Aloe vera Leaf Gel in Treatment of Advanced Type 2 Diabetes Mellitus Needing Insulin Therapy: A Randomized Double-Blind Placebo-Controlled Clinical Trial

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Abstract

Background: Advanced type 2 diabetes mellitus (T2DM) needing insulin therapy is a common disease. Previous studies indicate that aloe (*Aloe vera* L.) leaf gel may positively affect the blood glucose and lipid levels in patients with advanced T2DM needing insulin.

Objective: Evaluation of the efficacy and safety of aloe leaf gel in the treatment of type 2 diabetic patients resistant to oral synthetic anti-hyperglycemic drugs needing insulin.

Methods: In this randomized double - blind placebo-controlled clinical trial with the patients aged 40-60 years, the efficacy and safety of taking aloe leaf gel (one 300 mg capsule every 12 hours for 2 months) combined with oral synthetic anti-hyperglycemic drugs in treatment of 35 patients were evaluated and compared with the placebo group (n = 35).

Results: The aloe leaf gel lowered the blood levels of fasting glucose and glycosylated hemoglobin significantly (p = 0.041 and p = 0.023 respectively) without any significant effects on the lipid profile and liver/kidney function tests (p > 0.05) compared with placebo at the endpoint. No adverse effects were reported.

Conclusion: The results suggest that aloe leaf gel may safely improve glycemic control in patients with advanced T2DM needing insulin.

Keywords: *Aloe vera*, Glycemic control, Patient, Type 2 diabetes

Introduction

Type 2 diabetes mellitus (T2DM) is a common disease [1]. If T2DM is not duly treated, it will lead to microvascular, macrovascular and other complications. The complications are the main causes of morbidities and mortalities due to T2DM [2, 3]. While insulin and oral anti-hyperglycemic drugs such as sulfonylureas, biguanides, thiazolidinediones, meglitinides, alpha-glucosidase and DPP-4 inhibitors are the cornerstone of T2DM treatment, they have limited efficacies and important adverse effects. Thus, more efficacious and safer anti-hyperglycemic agents are needed [4, 5]. Multiple anti-hyperglycemic drugs with different mechanisms are often used for effective treatment of type 2 diabetic patients [6]. T2DM is a progressive disease, characterized by a progressive decline of β-cell function up to its exhaustion so that adequate glycemic control with a logical combination of oral therapies cannot be achieved which leads to the need of insulin as sole therapy. Up to 50% of type 2 diabetic patients initially treated with oral anti-hyperglycemics ultimately need insulin [7].

Plants have played a significant role in maintaining human health and improving the quality of life for thousands of years [8]. Herbal supplements may be effective in prevention and treatment of diseases [9, 10, 11]. Currently, there is renewed interest in plant-based medicines and functional foods modulating physiological effects in the prevention and cure of diabetes. The plant kingdom is a wide field to search for natural effective oral anti-hyperglycemic agents that have slight or no side effects. More than about 1200 plant species have been recorded to be used empirically for their alleged anti-hyperglycemic activity [12]. Aloe (*Aloe vera* L., Liliaceae family) leaves have long been used in the traditional medicines of various cultures to treat numerous diseases such as diabetes mellitus [13, 14]. A variety of pharmacological effects have been demonstrated for aloe leaf preparations [13]. For example, aloe gel lowered the blood triglyceride level in a mouse model of T2DM [15]. Further, Aloe gel reduced the blood cholesterol, triglyceride, low density lipoprotein (LDL) and very low density lipoprotein levels, but increased the blood high density lipoprotein (HDL) level in streptozocin-induced diabetic rats [16]. A controlled clinical trial (n = 60) indicated decreased blood total cholesterol, triglyceride and LDL levels with 12 weeks of two different doses of aloe gel in two groups of hyperlipidemic patients compared with baseline. Since this trial was available as an abstract only, neither intergroup comparisons nor randomization nor blinding were mentioned [17]. Reports concerning the effects of aloe on the blood glucose levels in animal models have been inconsistent [18 - 22]. Two nonrandomized clinical trials (n = 76 and n = 40) are available from the same investigator group that reported decreased fasting blood glucose and triglyceride levels, but no change in the cholesterol level with 6 weeks of juice made from aloe gel in type 2 diabetic patients. The blood lipid levels were not the primary endpoints in the trials [21, 23]. Case reports of five type 2 diabetic individuals reported decreases in fasting blood glucose and glycosylated hemoglobin (HbA1c) levels [24]. No adverse effects were reported in these trials. The trials had methodological drawbacks

such as small sample sizes and lack of randomization and double-blindness. Moreover, in a recent trial, aloe gel was a safe anti-hyperglycemic and anti-hypercholesterolemic agent in hyperlipidemic (hypercholesterolemic and/or hypertriglyceridemic) type 2 diabetic patients resistant to daily intake of two 5 mg glyburide and two 500 mg metformine tablets. Need for insulin to control glycemia was not among patients inclusion criteria [25]. Preliminary data suggest a potential effect of aloe gel on the glycemic control and lipid profile in type 2 diabetic patients [13, 14]. Little research has been conducted on the effects of aloe gel in the treatment of type 2 diabetic patients so that further and better trials are needed [13, 14]. Thus, the effects of aloe gel in the treatment of advanced stage of T2DM needing insulin therapy were evaluated in the present study. This work was conducted in parallel with the study reported earlier [25].

Methods

Aloe and preparation of the aloe leaf gel powder

The freshly harvested whole aloe leaves obtained from the Research Institute of Medicinal Plants (Karaj, Iran) were washed in a suitable bactericide (chlorhexidine). 1 inch of the leaf base, 2 inches of the tapering point and sharp spines located along the leaf margins were removed by a knife. The skin was carefully separated from the parenchyma. The filets were extensively washed with distilled water to remove the exudates from their surfaces, then the filets were ground to a liquid and the pulp was removed by filtering. The gel obtained was treated with activated carbon to decolorize the gel and remove aloin and anthraquinones, which have laxative effects. The resultant gel was then freeze-dried so that a pure powder was produced [26].

Determination of the acemannan content of the gel powder by HPTLC analysis

The acemannan content of the gel powder was analyzed by HPTLC according to the method described previously [27]. A stock solution of acemannan (1000 mg/mL) was prepared in water. Different concentrations of the stock solution (10, 20, 40 and 80 mg/mL) were obtained by water dilution. 10 µL of each of them were spotted in triplicate on TLC plates so as to obtain concentrations of 100, 200, 400 and 800 ng per spot of acemannan, respectively.

The data of peak areas versus acemannan masses were treated by the linear least square regression method. 1mg of aloe powder dissolved in 10 mL of water was used for quantification of acemannan. 10 µL of each of the concentrations of standard solutions were spotted in triplicate on HPTLC plates. Chromatograms were developed for 10 cm using n-butanol: n-propanol: glacial acetic acid: water (30: 15: 10: 5 v/v/v/v). After development, the plates were sprayed with anisaldehyde sulfuric acid reagent and the spots were detected by heating the plate at 105-110°C for 3 min. The sprayed plates were scanned at 600 nm. The calibration curve of acemannan was obtained by plotting peak areas for different concentrations of acemannan applied. 10 µL of each of the sample solutions was spotted in triplicate on HPTLC plates. Chromatograms were developed, scanned and the peak areas recorded. The amount of acemannan in the sample was calculated by the calibration curve of acemannan.

Preparation of the aloe gel powder and placebo capsules

The aloe gel powder as the drug and toast powder as the placebo were separately filled into oral gelatin capsules with identical appearance by using a hand-operated capsule-filling machine (Scientific Instruments and Technology Corporation, USA). The aloe capsules contained 300 mg of the aloe gel powder. Toast powder was chosen as the placebo, because its appearance was relatively similar to the aloe gel powder.

Patients
Inclusion criteria

Iranian male and female type 2 diabetic patients; patients aged 40 to 60 years; patients with fasting serum glucose levels from 150 mg/dL to 250 mg/dL; patients with blood glycosylated hemoglobin levels from 7.5% to 10%; patients resistant to the combination of oral synthetic anti-hyperglycemic drugs (glyburide, metformin, gliclazide, acarbose, pioglitazone and repaglinide) needing insulin therapy but refusing it.

Exclusion criteria

Patients taking other anti-hyperglycemic and anti-hyperlipidemic agents; patients receiving insulin therapy; patients with cardiac, renal, hepatic, hematological diseases, hypothyroidism, tachycardia, vertigo and seizure; patients with a history of gallstones or gall bladder surgery; patients using estrogen, steroid, beta-blocker and thiazide; pregnant women; women planning pregnancy; breast-feeding women.

Protocol

Seventy Iranian male and female outpatients who were eligible according to the inclusion and exclusion criteria participated in this study. The demographical data of the subjects are given in the table 1. A group of thirty five patients took the aloe capsules at the dose of one 300 mg capsule every 12 hours by the oral rout for 2 months and another concurrently parallel group of thirty five patients took the placebo capsules orally every 12 hours for 2 months. The dosage of the aloe gel was based on the results of a dose finding study. Block randomization was used for treatment allocation. The study was double-blind. Further, the patients were recommended to restrict intake of carbohydrates such as rice and confectionery from two months before the beginning of the trial onward. All the subjects recorded the names and amounts of the daily consumed foods for 3 days every week. To monitor the patients' compliance with the allocated treatments, the patients returned any capsules left and were asked questions about taking the capsules on their monthly visit. The

Table 1- The demographical data of the subjects who participated in the trial. The data are given as mean ± SD.

Parameter	Aloe group	Placebo group
Age (years)	55.1 ± 11	52.9 ± 10.8
Gender	17 males, 18 females	15 males, 20 females
Duration of type 2 diabetes (years)	10.6 ± 9	9.2 ± 5.6
Body mass index (kg/m^2)	27.3 ± 6.2	29.1 ± 5.3

treatment, diet and physical activity of the patients remained unchanged throughout the study. At the beginning and also the end of the study, the fasting (after fasting for 12 hours) blood levels of glucose, HbA1c , creatinine, BUN (blood urea nitrogen), AST (aspartate aminotransferase), ALT (alanine aminotransferase), AP (alkaline phosphatase), GGT (γ-glutamyl transpeptidase), total bilirubin, direct and indirect bilirubins levels and fasting serum levels of triglycerides, total cholesterol, VLDL, LDL and HDL in the aloe and placebo groups were determined with standard enzymatic kits produced by the Pars Azmoon company (Tehran, Iran) and an auto analyzer (Hitachi 902, Japan). The baseline homogeneity of the blood parameter means across the aloe and placebo groups was analyzed by the independent samples t-test and P values below 0.05 were considered as significant. The data of the aloe and placebo groups at the end of the study were compared by independent samples t-test and P values below 0.05 were considered as significant. All participants were requested to report any adverse effects. Written informed consent was obtained from the patients. The medical ethics committee of the Ebne Sina Research Institute affiliated with the ACECR approved the protocol. Further, the trial was registered in the Iranian Registry of Clinical Trials with the number IRCT138706161157N2.

Results

Determination of the acemannan content of the gel powder by HPTLC analysis

Linearity was shown for acemannan between 100 and 800 ng/spot by 4 different concentrations of the acemannan standard. The equation $y = 1.943 \, x + 7.103$ ($R^2 = 0.999$) with the applied acemannan mass x and the area y was obtained (Fig. 1). 356 mg/g of acemannan was determined in the gel powder.

Patients

All the subjects finished the study. Further, they did not report any adverse effects. The demographical data of the subjects in the aloe and placebo groups were not significantly different from each other (Table 1). The baseline blood levels of all parameters were not significantly different between the two groups (Table 2) ($p > 0.05$).
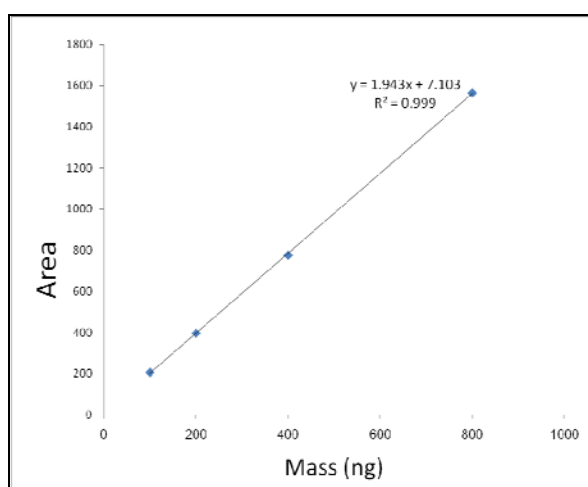

Fig. 1- Regression curve of acemannan between 100 and 800 ng/spot

Table 2- The fasting blood glucose and glycosylated hemoglobin (HbA1c) levels before and after intervention and their changes during the study. ^ap<0.05 significant (independent samples t-test). SD, standard deviation; ↓ decrease; ↑ increase

|		Mean (SD) before	P value	Mean (SD) after	P value	Percent change
Fasting blood glucose	Aloe	182.22 (35.76)	0.78	169.19 (36.84)	0.041a	3.67 (16.55) ↓
	Placebo	179.97 (34.83)		190.22 (48.12)		10.01 (22.80) ↑
HbA1c	Aloe	7.25 (1.10)	0.62	6.88 (1.05)	0.023a	6.46 (15.10) ↓
	Placebo	7.10 (1.35)		7.65 (1.66)		6.39 (22.40) ↑

The aloe gel lowered the fasting blood glucose and HbA1c levels significantly (p = 0.041 and p = 0.023 respectively) without any significant effects on the other parameter levels (p > 0.05) compared with the placebo group at the endpoint (Table 2). The percentages of endpoint reductions of the fasting blood glucose and HbA1c levels in the aloe group compared with the baseline levels were 3.67% and 6.46% respectively. The box plots of decreases (before intervention – after intervention) in the fasting blood glucose and HbA1c levels of the aloe and placebo groups are shown in the Figures 2 and 3.

Discussion

The results suggest that aloe gel improves glycemic control without any effects on the lipid profile and hepatic, renal or other adverse effects in the type 2 diabetic patients. The patients in the present trial were with advanced stage of diabetes and needed insulin which is in marked contrast to the trial reported earlier [25]. Thus, the results indicate that aloe gel has also beneficial effect on glycemic control in patients with advanced T2DM needing insulin. The improved glycemic control agree with the previous trials [21, 23, 24, 25], whereas the

lack of effect on the lipid profile disagree with the earlier trials [17, 21, 23, 25]. The lack of effect on the lipid profile may be because [1] hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) was not among the inclusion criteria and/or [2] the sample size of the present trial was small.

The only bioactive that was identified and quantified in the aloe gel used in the present trial was a mucopolysaccharide named acemannan. Further, the bioactives and mechanisms involved in the anti-hyperglymic action of the aloe gel were not studied in the clinical trial presented here. However, very few studies have been conducted on the characterization of the bioactives and mechanisms mediating the anti-hyperglycemic action of the aloe gel. Trace elements present in the gel and five phytosterols isolated from the gel were responsible for the anti-hyperglycemic effects of the gel in the streptozocin-induced diabetic rats and a mouse model of T2DM respectively [26, 27]. Further, aloe gel appeared to decrease insulin resistance in two mouse models of T2DM [15, 29]. Thus, considering the results of the present and previous trials, further clinical trials concerning the safety and efficacy of aloe gel
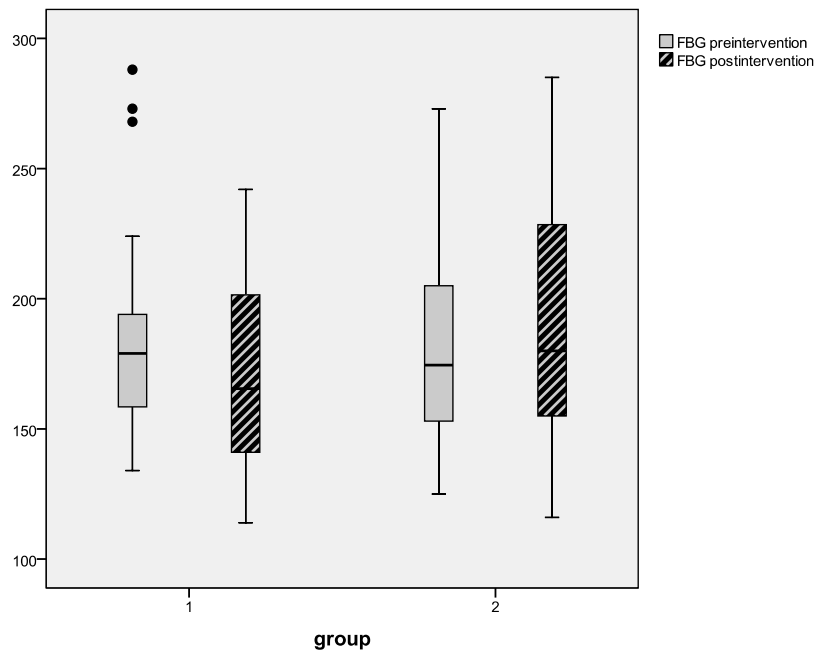
Fig. 2- Box plot of decreases (before intervention – after intervention) in the fasting blood glucose (FBG) levels (mg/dL) of the aloe (group 1) and placebo (group 2) groups
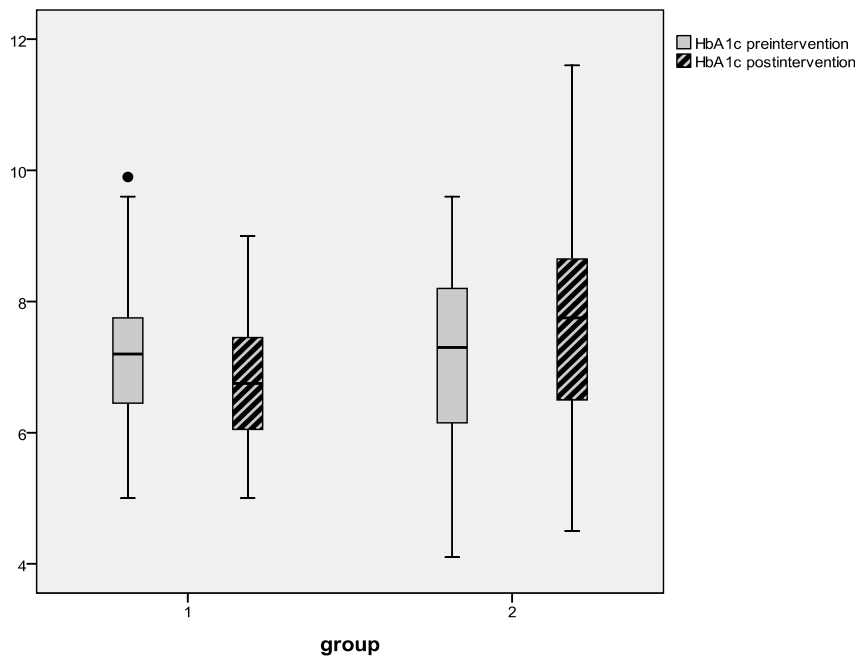
Fig. 3- Box plot of decreases (before intervention – after intervention) in the blood glycosylated hemoglobin (HbA1c) levels (percent) of the aloe (group 1) and placebo (group 2) groups

in the treatment of patients with T2DM and/or hyperlipidemia as well as more studies addressing the bioactives and mechanisms involved in the anti-hyperglycemic and ant-hyperlipidemic actions of aloe gel seem necessary.

Acknowledgements

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