ORIGINAL ARTICLE

Beneficial effects of *Aloe* vera in treatment of diabetes: Comparative *in vivo* and *in vitro* studies

Amira Mourad Hussein Abo-Youssef *, **Basim Anwar Shehata Messiha**

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Sueif University, Egypt

Abstract In the present investigation, the antidiabetic effect of *Aloe* vera leaf pulp extract was studied *in vivo* and *in vitro* as compared to glimiperide. Diabetes was induced experimentally in adult male albino rats by single-dose intraperitoneal injection of streptozotocin (50 mg/kg body weight). The *in vitro* study was performed using isolated islets of pancreas from adult female albino rats.

Both aloe extract (10 ml/kg, p.o.) and glimiperide (10 mg/kg, p.o.) significantly decreased serum glucose and significantly increased serum insulin levels as compared to control diabetic rats. Serum levels of malondialdehyde (MDA) and superoxide dismutase (SOD) were significantly decreased while blood glutathione (GSH) was significantly increased by aloe treatment as compared to diabetic rats. Effect of aloe was better than the effect of glimiperide. Regarding the *in vitro* study, both aloe (10 μl/l) and glimiperide (10 μmol/l) significantly increased both basal and stimulated insulin secretion from the isolated islets of pancreas as compared to control. These results show a promising antidiabetic effect of aloe for further clinical trials regarding clinical use of aloe extract for treating type II diabetes.

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1. Introduction

Diabetes mellitus is a serious chronic metabolic disorder characterized by a decrease or cessation of insulin secretion in response to normal physiological stimuli, or reduced responsiveness of peripheral tissues to insulin.[1] The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, as well as in the myocardium. Therefore, oxidative stress plays a major role in the development of diabetes complications, both microvascular and cardiovascular complications.[2,3]

Many authors claimed for antidiabetic and antioxidant potential of aloe in experimentally-induced diabetes.[4,5] The glucose lowering effects of *Aloe vera* were suggested to be mediated, at least partially, through its potent anti-oxidant effect.[6,7] Recent approaches suggest that treatment of diabetes should not only focus on insulin secretion but also on antioxidant protection of the β-cell. This may facilitate the repair of β-cells undergoing damage by oxidative stress secondary to hyperglycemia.[8]

* Corresponding author. Address: 14 Bashar Ibn Bord St.Nasr City, Cairo, Egypt. Tel.: +20 022748371, mobile: +0122 24 24 884.
E-mail address: dramira73@hotmail.com (A.M.H. Abo-Youssef).

Based on these facts, this research is designed to correlate antioxidant and antidiabetic effects of aloe in experimentally-induced diabetes *in vivo*. The results will also be supported by an *in vitro* study on isolated pancreatic islets.

2. Materials and Methods

2.1. Animals

Adult male albino rats of Wistar strain weighing about 200–250 g were used for the *in vivo* experiments. Adult female albino rats of Wistar strain weighing 150–200 g were used for the *in vitro* experiments. Animals were obtained from the National Research Center, Cairo, Egypt. The animals were housed in plastic cages (28 cm × 43 cm × 18 cm) and were maintained under conventional laboratory conditions throughout the study. They were fed standard pellet chow (El-Nasr Chemical Co., Cairo, Egypt) and were allowed water *ad libitum*. Animals described as fasted had been deprived of food for 18 h but had been allowed free access to water. This design is accepted by the ethics committee in the Faculty of Pharmacy Beni Sueif University.

2.2. Preparation of A. vera leaf pulp extract

A. vera leaves, over 3 years old, were washed, weighed, peeled and the leaf pulp (gel together with latex) was scratched with a spoon.[5] The pulp was homogenized with a homogenizer (Ultra-Turrax T25, IKA Labortechnik, Germany), mixed with an equal volume of phosphate buffered saline (0.1 M, pH = 7), homogenized again, kept at 4 °C overnight then filtered through cloth. The clear filtrate was kept at −20 °C in small portions until use. The yield of fresh aloe pulp was about 35% v/w in terms of starting fresh leaf weight.

2.3. Induction of experimental diabetes

After fasting for 18 h, diabetes was induced by intraperitoneal injection of streptozotocin (Sigma, St. Louis, MO, USA) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 50 mg/kg.[9] The control rats received the vehicle alone. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. After 96 h of streptozotocin injection, rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

2.4. Isolation of rat pancreatic islets

Pancreatic islets from adult female albino rats[10] were isolated according to the collagenase digestion technique.[11] Batches of five islets were picked up using stereomicroscope and transferred into small tubes each containing 1 ml KRH buffer supplemented with 0.5% bovine serum albumin and glucose either 3 mmol/l (basal concentration) or 16.7 mmol/l (stimulatory concentration) and the test agent under study was added. The tubes were covered and incubated at 37 °C in a shaking water bath for 1 h with intermittent hand shaking every 15 min. At the end of incubation period the tubes were transferred into ice bath and mixed with vortex mixer and then aliquots of 0.5 ml were for insulin determination.

2.5. Experimental design in vivo

The rats were divided into four groups, each of six animals, as follows:

Group I: Normal control rats.
Group II: STZ-induced diabetic control rats.
Group III: Diabetic rats given glimiperide (10 mg/kg) in aqueous solution daily using an intragastric tube for 14 days.
Group IV: Diabetic rats given *A*. vera (10 ml/kg) daily using an intragastric tube for 14 days.

After 14 days of the treatment, Blood samples were collected from the retro – orbital venous plexus following the technique described by Coccheto and Bjornsson (1983).[12] Briefly, rats were subjected to light ether anesthesia then blood was collected using heparinized microhematocrit capillary tubes into Wassermann tubes. Serum was separated by centrifugation at 3000 rpm for 10–15 min for the determination of serum glucose, insulin, SOD and MDA levels. For the assessment of blood GSH level blood samples were hemolyzed by the addition of cold distilled water.

2.6. Experimental in vitro

Using isolated rat pancreatic islets, two main groups of experiments were performed to study the effects of *A*. vera (20 μg/ml) and glimiperide (10 μmol/l) on basal (3 mmol/l glucose) and stimulated- insulin secretion (16.7 mmol/l glucose).

2.7. Determination of serum glucose and insulin

Fasting serum glucose was estimated by glucose oxidase method.[13] Insulin in samples, either from *in vitro* or *in vivo* experiments was estimated using Enzyme Linked Immunosorbent Assay (ELISA).[14]

2.8. Determination of serum malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH)

Serum MDA was estimated by the method of Mihara and Uchiyama (1978).[15] SOD activity was measured based on the ability of the enzyme to inhibit the autoxidation process of pyrogallol method of Marklund and Marklund (1974).[16] Glutathione was estimated by the method of Beutler et al. (1963).[17]

2.9. Statistical analysis

Data were expressed as the mean ± standard error of the mean (s.e.m); and comparison between the different treatments was carried out using one way ANOVA followed by Tukey–Kramer multiple comparisons test. Significance was accepted at $p < 0.05$.

3. Results

3.1. Effect of two weeks daily dose administration of A. vera and glimiperide on serum glucose and insulin levels of streptozotocin-induced diabetic male rats

Table 1 shows the level of serum glucose and serum insulin in normal and STZ induced diabetic rats. There was a significant

Table 1 Effect of two weeks daily dose administration of *Aloe vera* and glimiperide on serum glucose and insulin levels of streptozotocin-induced diabetic male rats.

Groups	Serum glucose level (mg/dl)	Serum insulin level (ng/ml)
Normal control	82.24 ± 5.17	7.05 ± 0.76
Diabetic control	$331.88 \pm 29.72^{*}$	$2.82 \pm 0.66^{*}$
Diabetic + *A. vera*	93.66 ± 26.92^{a}	6.78 ± 0.98^{a}
Diabetic + glimiperide	117.43 ± 21.96^{a}	6.26 ± 0.65^{a}

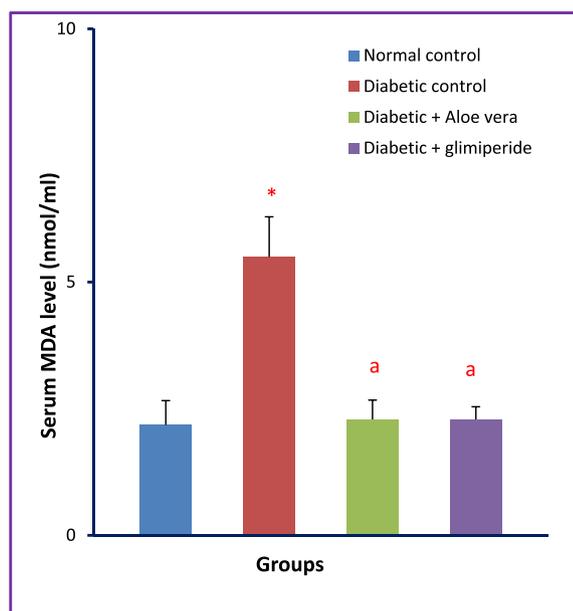Values are given as mean $\pm$ s.e.m for groups of six animals each.
Values are statistically significant at $p < 0.05$.
Diabetic control was compared to normal control rats.
Diabetic + *A. vera* and diabetic + glimiperide were compared with diabetic control.
[*] Significantly different from the normal control.
[a] Significantly different from the diabetic control.

Values are given as mean ± s.e.m for groups of six animals each.

Values are statistically significant at p < 0.05.

Diabetic control was compared to normal control rats.

Diabetic + Aloe vera and diabetic + glimiperide were compared with diabetic control.

[*] significantly different from the normal control.

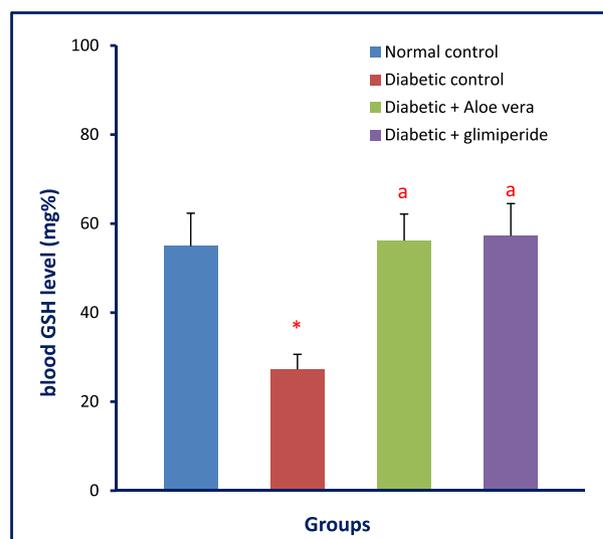[a] significantly different from the diabetic control.

Figure. 1 Effect of two weeks daily dose administration of *Aloe vera* and glimiperide on serum MDA level of streptozotocin-induced diabetic male rats.

Values are given as mean ± s.e.m for groups of six animals each.

Values are statistically significant at p < 0.05.

Diabetic control was compared to normal control rats.

Diabetic + Aloe vera and diabetic + glimiperide were compared with diabetic control.

[*] significantly different from the normal control.

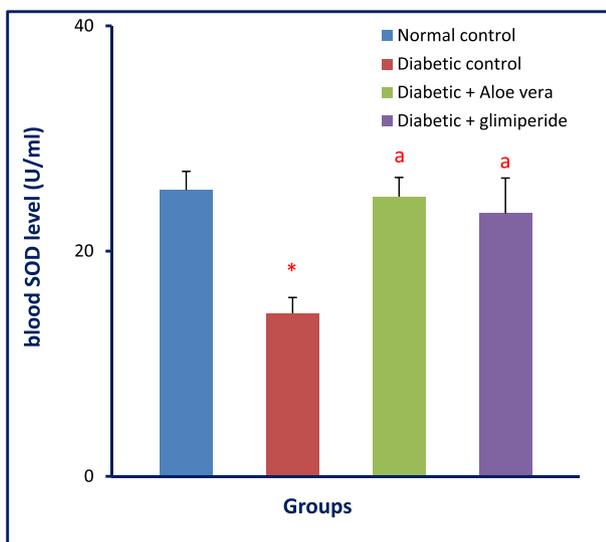[a] significantly different from the diabetic control.

Figure. 2 Effect of two weeks daily dose administration of *Aloe vera* and glimiperide on blood GSH level of streptozotocin-induced diabetic male rats.

elevation in serum glucose level and significant decrease in serum insulin level during diabetes when compared with corresponding control group.

Administration of *A. vera* and glimiperide tended to bring the values of serum glucose and insulin levels to near normal.

3.2. Effect of two weeks daily dose administration of A. vera and glimiperide on serum MDA, blood GSH and blood SOD levels of streptozotocin-induced diabetic male rats

Results are graphically illustrated in Figs. 1–3. There was a significant increase in serum MDA and significant decrease in blood GSH and SOD during diabetes as compared to

corresponding control group. Administration of *A. vera* gel extract and glimiperide significantly decreased serum MDA level and significantly elevated blood GSH and SOD levels.

3.3. Effect of A. vera and glimiperide on basal and stimulated-insulin secretion from isolated pancreatic islets of female rats

Results are graphically illustrated in Figs. 4 and 5. These graphs show the effect of *A. vera* and glimiperide on basal and stimulated- insulin secretion from isolated pancreatic islets of female rats.

Both *A. vera* and glimiperide significantly raised both basal and stimulated- insulin secretion as compared to the normal control value (3 mmol/l glucose).

Values are given as mean ± s.e.m for groups of six animals each.

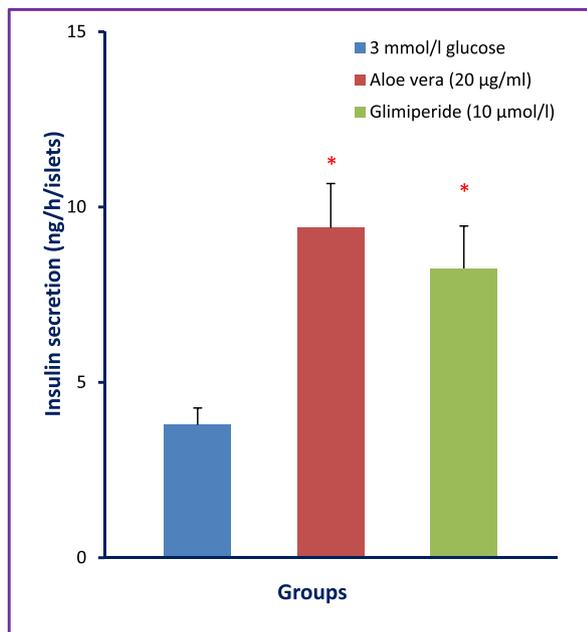Values are statistically significant at p < 0.05.

Diabetic control was compared to normal control rats.

Diabetic + Aloe vera and diabetic + glimiperide were compared with diabetic control.

* significantly different from the normal control.

a significantly different from the diabetic control.

Figure. 3 Effect of two weeks daily dose administration of *Aloe vera* and glimiperide on blood SOD level of streptozotocin-induced diabetic male rats.

Values are expressed as mean ± s.e.m for groups of six animals each.
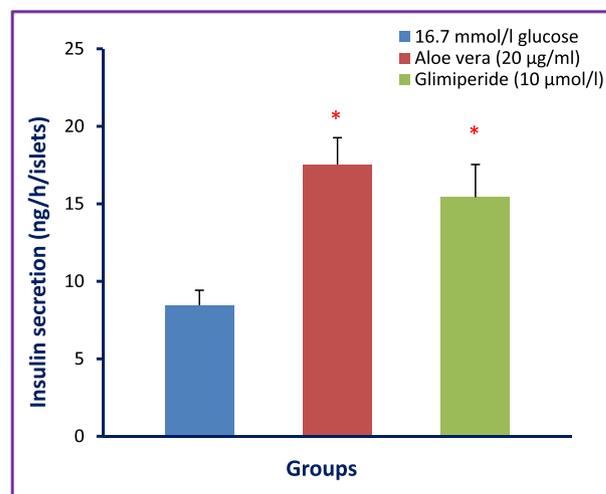
Values are statistically significant at p < 0.05.

*Significaly different from normal control.

Figure. 4 Effect of *Aloe vera* and glimiperide on basal insulin secretion from isolated pancreatic islets of female rats.

4. Discussion

Results of the present investigation strongly suggest an antidiabetic potential for aloe. *In-vivo* results show decreased serum glucose and increased serum insulin levels in aloe-treated rats compared to diabetic controls (Table 1). In agreement, previous authors claimed for such antidiabetic effect for aloe extract on experimental animals.[18,19] Our *in vitro* results support this finding, where aloe was found to increase the rate of insulin secretion from pancreatic islets (Figs. 4 and 5).

Many explanations were suggested for this antidiabetic effect of aloe. The first explanation is the potent antioxidant effect of aloe extract. Aloe is long known to have antioxidant potential via suppression of free radical formation and enhancement of cellular thiol status.[20–22] It is also reported to stimulate glutathione-S-transferase enzyme activity.[23] Our results strongly supported the antioxidant potential of aloe, where it was found to suppress elevated serum MDA levels and increase blood GSH and SOD levels. Recent approaches focus on the role of oxidative stress in pancreatic beta cell damage.[8,24] That is, oxidative stress is involved as a causative factor in the pathogenesis of diabetes, and hence antioxidants like aloe may have a true antidiabetic effect via antioxidant potential.

The anti-inflammatory potential of aloe may be the second explanation for its antidiabetic effect. Diabetes may be considered as an inflammatory disease where inflammation participates in the progression of diabetes, where tumor necrosis factor-α was found to decrease peripheral insulin sensitivity.[25] Many authors claimed for the anti-inflammatory potential of aloe due to many of its components like emodin and mannose-6-phosphate.[26,27] It was reported that the anti-inflammatory effect of aloe extract is comparable to that of hydrocortisone.[28]

Finally, aloe may act as a hypoglycemic agent through potent inhibition of pancreatic α-amylase activity.[29] This action decreases starch breakdown and offers good postprandial glycemic control.

These findings are promising for further clinical studies on aloe extract or extract components in the management of diabetes mellitus.

Values are expressed as mean ± s.e.m for groups of six animals each.

Values are statistically significant at p < 0.05.

*Significaly different from normal control.

Figure. 5 Effect of *Aloe vera* and glimiperide on stimulated-insulin secretion from isolated pancreatic islets of female rats.

5. Conflict of interest

None.

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