J Herbmed Pharmacol. 2023; 12(3): 413-418.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2023.45

Journal of Herbmed Pharmacology

Ameliorating effects of *Astragalus maximus* methanolic extract on inflammation and oxidative stress in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 13 February 2023
Accepted: 22 April 2023

Keywords:
Herbal medicines
Phenolic compounds
Flavonoids
Insulin
Diabetes mellitus

ABSTRACT

Introduction: Recent studies have reported that *Astragalus* spp. can display various biological effects, e.g., anticancer, antioxidant, antimicrobial, neuroprotective, and hepatoprotective activities. Here we decided to assess the ameliorating effects of *Astragalus maximus* methanolic extract (AMME) on inflammation and oxidative stress in streptozotocin-induced diabetic rats.
Methods: The dried aerial parts were extracted by maceration technique with 70% methanol. Diabetes was induced in rats via intraperitoneal injection of streptozotocin at 65 mg/kg. Diabetic rats orally received AMME at 75-30 mg/kg for 28 days. The serum levels of glucose, insulin, liver enzymes, bilirubin, creatinine (Cr), urea (Ur), triglyceride, and cholesterol, as well as the tissue levels of oxidant/antioxidant enzymes and pro-inflammatory cytokines were evaluated by the diagnostic kits. The level of α-amylase inhibition by AMME was also determined.
Results: AMME (150 and 300 mg/kg) treatment significantly reduced ($P<0.001$) the serum levels of glucose, cholesterol, triglyceride, Cr, Ur, liver enzymes, and oxidative enzymes in diabetic rats. The tissue levels of antioxidant enzymes in diabetic rats treated with AMME (150 and 300 mg/kg) were significantly increased ($P<0.01$). Treatment of diabetic rats with either 150 or 300 mg/kg AMME for 28 days significantly reduced interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) levels in the pancreas. AMME inhibited α-amylase in a dose-dependent manner with an IC_{50} value of 18.1 µg/mL.
Conclusion: This study showed that the oral administration of AMME in diabetic rats displayed a potent anti-diabetic activity through increasing insulin release and ameliorating effects on inflammation and oxidative stress; however, more investigations are desired to determine the action mechanism of the extract.

Implication for health policy/practice/research/medical education:
Our results revealed that the oral administration of *A. maximus* methanolic extract might be considered a natural product for controlling and treating diabetes mellitus; however, more investigations are desired to determine the action mechanisms of its extract.
Please cite this paper as: Sadeghi H, Sadeghi N, Raziani Y, Sridhar AK, Ghasemian Yadegari J, Moradi MN. Ameliorating effects of *Astragalus maximus* methanolic extract on inflammation and oxidative stress in streptozotocin-induced diabetic rats. J Herbmed Pharmacol. 2023;12(3):413-418. doi: 10.34172/jhp.2023.45.

Introduction

Diabetes mellitus is a metabolic disorder in the body in which the ability to produce insulin is lost or the body becomes resistant to insulin and the produced insulin cannot perform its normal function, thus, many tissues such as the pancreas, kidney, liver, brain, and heart are affected (1,2). It has been proven that insulin resistance and decreased insulin discharge are the principal causes of

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type 2 diabetes, which result in impaired glucose oxidation and hyperglycemia (3,4). Along with many chemical drugs that are used to control diabetes, the use of therapeutic supplements such as herbal supplements can be effective in reducing diabetes and its complications (5,6). It has been customary to use plants in the treatment of diseases in almost all countries of the world (7-9). Having minimal side effects, low cost, and availability of these plants have caused the gradual replacement of chemical drugs by plants (8,9).

Herbs belonging to the *Astragalus* genus from the Fabaceae family have nearly 3000 species, with two main distribution centers in America and Eurasia (10). *Astragalus* spp, due to the presence of compounds such as flavonoids, saponins, and polysaccharides, has various activities such as strengthening the body's immune system, regulating blood cholesterol, preventing diabetes, treating digestive disorders, treating allergies, and strengthening memory (11,12). Recent studies have reported that *Astragalus* spp. displayed various biological effects in modern pharmacology, e.g., anticancer, antioxidant, antimicrobial, neuroprotective, and hepatoprotective activities (12, 13). This work was designed to study the effect of *Astragalus maximus* methanolic extract (AMME) in streptozotocin-diabetic rats.

Materials and Methods

Plant

The plant materials (aerial parts) were collected from the rural regions of Noorabad city in the western regions of Iran, Lorestan province, and were identified by a botanist from the Department of Botany, Lorestan University, Khorramabad, Iran. A voucher specimen was stored at Lorestan University of Medical Sciences (No. 2827).

Preparing the methanolic extract

The dried aerial parts were extracted by maceration technique with 70% methanol. By a rotary evaporator, the alcoholic part was thrown out and the extract was reserved at minus 20°C for other tests (14).

Phytochemical study

The phytochemical examination of the AMME was accomplished to discover the presence of tannins, saponins, alkaloids, flavonoids, and glycosides, etc based on a previous investigation by means of some reagents, e.g., Mayer and Dragendorff 's reagents for alkaloids, Mg and HCl for flavonoids, 1% gelatin for tannins, $FeCl_2$ and H_2SO_4 for glycosides, and suds production for saponins (15).

Total phenolic and flavonoids compounds

The total phenolic and flavonoid compounds of AMME were determined based on the Folin- Ciocalteu's method and the aluminum chloride ($AlCl_3$) colorimetric assay based on the previous studies (16). The total phenolic compounds were expressed in terms of milligrams of gallic acid per gram (GAE/g) of extract, while the total flavonoid compounds were expressed in terms of milligrams of quercetin per gram (mg QE/g) of extract.

In vitro antidiabetic effects by evaluating α-amylase inhibition activity

The level of α-amylase inhibition by AMME was determined based on a previous study with some modifications (17). Briefly, sodium phosphate buffer (200 µL) was mixed with alpha-amylase (20 µL) and AMME at concentrations of 25–200 µg/ml. The combination was kept for 10 minutes at 37°C and then, 1% starch solution was added to the test tube, and kept again at 37°C. 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 without AMME. The % inhibition was considered based on the following 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.

Animals

Forty-eight adult male Wistar rats (200-250 g) were acquired from the care and reproduction of laboratory animals of Lorestan University of Medical Sciences (Khorramabad, Iran). Mice were stored in a room with a light/dark cycle of 12:12 hours at a temperature of 21±2°C.

Establishment of animal model of diabetes

To induce diabetes, streptozotocin (65 mg/kg/bw) was intraperitoneally injected to the tested mice. Three days after injection, the rats were fasted for 12 hours and blood samples were collected from the mice's tail. Then, the fasting blood sugar was studied by enzyme method. If the fasting blood sugar was equal to or >250 mg/dL, it was measured as a diabetic rat (18).

Study design

Mice were accidentally distributed into six groups containing 8 mice per each, including non-diabetic mice, diabetic mice that were orally treated with AMME at 75, 150, and 300 mg/kg/d for 28 days, and diabetic animals that were orally treated with glibenclamide (0.6 mg/kg/d).

Determination of the blood glucose and insulin

The blood glucose was measured via the commercial kits (Pars Azmon, Iran). Serum insulin level was also measured via a rat ELISA kit (Pars Azmon, Iran).

Measurement of the biochemical factors

On the 29th day after the induction of diabetes, blood

sampling was collected from the hearts of the mice and centrifuged for 10 minutes at 15 000 rpm and the serum specimens were collected. The serum levels of total proteins, triglyceride, cholesterol, as well as kidney and liver enzymes were examined by Pars Azmon diagnostic kits.

Measurement of oxidant/antioxidant enzymes

The tissue levels of oxidant/antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and lipid peroxidation (LPO) were determined in the pancreatic homogenates by Pars Azmon diagnostic kits.

Assessment of the pro-inflammatory cytokines

The pancreatic levels of some pro-inflammatory cytokines, e.g., interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the tested rats were assessed according to the commercial ELISA kits (Carmania Parsgen Co, Iran) based on the producer instructions.

Statistical analysis

Data were analyzed by SPSS software version 26.0. The differences between groups were assessed by one-way analysis of variance and the results were reported at a significance level of 5%.

Results

Phytochemical analysis

The phytochemical examination exhibited the appearance of flavonoids, saponins, terpenoids, and polysaccharides in AMME. The total phenolic and flavonoid contents were 3.41 (mg GAE/g DW) and 1.46 (mg QE/g DW) respectively.

Evaluating α-amylase inhibition activity

AMME inhibited the α-amylase as a dose-dependent response. The IC_{50} values for AMME and acarbose as the control drug were 18.1 and 4.2 µg/mL, respectively (Figure 1).

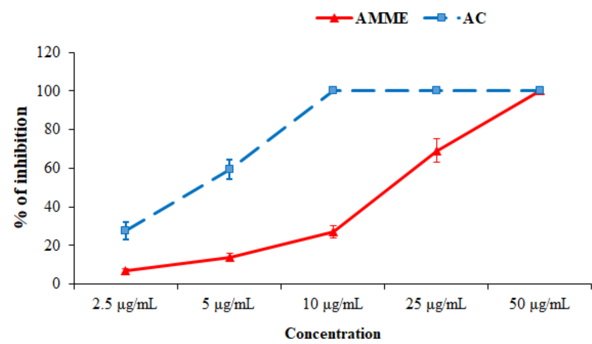

Figure 1. α-Amylase inhibition by methanolic extract of *Astragalus maximus* (AMME) and standard drug (acarbose; AC) (n=3).

Effects of AMME on the biochemical factors

In diabetic rats, the levels of glucose, cholesterol, and triglyceride were considerably ($P < 0.001$) elevated compared to the non-diabetic rats. However, the level of insulin was considerably ($P < 0.001$) declined in diabetic rats compared to the non-diabetic rats. In the diabetic rats receiving the AMME at doses of 150 and 300 mg/kg a significant reduction ($P < 0.05$) in the levels of glucose, cholesterol, and triglyceride and an elevation in the level of insulin were observed (Table 1). In diabetic rats, the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin were elevated when compared with the non-diabetic rats; nevertheless, diabetic rats treated with AMME at doses of 150 and 300 mg/kg for 28 days displayed improved levels of AST, ALT, ALP, and bilirubin compared with diabetic rats treated with normal saline ($P < 0.001$) (Table 2).

In diabetic rats, the levels of kidney function parameters of creatinine (Cr) and urea (Ur) were significantly raised when compared with the non-diabetic rats; however, diabetic rats treated with AMME at doses of 150 and 300 mg/kg for 28 days displayed improved levels of Cr, Ur, uric acid, total protein, and albumin compared with diabetic rats treated with normal saline ($P < 0.001$) (Table 3).

Effects of AMME on the oxidant/antioxidant enzymes

Diabetes resulted in a significant reduction in the tissue activity of antioxidant enzymes ($P < 0.01$). However, diabetic rats treated with AMME at doses of 150 and 300 mg/kg for 28 days exhibited noticeable enhancement

Table 1. Antidiabetic effects of *Astragalus maximus* methanolic extract and glibenclamide on the serum levels of some biochemical parameters in the diabetic rats

Group	Glucose (mg/dL)	Insulin (mU/mL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Non-diabetic rats	83.6 ± 2.36	3.81 ± 0.89	76.6 ± 5.51	83.4 ± 5.21
Diab+Normal saline	236 ± 8.56	0.61 ± 0.071	137.3 ± 4.46	146.5 ± 6.65
Diab+GLB (0.6 mg/kg)	92.3 ± .4.62***	2.89 ± 0.31***	82.4 ± 3.72***	79.8 ± 5.48***
Diab+AMME 75 mg/kg	196.3 ± 5.12	1.07 ± 0.084	102.4 ± 6.12	91.4 ± 5.48***
Diab+AMME 150 mg/kg	132.3 ± 4.12***	1.89 ± 0.23***	85.6 ± 5.44***	89.3 ± 6.87***
Diab+AMME 300 mg/kg	102.6 ± 5.46***	2.71 ± 0.34***	76.5 ± 6.66***	71.3 ± 5.41***

AMME, *Astragalus maximus* methanolic extract; GLB, glibenclamide; Diab, diabetic.
*** $P < 0.001$ in comparison with the diabetic rats treated with normal saline.

Table 2. The effects of *Astragalus maximus* methanolic extract and glibenclamide on the serum levels of liver enzymes in healthy and diabetic rats

Group	ALT (Unit/L)	AST (Unit/L)	ALP (Unit/L)	Bilirubin (mg/dL)
Non-diabetic rats	126.6 ± 2.36	134.4 ± 4.73	196.5 ± 6.73	0.86 ± 0.04
Diab+Normal saline	174.3 ± 3.46	192.5 ± 3.86	278.6 ± 4.76	1.78 ± 0.07
Diab+GLB (0.6 mg/kg)	129.4 ± .1.62***	137.3 ± 2.67***	191.3 ± 4.67***	0.91 ± 0.08***
Diab+AMME 75 mg/kg	158.4 ± 7.68	178.4 ± 5.41	247.6 ± 8.12	1.59 ± 0.13
Diab+AMME 150 mg/kg	144.4 ± 5.42	154.6 ± 7.23	227.7 ± 9.5	1.26 ± 0.21
Diab+AMME 300 mg/kg	124.4 ± 6.24***	131.4 ± 6.47***	178.7 ± 8.63***	0.89 ± 0.07***

AMME, *Astragalus maximus* methanolic extract; GLB, glibenclamide; Diab, diabetic; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
*** $P < 0.001$ in comparison with the diabetic rats treated with normal saline.

Table 3. The effect of *Astragalus maximus* methanolic extract and glibenclamide on the serum level of kidney parameters in non-diabetic and diabetic rats

Group	Total protein (mg/dL)	Cr (mg/dL)	Ur (mg/dL)
Non-diabetic rats	6.31 ± 0.65	0.66 ± 0.04	21.6 ± 4.21
Diab+Normal saline	2.21 ± 0.36	2.71 ± 0.21	44.3 ± 5.32
Diab+GLB (0.6 mg/kg)	5.98 ± 0.72***	0.72 ± 0.09***	24.2 ± 5.48***
Diab+AMME 75 mg/kg	3.26 ± 0.51	2.13 ± 0.21	34.2 ± 3.62
Diab+AMME 150 mg/kg	4.46 ± 0.72***	1.87 ± 0.23	28.3 ± 3.46***
Diab+AMME 300 mg/kg	5.56 ± 0.82***	0.94 ± 0.07***	21.5 ± 2.33***

AMME, *Astragalus maximus* methanolic extract; GLB, glibenclamide; Diab, diabetic; Cr, creatinine; Ur, urea.
*** $P < 0.001$ in comparison with the diabetic rats treated with normal saline.

in the tissue activity levels of SOD, CAT, and GST ($P < 0.001$). Diabetes also caused an elevation in tissue malondialdehyde (MDA) level in the tested mice; however, after treatment of diabetic rats with AMME at doses of 150 and 300 mg/kg for 28 days, the tissue level of MDA was declined significantly ($P < 0.001$) when compared with diabetic rats (Table 4).

Following the treatment of diabetic rats with either 150 or 300 mg/kg AMME for 28 days, a significant reduction in the pancreas levels of TNF-α and IL-1β were observed when compared with diabetic rats (Figure 2).

Discussion

This work was designed to study the effect of *A. maximus* methanolic extract in streptozotocin-diabetic rats. The phytochemical examination exhibited the appearance of flavonoids, saponins, terpenoids, and polysaccharides in AMME. The total phenolic and flavonoid contents were 3.41 (mg GEA/g DW) and 1.46 (mg QE/g DW), respectively. In line with our results, in previous studies these compounds have been the main compounds of *Astragalus* spp. (19-21). Flavonoids and phenols from herbal medicines are valuable alternative medications for diabetes mellitus. These compounds are promising molecules that might produce novel drug discoveries (22). Phenolic compounds have exhibited their anti-diabetic effects through various mechanisms such as improving the insulin sensitivity and glucose uptake, inhibition of α-glucosidase/α-amylase enzymes, activation of AMPK and PPAR pathways, and modulating the oxidative stress and inflammation (23).

Table 4. The effect of *Astragalus maximus* methanolic extract and glibenclamide on the pancreatic tissue levels of oxidant/antioxidant enzymes (SOD, CAT, GPX and MDA) in non-diabetic and diabetic rats

Group	CAT (nmol/L)	GPX (nmol/mg)	SOD (nmol /mg)	MDA (nmol/mg)
Non-diabetic rats	3.01 ± 0.31	43.2 ± 4.42	5.78 ± 0.61	2.54 ± 0.31
Diab+Normal saline	1.64 ± 0.12	24.6 ± 2.56	0.92 ± 0.078	8.46 ± 0.89
Diab+GLB (0.6 mg/kg)	2.76 ± 0.14***	39.8 ± 3.23***	5.12 ± 0.61***	3.23 ± 0.76***
Diab+AMME 75 mg/kg	1.84 ± 0.19	26.1 ± 2.15	1.68 ± 0.32	7.12 ± 1.46
Diab+AMME 150 mg/kg	2.47 ± 0.37***	36.2 ± 2.68***	3.21 ± 0.36***	5.01 ± 0.68***
Diab+AMME 300 mg/kg	2.72 ± 0.12***	43.1 ± 3.11***	4.23 ± 0.73***	3.78 ± 0.51***

AMME, *Astragalus maximus* methanolic extract; GLB, glibenclamide; Diab, diabetic; SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; MDA, malondialdehyde.
*** $P < 0.001$ in comparison with the diabetic rats treated with normal saline.

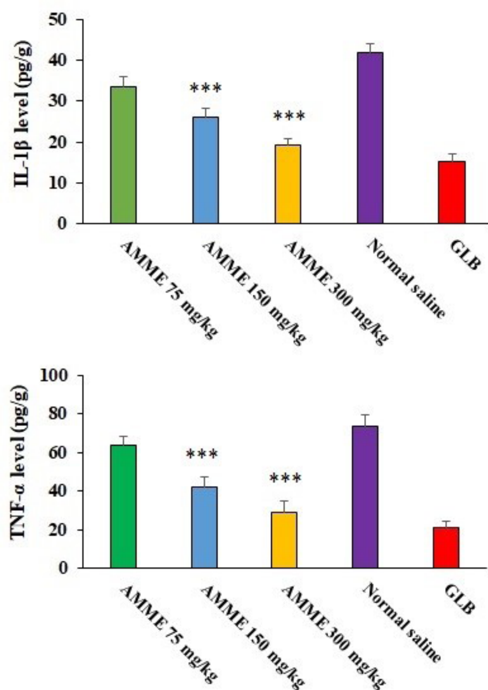

Figure 2. Pancreas levels of IL-1β and TNF-α after treatment of the diabetic rats with various doses of Astragalus maximus methanolic extract and glibenclamide (0.6 mg/kg) for 28 days. ****P*<0.001 compared to the normal saline group. AMME, *Astragalus maximus* methanolic extract; GLB, glibenclamide; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α.

Previous investigations reported the antidiabetic effects of *A. membranaceus* and its phytochemical ingredients, e.g., phenols, flavonoids, saponins, and polysaccharides for treating the types 1 and 2 diabetes mellitus through the modulation of inflammatory and apoptotic cytokines, as well as increasing the antioxidant activities and insulin sensitivity (24).

AMME inhibited α-amylase dose-dependently. In the diabetic rats receiving the AMME at doses of 150 and 300 mg/kg a significant reduction ($P < 0.05$) in the levels of glucose, cholesterol, and triglyceride was observed, while they displayed an elevated level of insulin. Consequently, AMME may be displayed an insulin-like activity through releasing insulin from the pancreas and improving the serum glucose level of diabetic rats. It has been previously proven that herbal extracts most likely increase the consumption of glucose by the adjacent tissues, prevent glucose absorption in the kidneys, and increase the release of insulin from the islets cells of the pancreas (25). We reported that diabetic rats treated with AMME at doses of 150 and 300 mg/kg for 28 days displayed improved levels of AST, ALT, ALP, bilirubin, Cr, Ur, uric acid, total protein, and albumin compared with diabetic rats treated with normal saline. These promising effects on liver and kidney function are probably due to the positive effects of herbal extract in ameliorating the liver and kidney injuries induced by streptozotocin (26).

Our results revealed that diabetes results in a significant reduction in the tissue activity of antioxidant enzymes. However, diabetic rats treated with AMME at doses of 150 and 300 mg/kg for 28 days exhibited that the tissue activity levels of SOD, CAT, and GST were noticeably enhanced. Diabetes also caused an elevation in tissue MDA level in the tested mice; however, after treatment of diabetic rats with AMME at doses of 150 and 300 mg/kg for 28 days, the tissue level of MDA was declined when compared with diabetic rats. Diabetes results in an elevation in oxidative stress and lipid peroxidation; where there is a direct association between diabetes symptoms and lipid peroxidation. In addition, it has been proven that elevated blood sugar results in a significant reduction in the activity of antioxidant enzymes and subsequently a rise in free radicals (27-29). Therefore, we can suggest that AMME, through increasing the antioxidant enzymes and reducing the oxidative stress, is able to control diabetes in rats. 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 (30). The treatment of diabetic rats with either 150 or 300 mg/kg AMME for 28 days resulted in significant reduction of TNF-α and IL-1β levels in the pancreas, when compared with diabetic, indicating that AMME through its anti-inflammatory effects may control diabetes in rats.

Conclusion

This study showed that the oral administration of *A. maximus* methanolic extract in diabetic rats with streptozotocin displayed a potent anti-diabetic activity through increasing insulin release and ameliorating inflammation and oxidative stress; however, more investigations are desired to determine the mechanism effects of the extract.

Authors' contribution

HS designed, HS, NS, and HRM performed experiments and collected data, RR wrote the draft, HRM and JGY discussed the results and strategy, and MNM supervised, directed, and managed the study. All authors approved the final version to be published.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

This study was approved by the ethics committee of Lorestan University of Medical Sciences, Khorramabad, Iran, with the ethics number of IR.LUMS.REC.1401.219.

Funding/Support

The authors declare that they have not received any financial support from any organization.

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