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Astragalus adscendens extract shows antidiabetic effects through controlling oxidative stress, inflammation and apoptosis in streptozotocin−induced diabetic rats

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ABSTRACT

Objective: To assess the effect of oral treatment of methanolic extract of the aerial parts of *Astragalus adscendens* in streptozotocin-induced diabetic rats.

Methods: In order to induce diabetes, rats intraperitoneally received streptozotocin at 65 mg/kg. Sixty adult male Wistar rats were allocated into six groups (10 rats per each) including the healthy control group, the diabetic group as well as the diabetic group treated with *Astragalus adscendens* methanolic extract at 50, 100, and 200 mg/kg per day or glibenclamide (0.6 mg/kg/day) for 28 d. The effects of *Astragalus adscendens* methanolic extract on the levels of glucose, insulin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, bilirubin, creatinine, urea, uric acid, total protein, albumin, triglyceride, cholesterol, α-amylase, oxidant/antioxidant enzymes, and inflammatory cytokines were evaluated. Real time-PCR was also used for measuring the gene expression of *caspase−3*, *Bcl2*, and *Bax*.

Results: The levels of glucose, cholesterol, triglyceride, creatinine, urea, uric acid, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, and malondialdehyde considerably declined (P<0.001) in diabetic rats after treatment with *Astragalus adscendens* methanolic extract especially at a dose of 200 mg/kg. In addition, treatment with *Astragalus adscendens* methanolic extract noticeably increased the level of insulin, total protein, and albumin as well as improved the activities of catalase, glutathione peroxidase, and superoxide dismutase, as well as the expression levels of TNF-α, IL-1β, *caspase−3*, *Bcl2* and *Bax* (P<0.001) compared to the diabetic control group. The extract also inhibited α-amylase in a dose-dependent manner with an IC_{50} value of 19.6 μg/mL.

Conclusions: *Astragalus adscendens* methanolic extract shows potent antidiabetic, anti-inflammatory, anti-apoptotic, and antioxidant effects in diabetic rats. However, more studies are needed to verify the underlying mechanism of the effect of this plant extract and test its efficacy in clinical trials.

KEYWORDS: Herbal medicines; *Astragalus adscendens*; Diabetes; Antioxidant; Antidiabetes; Streptozotocin; Antiinflammation

1. Introduction

Diabetes mellitus is well-known as the most prevalent endocrine illness, during which, due to a lack of insulin or a decrease in the sensitivity of the target tissues to its effects, there are disturbances

Significance

Various chemical drugs are used to control and treat diabetes, whereas, the use of herbal drugs can have a complementary effect on these drugs or even be a suitable alternative to them with fewer side effects and lower cost. The present study showed that *Astragalus adscendens* had promising antidiabetic effects in streptozotocin-induced diabetic rats. However, more studies are needed to verify the underlying mechanism of the effect of this plant extract.

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in the metabolism of proteins, fats, and carbohydrates[1]. Due to the disturbance in the secretion and effect of insulin, hyperglycemia is created, which in the long term has a wide range of effects on all the organs of the body, including the occlusion of small blood vessels and the thickening of the basement membrane of capillaries, involvement of large vessels, rapidly progressive atherosclerosis, and involvement of nerves[2–5]. Insulin resistance and reduced insulin release are the main causes of type 2 diabetes, which lead to impaired glucose oxidation and hyperglycemia[6]. Diabetes mellitus is linked with metabolic disorders, *e.g*., an increase in cholesterol, triglycerides, and low-density lipoprotein (LDL), and a decrease in high-density lipoprotein (HDL). Evidence shows that hyperglycemia and hyperlipidemia play an important role in causing diabetes complications[7].

Along with many chemical drugs that are used to control and treat diabetes, the use of herbal drugs can have a complementary effect on these drugs or even be a suitable alternative to them with fewer side effects and lower cost[8]. *Astragalus* L. is a genus of perennial plants belonging to the Fabaceae family, with approximately 2 900 species, which has two main distribution centers in America and Eurasia[9,10]. In modern medicine, *Astragalus* spp. showed several pharmacological effects, *e.g*., anticancer, antioxidant, antimicrobial, neuroprotective, and hepatoprotective[11–13]. Considering the necessity of revitalizing folk medicine and identifying the antidiabetic effects of medicinal herbs in nature that have valuable therapeutic effects, the present study aimed to assess the effect of oral treatment of methanolic extract of the aerial parts of *Astragalus adscendens* (*A. adscendens*) in streptozotocin-induced diabetic rats.

2. Materials and methods

2.1. Plant

The aerial parts of *A. adscendens* were collected from the mountainous regions of Noorabad city in the western regions of Iran, Lorestan province (34°04'33.2"N 47°56'55.3"E), which were identified by a botanist (Dr. Mohammad Mehrnia) from the Department of Botany, Lorestan University, Khorramabad, Iran. The control samples were stored in the herbarium, Faculty of Pharmacy, Lorestan University of Medical Sciences (No.2022.219).

2.1.1. Extraction

The dried aerial parts were ground and defatted with *n*-hexane. Then, the extraction was carried out by maceration method with 70% methanol. By means of the rotary evaporator, the alcoholic part was discarded, and the extract was kept at –20 ℃ for further tests[14].

2.1.2. Phytochemical study

The major phytochemical examination of *A. adscendens* methanolic extract was achieved for detecting the presence of some phytochemicals, *e.g*., tannins, saponins, alkaloids, flavonoids, and glycosides based on the previous investigation[14].

2.1.3. Total phenolic compounds

Folin-Ciocalteau's method was used to determine the total content of phenolic compounds based on the methods described elsewhere[15]. Briefly, in this method, 20 μL of the extract was mixed with distilled water and 100 μL of Folin Ciocalto reagent. After adding sodium carbonate solution (20%, 300 μL) to the test tube, the absorbance of tubes was measured with a spectrophotometer at a wavelength of 760 nm. The results were expressed as milligrams of gallic acid per gram of extract.

2.1.4. Total flavonoid compounds

Aluminum chloride ($AlCl_3$) colorimetric assay was performed to assess the total flavonoid content of *A. adscendens* methanolic extract based on the methods explained previously[16]. Briefly, after mixing the extract with $AlCl_3$ (0.1%), potassium acetate (0.1%), ethanol (95%), and distilled water, the resultant mixture was kept at 21 ℃ for 30 min. The absorbance of the combination was recorded at 415 nm. The results were expressed as milligrams of quercetin per gram of extract.

2.2. Establishment of diabetes

2.2.1. Ethical statement

This study was permitted by the Ethics Committee of Lorestan University of Medical Sciences, Khorramabad, Iran, (No. IR.LUMS.REC.1401.219).

2.2.2. Animals

Since the minimum number of animals in *in vivo* assay is eight animals[17], sixty adult male Wistar rats with a weight range of 200-250 g were obtained from the care and reproduction of laboratory animals of Razi Institute (Karaj, Iran) and the animals were housed in a room with a light/dark cycle of 12∶12 h at a temperature of (21±2)℃. The animal experiments were performed in line with the procedures for the Care and Use of Laboratory Animals[18].

2.2.3. Study design

Rats were allocated into six groups (10 rats per each) as follows: Ⅰ: rats in the healthy control group orally received 1 mL of distilled water daily; Ⅱ-Ⅳ: Diabetic rats orally received *A. adscendens* methanolic extract at 50, 100 and 200 mg/kg (the selection of these doses was based on the primary experiment conducted by the present authors, which showed that this extract does not have any toxicity to animals at these doses) per day for 28 d, respectively; Ⅴ: Diabetic rats orally received 1 mL of distilled water daily; Ⅵ: Diabetic rats orally received glibenclamide (0.6 mg/kg/day).

Streptozotocin (65 mg/kg/bw) was injected intraperitoneally to induce diabetes. After weighing, the amount of streptozotocin needed was dissolved in citrate buffer (pH 4.5) and used. Subsequently, the rats were fasted for 12 h, and the blood was taken from the tail of the rats. Their fasting blood sugar was then measured by an enzyme method. Rats with fasting blood sugar equal to or >250 mg/dL were used for further experiments[19].

2.3. Determination of blood glucose and insulin

The level of blood glucose was determined by means of commercial kits (Pars Azmon, Iran). Serum insulin was determined by means of a rat ELISA kit (ALPCO Diagnostics).

2.4. Determination of biochemical parameters

After centrifuging the collected blood for 10 min at 15 000 rpm and obtaining the serum samples, the serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine (Cr), urea (Ur), uric acid, total protein, albumin, triglyceride, and cholesterol were examined by using diagnostic kits (ParsAzmon, Iran) according to the manufacturer's instructions.

2.5. Determination of oxidative enzymes activities

After preparing the pancreatic tissue homogenates by a cold buffer at 4 ℃, the tissue levels of oxidant/antioxidant enzymes, *e.g.*, glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase were measured according to the commercial kits (ParsAzmoon, Iran). Tissue lipid peroxidation (LPO) level was measured based on the Nalondi™-Lipid Peroxidation Assay Kit-MDA. The basis of this method is the reaction of thiobarbituric acid with peroxidized lipids. This acid breaks the lipid peroxide molecules in MDA, and then MDA reacts with thiobarbituric acid, which produces substances that are spectrophotometrically compared with the standard curve.

2.6. Measurement of α-amylase inhibition

Briefly, sodium phosphate buffer (200 µL), was mixed with α-amylase (20 µL), and the extract at concentration of 2.5-50 µg/mL; then the combination was kept for 10 min at 37 ℃. In the next step, 1% starch solution was mixed in the test tubes and was kept again at 37 ℃. By adding the dinitrosalicylic acid reagent and stopping the reaction, the optical density of the combination was determined at 540 nm. The control tube was with no extract. The % inhibition was calculated based on the formula:

% of inhibition= (Absorbance of control – Absorbance of extract)/ Absorbance of control.

All tests were completed in triplicate and the IC_{50} was calculated by Probit test.

2.7. Oral glucose tolerance test in rats

At first, 30 rats were fasted for 16 h, and allocated into 5 groups including: (i): negative control group treated with normal saline; (ii): positive control group orally treated with glibenclamide (0.6 mg/kg) in a single dose; (iii, iv, and v): tested groups orally treated with 50-200 mg/kg of *A. adscendens* methanolic extract in a single dose, respectively. Thirty minutes after receiving drugs, rats received 2 g/kg of glucose solution. After collecting the blood specimen from the tail tip of each rat, the blood glucose level was measured directly before treatment and after 30-180 min of glucose consumption[20].

2.8. Measurement of the pro-inflammatory cytokines

The levels of some pro-inflammatory cytokines, *e.g.*, IL-1β and TNF-α in the pancreas were measured based on the commercial ELISA Kit (Carmania Parsgen Co, Iran) kits as per manufacturer's protocols.

2.9. Real-time PCR analysis for measuring the apoptosis genes (caspase-3, Bcl2, and Bax)

Total RNA was isolated from the pancreas tissue specimens by means of an RNeasy kit based on the kit protocol (Qiagen, USA). Next, reverse transcription was performed by means of a commercial kit (Fermentas, USA). Sequences of primers used for real-time PCR (5'-3') were as follows: *Bax* F: GGCTGGACACTGGACTTCCT, R: GGTGAGGACTCCAGCCACAA; *Bcl2* F: CATGCCAAGAGGGAAACACCAGAA, R: GTGCTTTGCATTCTTGGA TGAGGG; *Caspase-3* F: TTCATTATTCAGGCCTGCCGAGG, R: TTCTGACAGGCCATGTCATCCTCA; *β-actin* F: GTGACGTTGACATCCGTAAAGA, R: GCCGGACTCATCGTACTCC. The thermal conditions were performed with primary denaturation at 94 ℃ for 10 min, 40 amplification cycles (denaturation at 94 ℃ for 10 s, annealing at 55 ℃ for 30 s, and elongation at 70 ℃ for 30 s), and then a single cycle at 70 ℃ for 5 min. The mRNA expression level was determined using the $2^{-\Delta\Delta Ct}$ method and normalized against *β-actin*.

2.10. Statistical analysis

The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test using SPSS statistical software (26.0 ver). $P<0.05$ was considered significantly different.

3. Results

3.1. Phytochemical analysis of A. adscendens methanolic extract

The phytochemical study revealed the presence of flavonoids, saponins, terpenoids, and polysaccharides in *A. adscendens* methanolic extract.

3.2. Secondary metabolites contents of A. adscendens methanolic extract

The total phenolic and flavonoid content was 0.94 mg gallic acid equivalent/g dry weight and 0.67 mg quercetin equivalent/g dry weight, respectively.

3.3. Effect of A. adscendens methanolic extract on biochemical parameters

The serum levels of glucose, cholesterol, and triglyceride were noticeably raised in diabetic rats compared to healthy rats (P<0.001); whereas, a significant reduction was observed in the serum level of insulin in diabetic rats (P<0.001). Treatment of diabetic rats with *A. adscendens* methanolic extract, especially at doses of 100 and 200 mg/kg considerably declined the serum level of glucose, cholesterol, and triglyceride, while increasing insulin level (P<0.001) (Table 1).

3.4. Effect of A. adscendens methanolic extract on the serum level of liver enzymes

The serum levels of AST, ALT, ALP, and bilirubin were significantly increased in diabetic rats compared to healthy rats. However, treatment with *A. adscendens* methanolic extract especially at doses 100 and 200 mg/kg for 4 weeks caused a considerable improvement in the serum levels of AST, ALT, ALP, and bilirubin in comparison with the diabetic control (P<0.001) (Table 1).

3.5. Effect of A. adscendens methanolic extract on the serum level of kidney enzymes

The results showed that the serum levels of Cr, Ur, and uric acid as the main kidney functional parameters were elevated in diabetic rats compared to healthy rats. *A. adscendens* methanolic extract at 100 and 200 mg/kg markedly declined the serum levels of Cr, Ur, uric acid; whereas the levels of total protein and albumin were increased in comparison with the diabetic control group (P<0.001) (Table 1).

3.6. Effect of A. adscendens methanolic extract on oxidant/antioxidant enzymes

In diabetic rats, the activities of antioxidant enzymes including SOD, GPx, and catalase were reduced and MDA level was increased (P<0.001). *A. adscendens* methanolic extract at 100 and 200 mg/kg reversed streptozotocin-induced changes in antioxidant enzymes and MDA in diabetic rats (P<0.001) (Table 2).

3.7. Effect of A. adscendens methanolic extract on α–amylase inhibition

A. adscendens methanolic extract inhibited α-amylase in a dose-dependent manner. The IC_{50} value for *A. adscendens* methanolic extract was 19.6 μg/mL (Figure 1).

Table 1. Effect of oral treatment of the methanolic extract of *Astragalus adscendens* (MEAA) on the serum levels of some biochemical parameters in streptozotocin-induced diabetic rats.

Parameters	Healthy control	Diabetes				
		-	GLB (0.6 mg/kg)	MEAA 50 mg/kg	MEAA 100 mg/kg	MEAA 200 mg/kg
Glucose (mg/dL)	83.60±2.36	236.00±8.56$^{\#}$	92.30±4.62*	206.30±6.78	141.30±6.12*	97.60±6.66*
Insulin (mlU/mL)	3.81±0.89	0.61±0.07$^{\#}$	2.89±0.31*	0.98±0.08	1.72±0.23*	2.34±0.34*
Cholesterol (mg/dL)	76.60±5.51	137.30±4.46$^{\#}$	82.40±3.72*	119.30±5.42	101.60±5.23*	86.30±6.66*
Triglyceride (mg/dL)	83.40±5.21	146.50±6.65$^{\#}$	79.80±5.48*	123.20±6.78	97.20±6.87*	76.60±5.87*
ALT (Unit/L)	126.60±2.36	174.30±3.46$^{\#}$	129.40±1.62*	166.30±2.78	148.60±3.12*	132.40±2.66*
AST (Unit/L)	134.40±4.73	192.50±3.86$^{\#}$	137.30±2.67*	173.50±2.58	154.60±3.12*	135.40±2.26*
ALP (Unit/L)	196.50±6.73	278.60±4.76$^{\#}$	191.30±4.67*	225.30±5.67	207.70±6.23*	189.40±7.23*
Bilirubin (mg/dL)	0.86±0.04	1.78±0.07$^{\#}$	0.91±0.08*	1.56±0.17	1.14±0.12*	0.95±0.08*
Total protein	6.31±0.65	2.21±0.36$^{\#}$	5.98±0.72*	3.12±0.47	4.28±0.61*	5.88±0.73*
Albumin	3.41±0.21	1.68±0.07$^{\#}$	3.01±0.31*	1.98±0.24	2.41±0.69*	3.12±0.65*
Creatinine	0.66±0.04	2.71±0.21$^{\#}$	0.72±0.09*	1.74±0.12	1.21±0.17*	0.83±0.07*
Uric acid	1.49±0.21	4.56±0.36$^{\#}$	1.72±0.21*	3.54±0.74	2.13±0.65*	1.69±0.13*
Urea	21.6±4.21	44.30±5.32$^{\#}$	24.20±5.48*	39.30±4.14	30.10±3.21*	23.40±2.65*

The data are expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA). $^{\#}P$<0.001 compared with healthy control; $^{*}P$<0.001 compared with the diabetic control group. GLB: glibenclamide.

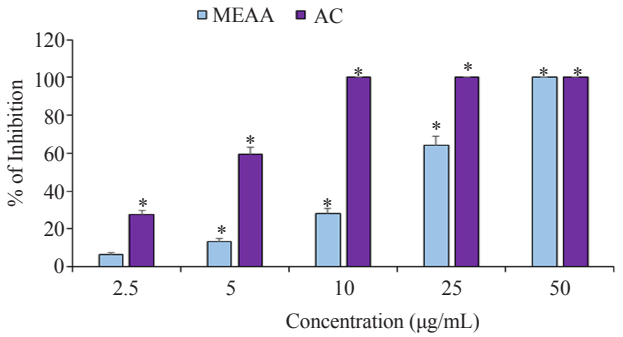

Figure 1. Effect of the methanolic extract of *Astragalus adscendens* (MEAA) on α-amylase inhibition (n=3). AC: acarbose.

3.8. Oral glucose tolerance test

There was no significant difference in blood glucose level among all the tested groups (P>0.05) before glucose administration. The glucose administration-induced hyperglycemia significantly declined by 100 mg/kg *A. adscendens* methanolic extract after 60 and 120 min and 200 mg/kg after 60-180 min of administration (P<0.001) (Table 3).

3.9. Effect of A. adscendens methanolic extract on pro-inflammatory cytokines

The inflammatory responses in the pancreas of diabetic rats were increased through the elevation of pro-inflammatory cytokines TNF-α and IL-1β. Treatment with either 100 or 200 mg/kg *A. adscendens* methanolic extract caused a significant reduction in TNF-α and IL-1β levels in diabetic rats (P<0.001) (Figure 2).

3.10. Effect of A. adscendens methanolic extract on apoptosis gene expression

The results showed that in diabetic rats, the expression level of *caspase-3* and *Bax* genes was markedly (P<0.001) elevated in pancreas tissues. Treatment of diabetic rats with either 100 or 200 mg/kg *A. adscendens* methanolic extracts considerably downregulated (P<0.001) the expression level of *caspase-3* and *Bax* genes in pancreas tissues. Moreover, the gene expression of *Bcl2* was significantly upregulated (P<0.001) after treatment of diabetic rats with 100 and 200 mg/kg *A. adscendens* methanolic extracts (Figure 3).

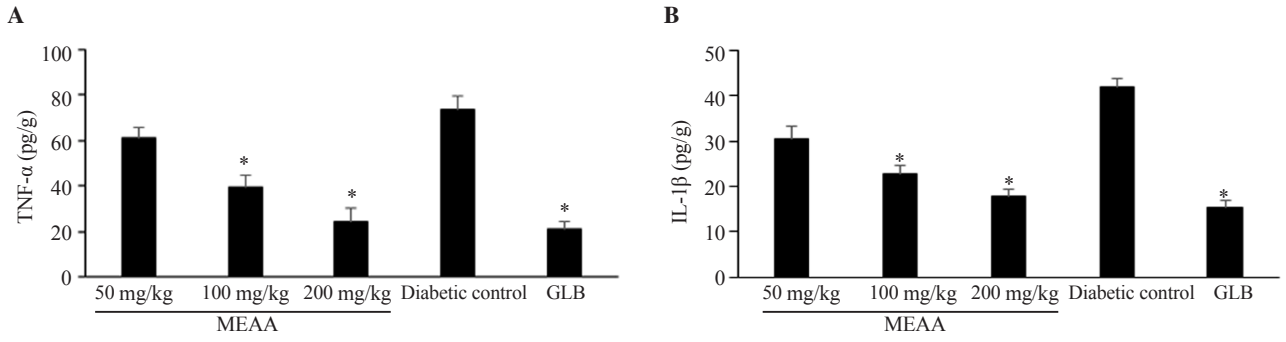

Figure 2. Effect of MEAA on (A) TNF-α and (B) IL-1β levels. The data are expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA). *P<0.001 compared with the diabetic control group.

Table 2. Effect of oral treatment of MEAA on the serum levels of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and malondialdehyde (MDA) in streptozotocin-induced diabetic rats.

Parameters	Healthy control	Diabetes				
		-	GLB (0.6 mg/kg)	MEAA 50 mg/kg	MEAA 100 mg/kg	MEAA 200 mg/kg
Catalase (nmol/L)	3.01±0.31	1.64±0.12#	2.76±0.24*	1.98±0.26	2.32±0.41*	2.86±0.36*
GPx (nmol/L)	43.20±4.42	24.60±2.56#	39.80±3.23*	29.30±3.35	34.40±3.29*	41.70±2.54*
SOD (nmol/L)	5.78±0.61	0.92±0.07#	5.12±0.61*	1.49±0.44	2.92±0.56*	3.45±0.61*
MDA (nmol/L)	2.54±0.31	8.46±0.89#	3.23±0.76*	6.78±1.12	5.26±0.84*	3.46±0.64*

The data are expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA). #P<0.001 compared with healthy control; *P<0.001 compared with the diabetic control group.

Table 3. Effect of MEAA on blood glucose level of rats administered oral glucose.

Time	Diabetic control	GLB (0.6 mg/kg)	MEAA 50 mg/kg	MEAA 100 mg/kg	MEAA 200 mg/kg
0 min	131.30±4.23	129.30±3.28	126.00±5.21	130.30±4.87	133.60±5.21
30 min	281.30±5.64	168.30±6.12*	271.30±4.23	243.60±8.30	233.50±7.23
60 min	242.30±8.60	107.30±7.40*	226.30±7.40	179.60±6.50*	132.40±6.20*
120 min	169.30±8.10	86.40±4.60*	132.30±6.40	114.60±6.56*	98.40±4.61*
180 min	132.30±5.89	78.20±4.76*	119.60±6.12	93.60±5.84	84.30±3.64*

The data are expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA). *P<0.001 compared with the diabetic control group.

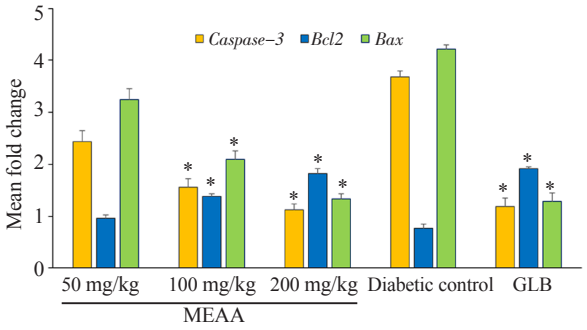

Figure 3. Effect of MEAA on the expression level of apoptosis-regulatory genes in diabetic rats. The data are expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA). $^{*}P<0.001$ compared with the diabetic control group.

4. Discussion

Streptozotocin increases the level of glucose in animals by damaging the membrane of beta cells of the pancreas, breaking DNA, and reacting with the enzymes called glucokinase[2]. Streptozotocin also enhances the expression of liver glucose 6-phosphatase mRNA and thereby increases blood glucose[2,3]. The results of the present study showed that oral treatment of the streptozotocin-induced diabetic rats with *A. adscendens* methanolic extract significantly reduced the amount of glucose, which caused a significant increase in serum insulin levels. Therefore, the plant extract may have an insulin-like effect by releasing insulin from the pancreas and affecting the serum glucose level of diabetic rats, or by an unknown mechanism, it causes a decrease in serum glucose.

Many researchers, by studying plant extracts and their antidiabetic effects, showed that most of these plants did not interact with the insulin receptor; rather, they stimulate the action of insulin by cross-reacting with insulin receptor tyrosine kinase[4]. There are no documented reports about the effect of plant extracts on lowering blood sugar levels; however, it suggested that the plant extracts probably enhance the consumption of glucose by the surrounding tissues, inhibit the absorption of glucose in the kidneys, and increase the release of insulin from the cells in the islets of the pancreas[4].

In this study, treatment with *A. adscendens* methanolic extract initiated a considerable decrease in the level of plasma lipids in diabetic rats. Although the mechanism of the effect of the extract on cholesterol and blood lipid levels has not been determined yet, previous studies showed that plant extracts probably interfere with the uptake of triglycerides by adipose tissue by increasing the level of insulin, and by stopping hydroxymethylglutaryl coenzyme A reductase. The plant extracts may cause the regeneration of plasma lipoproteins by inhibiting the activity of hydroxymethylglutaryl coenzyme A reductase[21].

We also found that *A. adscendens* methanolic extract effectively reduced the serum level of Ur, Cr, and uric acid in diabetic rats. It has already been proven that plant extracts reduced the catabolism of proteins, and reduced the loss of water, salt, and kidney damage by reducing blood glucose and urea. On the other hand, with a decrease in kidney function damage, they can cause a decrease in serum creatinine in diabetic animals[22].

In the present study, the effect of *A. adscendens* methanolic extract on serum levels of ALT, AST, and ALP in diabetic rats was investigated. The serum level of liver enzymes in the diabetic control group was increased significantly compared to the healthy control group. Under normal conditions, the serum concentration of liver enzymes is low, and the increase in their serum concentration is caused by the destruction of hepatocytes and the transfer of enzymes from the cytosol into the bloodstream. The extract decreased the activities of ALT and AST possibly by inhibiting the damage induced by streptozotocin[23].

There are several reports that flavonoids, tannins, and other polyphenolic compounds (such as coumarins), triterpenoids, and plant secondary metabolites have hypoglycemic and antihypertensive effects in various experimental animal models[24]. The study conducted by Khodzhaieva *et al*. showed the hypoglycemic effect of free glycosylated flavonoids and complexes of flavonoids with vanadium on streptozotocin-induced diabetic rats[25].

In addition, treatment with *A. adscendens* methanolic extract, especially at concentrations of 100 and 200 mg/kg/day significantly increased the activities of antioxidant enzymes SOD, catalase, and GPx and lowered MDA levels. A previous study shows that diabetes increases oxidative stress and lipid peroxidation. It has also been found that there is a relationship between the complications of diabetes and lipid peroxidation, such that an increase in blood sugar causes a decrease in the amount of endogenous protective antioxidants and an increase in free radicals[26]. Considering that oxidative stress is due to the intensification of the formation of free radicals and these substances seek to complete their electron circuit, the constituents of the cell, including protein and lipid structures, are damaged, which reduces the level of the antioxidant enzymes (SOD, GPx, and catalase) in the blood and tissue. Studies have shown that the increase in blood sugar caused by diabetes is one of the causes of increased oxidative stress[27,28]. It has also been reported that the diabetes-induced increase in blood glucose increases the production of free radicals by autoxidation and non-enzymatic glycation of proteins, as well as stimulation of the production of H_2O_2 in cells[29]. It is also known that in all types of diabetes, oxidative stress increases due to increased oxygen free radicals and weakened antioxidant defense systems[30]. In this study, we also found that the antidiabetic activity of *A. adscendens* methanolic extract can be related to the inhibition of α-amylase, an enzyme that breaks α-1,4 glycosidic bonds present in the starch polysaccharide[31]. α-Amylase inhibition can delay the breakdown of carbohydrates which results in a reduction in blood glucose[32].

Inflammatory responses are well-known as key factors in the development of diabetes and are thus linked to elevated insulin

resistance and declined reaction in insulin target organs[33]. The present study showed that *A. adscendens* methanolic extract at either 100 or 200 mg/kg caused a significant reduction in TNF-α and IL-1β levels in the pancreas of diabetic rats. Therefore, inflammation was alleviated in diabetic rats treated with *A. adscendens* methanolic extract. Apoptosis or programmed cell death is a physiological and biological process to maintain homeostasis, which is caused by different pathways[34], including hyperglycemia, which causes oxidative stress and inflammation[35]. An increase in blood sugar can cause an unbalanced apoptotic process by the alternation in the function of the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl2[36]. Treatment with 100 and 200 mg/kg *A. adscendens* methanolic extract considerably downregulated the expression level of *caspase-3* and *Bax* genes as well as upregulated *Bcl2* gene expression. Therefore, it can be proposed that treatment with *A. adscendens* methanolic extract can control inflammation and apoptosis in diabetic rats and consequently improve diabetic complications.

In conclusion, *A. adscendens* methanolic extract showed potent antidiabetic, anti-inflammatory, anti-apoptotic, and antioxidant effects in diabetic rats. However, more studies are needed to understand the underlying mechanism of its antidiabetic effect and test its efficacy in clinical trials.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

YR designed the study. KK, HRM, and MNM performed experiments and collected data. HM and JGY drafted the manuscript. MNM and JGY discussed the results and strategy. MNM and JGY supervised, directed, and managed the study. All authors approved the final version to be published.

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