Anti-hyperglycemic and Anti-hypercholesterolemic Effects of *Aloe vera* Leaf Gel in Hyperlipidemic Type 2 Diabetic Patients: A Randomized Double-Blind Placebo-Controlled Clinical Trial

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Abstract
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Diabetes mellitus type 2 with dyslipidemia is a common disease. Previous studies suggest that aloe (*Aloe vera* L.) leaf gel may positively affect the blood glucose and lipid levels in dyslipidemic type 2 diabetic patients. Thus, in this randomized double-blind placebo-controlled clinical trial with hyperlipidemic (hypercholesterolemic and/or hypertriglyceridemic) type 2 diabetic patients aged 40 to 60 years not using other anti-hyperlipidemic agents and resistant to daily intake of two 5 mg glyburide tablets and two 500 mg metformin tablets, the efficacy and safety of taking aloe gel (one 300 mg capsule every 12 hours for 2 months) combined with the aforementioned drugs in treatment of 30 patients were evaluated and compared with the placebo group (n = 30). The aloe gel lowered the fasting blood glucose, HbA1c, total cholesterol, and LDL levels significantly (p = 0.036, p = 0.036, p = 0.006, and p = 0.004, respectively) without any significant effects on the other blood lipid levels and liver/kidney function tests (p > 0.05) compared with the placebo at the endpoint. No adverse effects were reported. The results suggest that aloe gel may be a safe anti-hyperglycemic and anti-hypercholesterolemic agent for hyperlipidemic type 2 diabetic patients.

Introduction
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Type 2 diabetes mellitus (T2DM) is common worldwide [1]. Type 2 diabetic patients frequently have dyslipidemia [increased low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and triglycerides, and decreased high-density lipoprotein (HDL)]. The lipid changes are attributed to increased free fatty acid flux secondary to insulin resistance. Dyslipidemia is one of the major risk factors for premature cardiovascular morbidity and mortality in the T2DM patients [2,3]. Multiple anti-hyperglycemic and anti-hyperlipidemic drugs with different mechanisms are often needed for effective treatment of hyperlipidemic type 2 diabetic patients [4,5]. Conventional anti-hyperglycemic and anti-hyperlipidemic drugs have limited efficacies and important adverse effects. Thus, more efficacious and safer anti-hyperglycemic and anti-hyperlipidemic agents are needed [6,7]. 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 [9–11]. Cur-

rently, there is renewed interest in the 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) is a popular traditional remedy for numerous diseases such as diabetes mellitus in several cultures [13,14]. A variety of pharmacological effects have been demonstrated for aloe leaf preparations [13]. For example, aloe gel lowered the blood triglycerides level in a mouse model of T2DM [15]. Further, aloe gel reduced the blood cholesterol, triglycerides, LDL and VLDL levels, but increased the blood HDL level in streptozocin-induced diabetic rats [16]. A controlled clinical trial (n = 60) indicated decreased blood total cholesterol, triglycerides, and LDL levels after 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 on the effects of aloe in the animal models of diabetes have been inconsistent [18–22]. Two nonrandomized clinical trials (n = 76 and n = 40) are available from the same investigation group that reported decreased fasting blood glucose and triglycerides levels, but no change in the cholesterol level after 6 weeks of juice made from aloe gel in type 2 diabetic patients. The blood lipid levels were not the primary outcomes in the trials [21, 23]. Case reports of five type 2 diabetic individuals reported decreases in fasting blood glucose and glycosylated hemoglobin (HbA1c) levels [18]. No adverse effects were reported in these trials [17, 18, 21, 23]. The trials had methodological drawbacks such as lack of randomization, lack of double-blindness, small sample size, and lack of power calculation [13, 14]. The preliminary data suggest a potential effect of aloe in glycemic control and hyperlipidemia; however, further information is needed [13, 14].

In conclusion, further and better trials are needed to define the clinical efficacy and safety of aloe in the treatment of diabetes mellitus and hyperlipidemia more precisely. Thus, the efficacy and safety of aloe gel in the treatment of hyperlipidemic (hypercholesterolemic and/or hypertriglyceridemic) type 2 diabetic patients were evaluated and compared with placebo in the study presented here.

Materials and Methods
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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 [24].

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 [25]. 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. 1 mg 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). 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 outpatients aged 40 to 60 years; patients with fasting blood levels of glucose between 150 mg/dL to 200 mg/dL and HbA1c between 7% to 9% despite taking two 5 mg glyburide tablets and two 500 mg metformin tablets every day; patients using two 5 mg glyburide tablets and two 500 mg metformin tablets every day; newly diagnosed patients with fasting blood LDL and/or triglycerides levels above 100 mg/dL and 150 mg/dL, respectively.
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
Sixty-seven Iranian male and female outpatients (thirty-three patients in the aloe group and thirty-four patients in the placebo group) were recruited according to the inclusion and exclusion criteria. Thirty patients in each group finished the trial which was the sample size calculated to estimate 25 mg/dL difference of total cholesterol between the groups, considering type I error = 0.05 and 80% power. The CONSORT flowchart describing the progress of the patients through the trial is shown in ● **Fig. 1**. The demographic data of the subjects who finished the trial are given in ● **Table 1**.
A group of thirty patients took the aloe capsules at the dose of one 300 mg capsule every 12 hours by the oral route for 2 months, and another concurrently parallel group of thirty 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 and fatty foods such as rice, confectionery, red meat, pies, cream pies, eggs, and fatty dairy products 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 treatment, diet, and physical activity of the patients completing the trial 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 nitro-

Fig. 1   The CONSORT flowchart describing the progress of the patients through the trial.
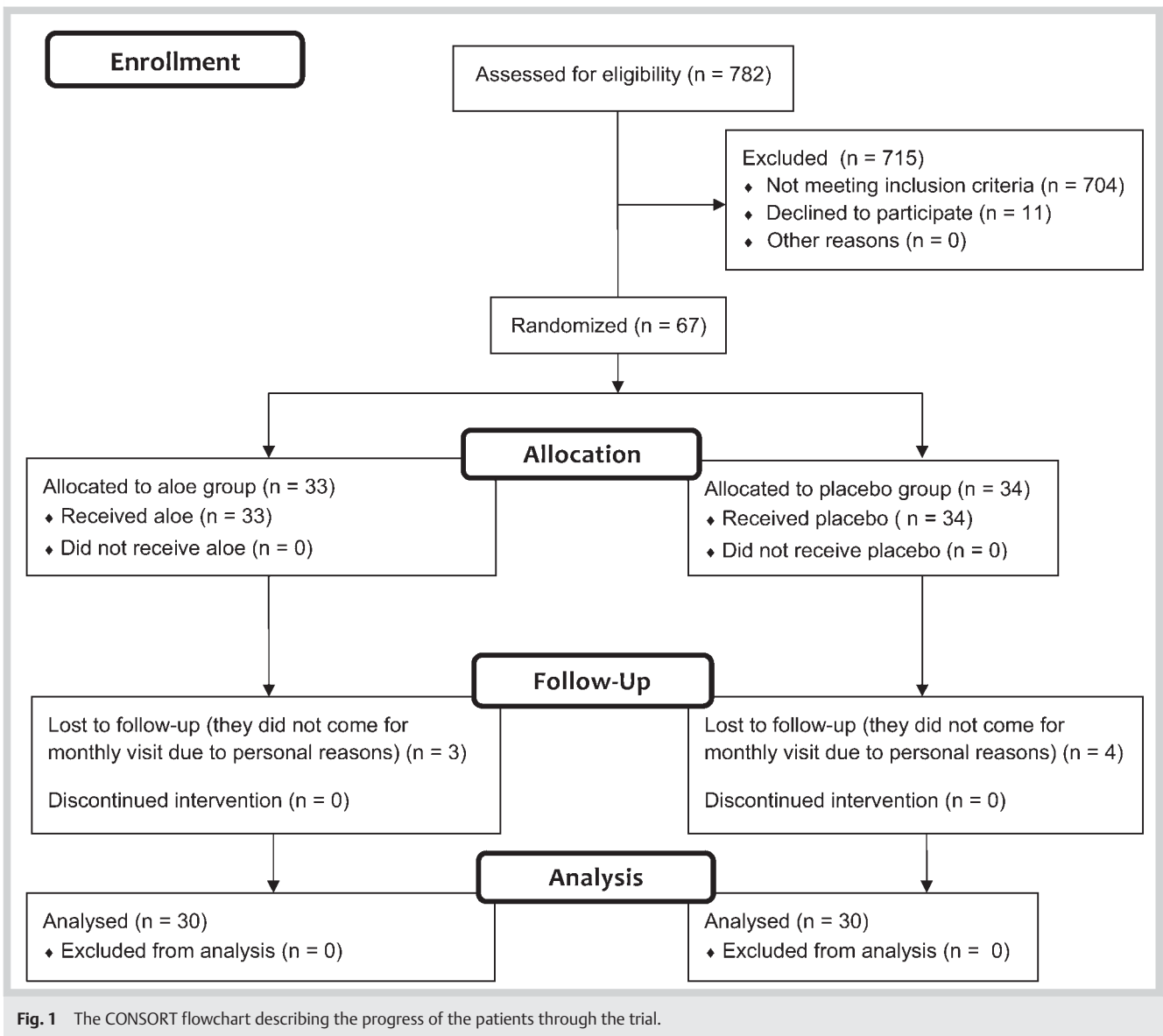
gen), AST (aspartate aminotransferase), ALT (alanine aminotransferase), AP (alkaline phosphatase), GGT (γ-glutamyl transpeptidase); total, direct, and indirect bilirubins; triglycerides, total cholesterol, VLDL, LDL, and HDL in the aloe and placebo groups were determined with standard enzymatic kits produced by the Pars Azmoon company and an auto analyzer (Hitachi 902). The glucose, HbA1c, and lipid levels were the primary outcome variables. The other blood parameter levels were the secondary outcome variables. The baseline homogeneity of the blood parameter means across the aloe and placebo groups was analyzed by the Mann-Whitney U test, and p values below 0.05 were considered as significant. At the end of the study, the data of the patients in the aloe and placebo groups were also compared by the Mann-Whitney U test and p values below 0.05 were considered as significant. All statistical analyses were per-protocol analyses. 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 (approval number and date: 492/51/29 and 23 Nov. 2008). Further, the trial was reg-

istered in the Iranian Registry of Clinical Trials with the number IRCT138706161157N2.

Results

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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. 2**). 356 mg/g of acemannan was determined in the gel powder.

No adverse effects were reported. The groups were matched in regard to demographic data (age, gender, duration of diabetes, and body mass index) (○ **Table 1**). The baseline blood levels of all parameters were not significantly different between the two groups ($p > 0.05$) (○ **Table 2**).

The aloe gel lowered the glucose, HbA1c, total cholesterol, and LDL levels significantly ($p = 0.036$, $p = 0.036$, $p = 0.006$, and $p = 0.004$, respectively) without any significant effects on the other parameter levels ($p > 0.05$) compared with the placebo group
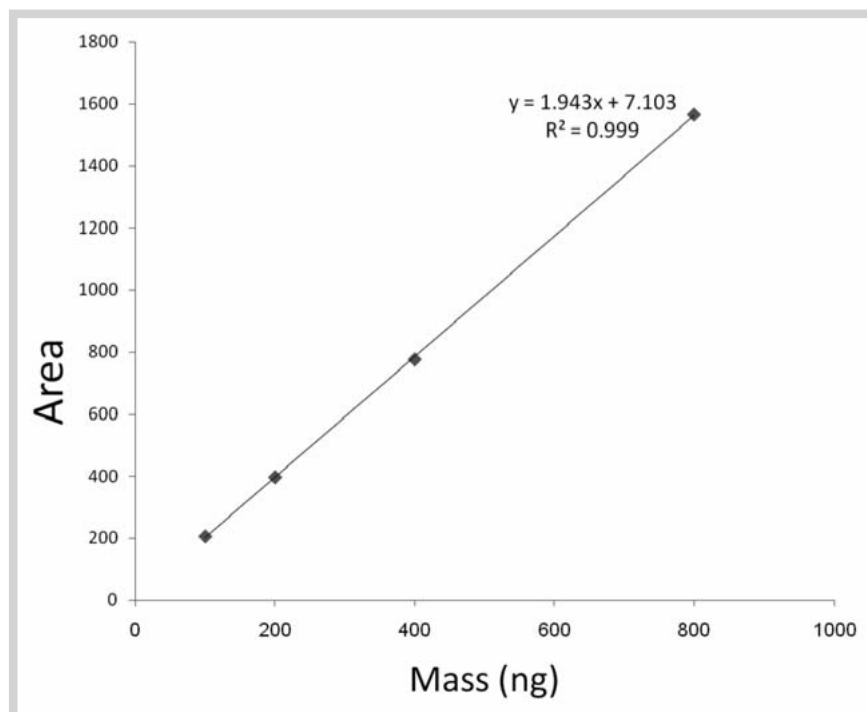
Fig. 2 Regression curve of acemannan between 100 and 800 ng/spot.

Equation on graph:
$$y = 1.943x + 7.103$$
$$R^2 = 0.999$$

Parameter	Aloe group	Placebo group
Age (years)	56.8 ± 10.7	52.3 ± 10.2
Gender	16 males, 14 females	13 males, 17 females
Duration of type 2 diabetes (years)	11.8 ± 9.3	8.5 ± 4.4
Body mass index (kg/m²)	28.7 ± 7.1	29 ± 6.4

Table 1 The demographic data of the subjects who finished the trial. The data are given as mean ± SD.

Table 2 The fasting blood parameter levels before and after intervention and their changes during the study.

Parameters	Groups	Mean (SD) before	p value	Mean (SD) after	p value	Percent (SD) change
Glucose	Aloe	173.0 (19.6)	0.15	167.8 (32.8)	0.036[a]	4.8 (14.7) ↓
	Placebo	184.6 (31.1)		191.2 (42.9)		5.2 (25.1) ↑
HbA1c	Aloe	7.3 (1.1)	0.88	6.6 (1.1)	0.036[a]	8.1 (18.0) ↑
	placebo	7.3 (1.4)		7.8 (1.8)		8.6 (24.0) ↑
Total cholesterol	Aloe	242.3 (39.4)	0.23	217.9 (28.2)	0.006[a]	8.3 (17.0) ↓
	Placebo	227.0 (31.4)		181.0 (42.9)		18.9 (22.2) ↓
LDL	Aloe	134.8 (20.2)	0.12	125.7 (15.2)	0.004[a]	4.5 (21.1) ↓
	Placebo	123.4 (20.2)		100.6 (25.4)		16.4 (25.5) ↓
Triglycerides	Aloe	308.0 (103.6)	0.714	210.8 (79.1)	0.143	30.0 (22.2) ↓
	Placebo	341.4 (128.0)		169.7 (76.9)		45.0 (26.7) ↓
HDL	Aloe	44.9 (13.8)	0.105	43.0 (10.9)	0.407	2.0 (31.6) ↓
	Placebo	48.4 (13.2)		44.2 (8.8)		0.4 (41.5) ↓

[a] $P < 0.05$, significant (Mann-Whitney U test); SD, standard deviation; ↓ decrease; ↑ increase

at the endpoint (○ **Table 2**). The percentages of endpoint reductions of the glucose, HbA1c, total cholesterol, and LDL levels in the aloe group compared with the baseline levels were 4.8%, 8.07%, 8.35%, and 4.48%, respectively. The box plots of decreases (before intervention – after intervention) in the glucose, HbA1c, total cholesterol, and LDL levels of the aloe and placebo groups are shown in ○ **Figs. 3 – 6**.

Discussion
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The results suggest that aloe gel improves glycemic control and lowers the blood levels of total cholesterol and LDL, but does not affect the other blood lipid levels and does not cause any hepatic, renal, or other adverse effects in the hyperlipidemic type 2 diabetic patients. The improved glycemic control agrees with the previous trials [18,21,23]. However, the effects on the lipid profile have some discrepancies with the earlier trials [17,21,23].
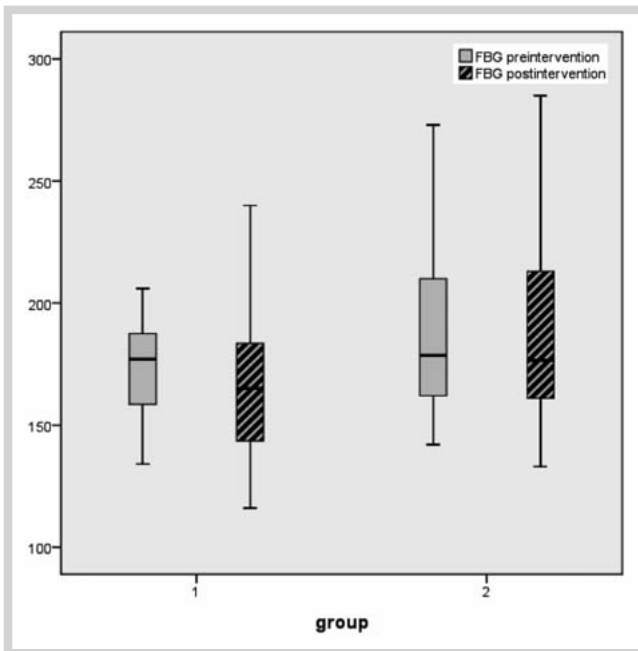
Fig. 3 Box plot of decreases (before intervention – after intervention) in the fasting blood glucose (FBG) levels (mg/dL) of the aloe (group 1) and placebo (group 2) groups.
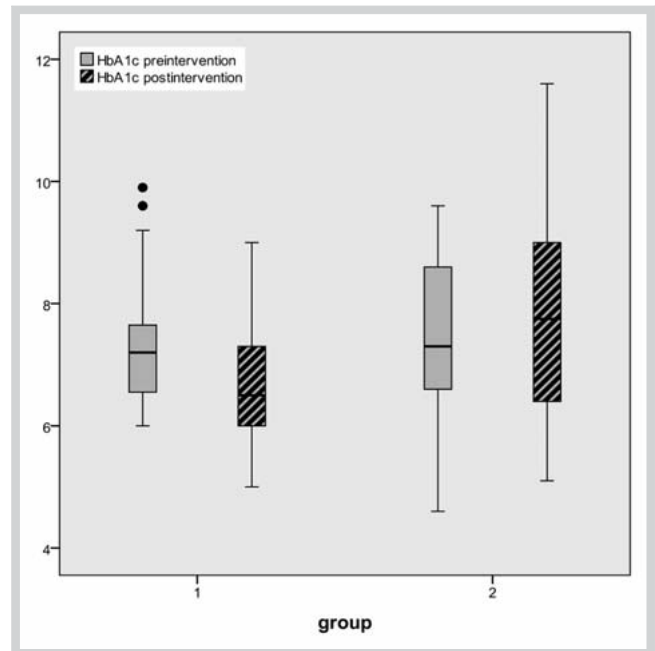

Fig. 4 Box plot of decreases (before intervention – after intervention) in the blood glycosylated hemoglobin (HbA1c) levels (percent) of the aloe (group 1) and placebo (group 2) groups.
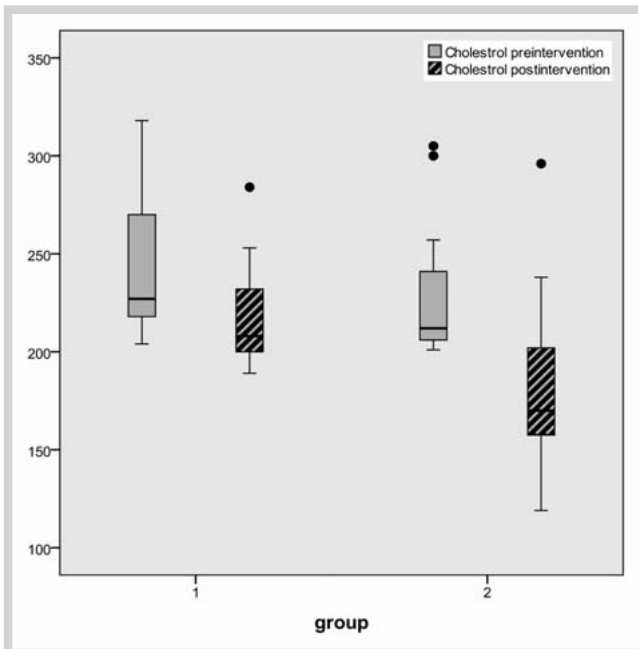

Fig. 5 Box plot of decreases (before intervention – after intervention) in the blood total cholesterol levels (mg/dL) of the aloe (group 1) and placebo (group 2) groups.
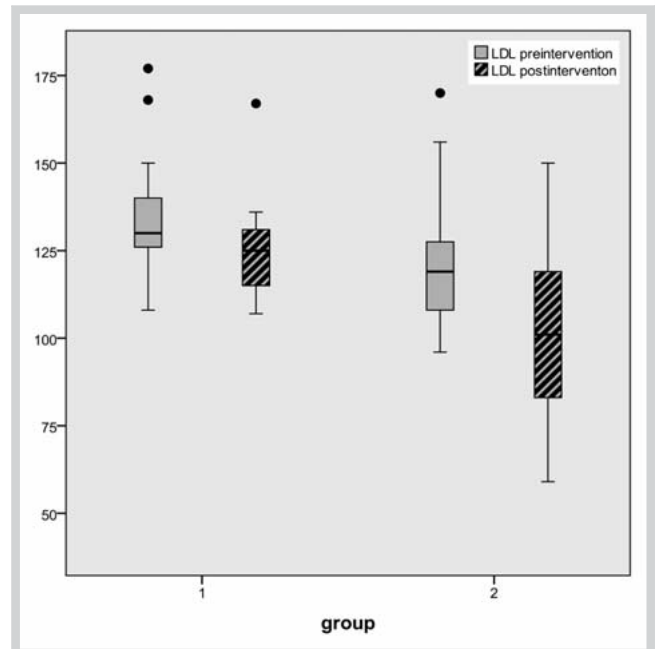

Fig. 6 Box plot of decreases (before intervention – after intervention) in the blood low-density lipoprotein (LDL) levels (mg/dL) of the aloe (group 1) and placebo (group 2) groups.

The total cholesterol and LDL lowering effects of the aloe gel in the present trial agree with the previous study reporting the total cholesterol and LDL lowering effect of aloe gel [17] but disagree with the earlier reports showing no effect of aloe gel on the cholesterol level [21,23]. Further, the lack of the aloe gel effect on the triglycerides level in the current trial is inconsistent with the pre-

vious studies reporting the triglycerides lowering effect of aloe gel [17,21,23]. The lack of the aloe gel effects on the triglyceride and HDL levels in the present trial could be due to the small number of the patients.

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-hyperglycemic and anti-hypercholesterolemic actions of the aloe gel were not investigated in the study presented here. 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]. Also, aloe gel appeared to decrease insulin resistance in two mouse models of T2DM [15,28]. It is notable that the bioactives and mechanisms underlying the effects of aloe gel on the lipid profile have not been studied so far. Finally, considering the results of the present and previous trials, further and larger clinical trials concerning the efficacy and safety of aloe gel in the treatment of patients with T2DM and/or hyperlipidemia as well as more studies addressing the bioactives and mechanisms involved in the effects of aloe gel on the blood glucose and lipid levels seem necessary.

Acknowledgements
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Conflict of Interest
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The authors do not have any financial/commercial conflicts of interest in the study presented here.

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