations time of protein incubation with glucose. Considering to the disadvantages of glycosilation of the proteins that was mentioned above the inhibiting this reactions is absolutely necessary to remove complications of the diabetes. The researchers conducted numerous studies in this area to find compounds that inhibit the glycosylation of proteins without the alarming side effects and some medicines have been introduced that can break the linkage between glycosilated proteins and have a positive effects on diabetes complications (Swamy and Abraham, 1989). But considering the side effects of these medicines the need for alternative with minimum side effects, high degree of confidence that can be used for a long time is felt more than before and much attention has been paid to herbal medicine in this area. Herbal medicines are natural materials that have less chance of side effects. Recent studies proved antioxidant, anti diabetic and hypoglycemic effects of herbal medicines (Alpers, 2009). Aloe vera is from the Liliaceous family that has about 360 species. The plant has lance-shaped and grayish leaves containing clear gel in a central glazed tissue (Rajasekaran *et al*., 2005). Aloe vera leaves have been used for thousands of years in different countries as a medicine (Vogler and Ernst, 1999). Aloe vera has numerous benefits, some of which are wound healing (Muller *et al*., 2003), anti inflammation (Langmead *et al*., 2004), anti cancer (Chen *et al*., 2004), antidiabetic (Tanaka *et al*., 2006) and anti oxidant (Rajasekaran *et al*., 2005). This herbal is rich in catalase enzyme, E and C vitamins so has an anti toxic effect (Can *et al*., 2004; Kuzuya *et al*., 2001). In this study we wanted to survey one of the probable mechanisms of useful hypoglycemic effects of this plant. For this goal the effect of Aloe vera extract on inhibiting the Albumin glycosylation reaction and breaking the Albumin-glucose bond *in vitro* was surveyed with the use of TBA, a common method for detection and measurement of the Albumin glycosylation (Dolhofer *et al*., 2001).

MATERIALS AND METHODS

Preparation of alcoholic extract of Aloe vera: The fresh leaves of Aloe vera were prepared and after botanical expert's confirmation, then wash them out with the water and extracted their gel. The gel was homogenized and increased its volume to four times in to 95% ethanol. Aloe vera gel and alcohol were mixed on the shaker for 4 days. The extract was purred using a filter and then concentrated with rotary evaporator in the 45°C. The extract completely dried in 45°C and changed to the powder. Then, the concentrations of 0.1, 0.2, 0.5 and 1 g dL^{-1} with distilled water were prepared.

Glycosylation reaction of albumin: One milliliter of 30 g 100 mL^{-1} glucose solution was added to 1 mL of 5 g 100 mL^{-1} albumin solution and genetamycin with 0.2 g L^{-1} in 0.01 M phosphate buffer with pH = 7.4 and 3 mM sodium azide was added to prevent every contamination and was placed 72 h in a constant state in room temperature. It was dialyzed in phoshphate buffer after incubation time (dialyzed pack was prepared in the 10 mmol L^{-1} EDTA).

Measurement of glycosilation of albumin: To verify if Albumin was glycosilated or not, TBA (thiobarbituric acid) test was used. One milliliter trichloro acetic acid (TCA) solution with 20% concentration was added to the mixture (glucose and albumin) and then centrifuged in 3000 rpm

for 10 min. The supernatant solution was discarded two times. After that it was added 1 mL phosphate buffer and 0.5 mL oxalic acid with 0.01 M and 0.3 N concentrations respectively and then put it 1 h in a boiling water bath. After cooling in the lab temperature, 0.5 mL trichloroacetic acid 40% was added to each tube and after centrifuging for 10 min in 3000 rpm, the upper solution was separated and 0.5 mL TBA with 0.05 M concentration was added to 1 mL of it and then it was put in the water bath with 40°C temperature for half an hour. Finally sample absorption in the 443 nm wavelength was measured. The more absorption means the more gycosilation of Albumin.

Effect of Aloe vera extract on inhibiting albumin glycosilation reaction: There was 0.1 mL of each Aloe vera extract with different concentration (0.1, 0.2 , 0.5 and 1 g dL^{-1}) was added to 1 mL 5% Albumin solution and 1 mL 30 g L^{-1} glucose (in buffer phosphate solution with genetamycin) simultaneously and incubated 72 h in the lab temperature. TBA test was done to determine the effect of each concentration. In this study we considered absorption of the Albumin and glucose without the existence of extract as a control group. Reduction of absorption rate compared with that of the control group indicates the impact of Aloe vera extract on glycosilation reaction of Albumin.

Effect of Aloe vera extract on breaking bond between albumin and glucose: In this part, the reaction of Albumin glycosilation was first done and then different concentrations of the extract were added to it. The effect of each concentration on breaking the bond between Albumin and Glucose was assayed by TBA method after 24, 48, 72 and 144 h following adding the extract to the glucose-albumin solution.

Statistical data analysis: After the data were stored into the computer, they were processed by One-way ANOVA and Tukey's test (by 14th version of SPSS software). Measurement was repeated three times and the average of results was used in statistical analysis. And p values less than 0.05 were considered statistically significant.

RESULTS

Effect of extract on inhibiting the reaction: The results of the effect of Aloe vera extract's different concentrations on inhibiting the glycosilation reaction is shown in Fig. 1. According to this Fig. 1, the Aloe vera extract has an inhibitory effect in all concentrations except in 1 g dL^{-1} concentration. This effect was more obvious for 0.1and 0.2 g dL^{-1} concentrations compared with Groups 1 and 2 and the difference was significant ($p<0.05$). Maximum inhibitory effect was related to the concentration of 0.1 g dL^{-1} which greatly inhibits the reaction of glycosilation. Effect of different concentrations of Aloe vera extracts on inhibition of non-enzymatic reaction of Albumin glycosilation was: 0.1>0.2>0.5 g dL^{-1}. As it is seen there was an inverse relation between the extract concentration and glycosilation reaction. The inhibitory effect was greatest when there was the lowest concentration of the extract.

Effect of extract on breaking the albumin-glucose bond: The results of the effect of Aloe vera extract on breaking bonds between Albumin and glucose (glycosilated Albumin) are shown on Fig. 2-5. At 24 h treatment with different concentrations of extracts of Aloe vera, all concentrations except 0.2 g dL^{-1} concentration can break the Albumin-glucose bond significantly. The 0.2 g dL^{-1} concentration was not effective (Fig. 2). Fourty eight hour treatment with different

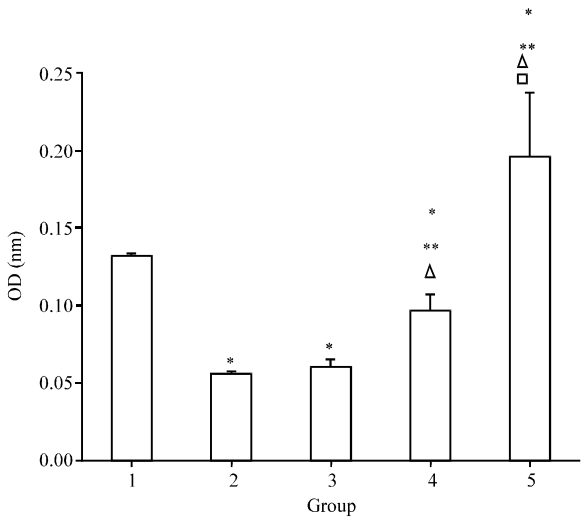

Fig. 1: Effect of different concentrations of Aloe vera extracts on inhibition of non-enzymatic reaction of Albumin glycosilation, Group 1: Control group (Albumin and glucose without the Aloe vera extract), Group 2: Simultaneous treatment (glucose, albumin and Aloe vera extract with 0.1 g dL^{-1} concentartion), Group 3: Simultaneous treatment (glucose, albumin and Aloe vera extract with 0.2 g dL^{-1} concentartion), Group 4: Simultaneous treatment (glucose, albumin and Aloe vera extract with 0.5 g dL^{-1} concentartion) and Group 5: Simultaneous treatment (glucose, albumin and Aloe vera extract with 1 g dL^{-1} concentartion), *: Significant difference with Group 1, **: Significant difference with Group 2, Δ: Significant difference with Group 3, □: Significant difference with Group 4

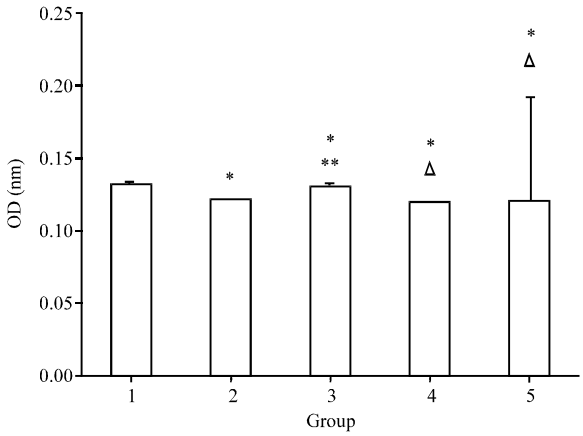

Fig. 2: Effect of different concentrations of Aloe vera extract on breaking the Albumin-glucose bond (24 h treatment of extract with glycosilated Albumin) ¥

concentrations of Aloe vera extract broke the bonds between Albumin and glucose significantly ($p<0.05$) and the maximum effect was in the 0.5 g dL^{-1} concentration (Fig. 3). Seventy two and 144 h treatment with different concentrations of extract of Aloe vera decreased the amount of glycosilated Albumin significantly (more bonds between Albumin and glucose were broken) ($p<00.05$) and the 0.1 and 0.5 g dL^{-1} concentrations have a more breaking rate ($p<0.05$) (Fig. 4, 5).

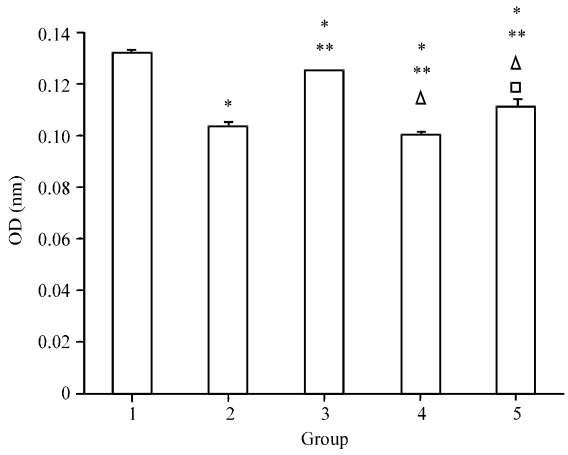

Fig. 3: Effect of different concentrations of Aloe vera extract on breaking the Albumin-glucose bond (48 h treatment of extract with glycosilated Albumin) ¥

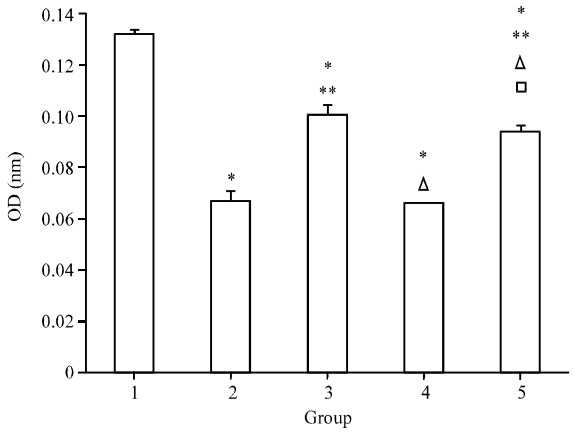

Fig. 4: Effect of different concentrations of Aloe vera extract on breaking the Albumin-glucose bond (72 h treatment of extract with glycosylated Albumin) ¥

According to these figures, its clear that breaking the bonds is time-dependent not concentration, it means that with increasing treatment time can be further broken link that 144 h treatment has a maximum effect and in other hands in all hours mentioned above, 0.1 and 0.5 g dL^{-1} concentrations have a maximum effect on breaking the bond between Albumin and glucose.

DISCUSSION

Diabetes mellitus is one of the common endocrine disorders in human societies and fighting against it imposes enormous costs to the healthcare system. Its most important and typical clinical sign is increase in the blood glucose which leads to non-enzymatic glycosilation of body proteins (free aldehyde groups of glucose or other sugars bind to free amine groups of non-conserved proteins) (Thorpe and Baynes, 1996). Since the cells and its extracellular matrix have a dynamic and reciprocal relationship, changes in matrix composition by glycosilation lead to changes in cell behavior such as those in cell trafficking and phosphorylation of key molecules in intracellular

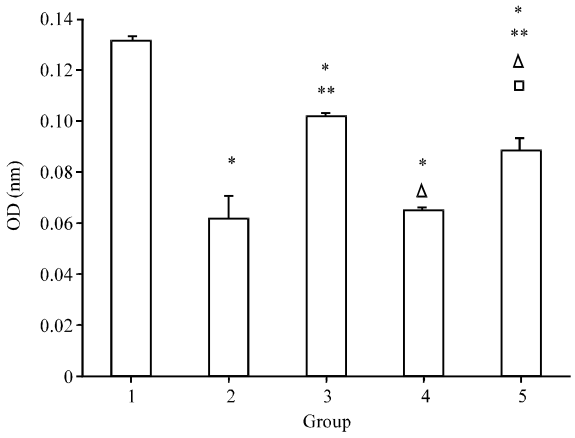

Fig. 5: Effect of different concentrations of Aloe vera extract on breaking the Albumin-glucose bond (144 h treatment of extract with glycosylated Albumin) ¥, Group 1: Control group (glycosylated Albumin without Aloe vera extract), Group 2: Effect of 0.1 g dL^{-1} concentration of extract on breaking the bond between Albumin and glucose, Group 3: Effect of 0.2 g dL^{-1} concentration of extract on breaking the bond between Albumin and glucose, Group 4: Effect of 0.5 g dL^{-1} concentration of extract on breaking the bond between Albumin and glucose and Group 5: Effect of 1 g dL^{-1} concentration of extract on breaking the bond between Albumin and glucose, *: Significant difference with Group 1, **: Significant difference with Group 2, Δ: Significant difference with Group 3, □: Significant difference with Group 4

signaling, expression of extracellular matrix proteins and their intermediaries. Extracellular matrix in patients with diabetes mellitus is much more glycosilated than that in non diabetic patients. Accumulation of sugar products and structural changes in the extracellular matrix in diabetes is associated with the development of performance problems (Rahbar *et al*., 1969). Considering these points, it seems the inhibition of non-enzymatic reaction of glycosilation is one of the most important ways to prevent long term complications of diabetes mellitus.

In the present study the effect of Aloe vera extract at 0.1, 0.2, 0.5 and 1 g dL^{-1} concentrations on the inhibition of Albumin glycosylation reaction (by adding the extract and glucose simultaneously to Albumin) was evaluated. Aloe vera extract has an inhibitory effect in all concentrations except in 1 g dL^{-1} concentration and the maximum effect was in the 0.1 g dL^{-1} concentration. Increasing the extract concentration lead to decrease in its effect on inhibiting Albumin glycosylation and 1 g dL^{-1} concentration had no effect on Albumin glycosylation reaction. As far as we know there are no similar published studies which can be compared with our findings. In another part of the study, the effect of Aloe vera extract at concentrations mentioned above on breaking bonds between albumin and glucose was evaluated. With increasing time of treatment with glycosylated albumin extract more links was broken which indicates that if Aloe vera extract consumption is continued, its impacts on the complications of diabetes will increase. So far, there have been several studies regarding factors that affect non-enzymatic glycosylation of serum proteins. In a study by Safari *et al*. (2002) that was performed on some herbal products, Volatile oils including pluegone, thymol, geraniol, linalool and limonene caused reduction in non-enzymatic glucosylation *in vitro*. In another study it was proved that turmeric, cardamom and ginger can

cause reduction in non-enzymatic Albumin glycosylation *in vitro* (Sheykh *et al.*, 2004). These studies are all consistent with present study findings. Because Aloe vera glycosidic compounds, are rich in catalase and vitamins E and C (Can *et al.*, 2004) and three anthranoids; barbaloin, isobarbaloin and aloenin maybe its hypoglycaemic effects is mediated by these components (Kuzuya *et al.*, 2001). Nowadays a lot of attention to the glycosylation reaction inhibitors for their therapeutic potential has been. Anti glycosylation compounds, probably by blocking carbonyl groups on reducing sugars, Amador products and 3-deoxyglucosones prevents the formation of developed sugar products that Aloe extract in this study probably act in this way. Medicines recently founded that break cross-links into advanced sugar products and reduce disturbances of diabetes mellitus (Ahmed, 2005; Hunt *et al.*, 1993). In this study Aloe vera also showed this effect, which to understanding the possible mechanisms needs subsequent studies. In other research the effect of different flavonoids in a non-enzymatic glycosilation reaction of proteins was evaluated. Flavonoids, routin, kaemferol, quercetin, apigenin, naringin, morin and biochanin A cause a decrease in non-enzymatic glycosilation of albumin, hemoglobin and insulin *in vitro* (Asgary *et al.*, 2004). Also in Delers *et al.* (1988) showed that some poly-phenolic compounds present in plants can reduce protein glycosylation in the blood, such as haptoglobin. One study indicates that S-allyl cysteine in garlic is an effective and major anti-glycosilation compound (Nakagawa *et al.*, 2002). In addition, other studies on anti-diabetic effects of Aloe vera have been done. First study on the hypoglycemic effect of Aloe species (Aloe vera) was conducted by Agarwal (1985) that was a special recipe which containes Aloe leaves to 31617 patients, twice daily for 5 years and signs of low blood sugar levels, triglycerides and total cholesterol reported.

CONCLUSION

In the present study the Aloe extracts imposed hypoglycemic and anti-glycosylation effects through the inhibition of non-enzymatic glycosylation reaction of albumin and through breaking the link between the Albumin and glucose that these effects can be attributed to the presence of compounds in this plant and with supplementary studies can use this plant as an effective medicine in treatment and prevention of diabetic disorders.

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