

Liver Dysfunction and Oxidative Stress in Streptozotocin-Induced Diabetic Rats: Protective Role of *Artemisia Turanica*

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Key Words

diabetes mellitus, artemisia turanica, metformin, oxidative stress

Abstract

Objectives: Oxidative stress plays a central role in diabetes-induced complications. In the present study, the protective effect of *Artemisia turanica* (*A. turanica*) was evaluated against diabetes-induced liver oxidative stress and dysfunction.

Methods: Fifty male Wistar rats were randomly divided into five groups: control, diabetic, diabetic + metformin, diabetic + *A. turanica* extract, and diabetic + *A. turanica* extract + metformin. Experimental diabetes was induced by a single-dose (55 mg/kg, intraperitoneally (ip)) injection of streptozotocin (STZ). Metformin (300 mg/kg) and *A. turanica* extract (70 mg/kg) were orally administered three days after STZ injection for four weeks. The levels of malondialdehyde (MDA), total thiol content and superoxide dismutase (SOD) and catalase activities were measured in the liver tissue. Serum glucose concentration, aspartate aminotransferase (AST)

and alanine aminotransferase (ALT) activities were also determined.

Results: In the diabetic group, serum glucose concentration, serum AST and ALT activities and liver MDA level were significantly higher while tissue total thiol content as well as catalase and SOD activities were lower, compared to the control group. Serum glucose in diabetic rats treated with metformin + *A. turanica* extract showed a significant decrease compared with the diabetic group. In all the *A. turanica* extract and metformin treated groups, serum ALT, tissue MDA level, total thiol content and SOD activity significantly improved compared with the diabetic rats. However, treatment of the diabetic rats only with metformin could not significantly change the activities of catalase and AST compared with the diabetic group.

Conclusion: These findings suggested that *A. turanica* extract had a therapeutic effect on liver dysfunction and oxidative stress induced by diabetes, that may be probably due to its antioxidant and antiinflammatory effects.

1. Introduction

Diabetes mellitus is a common health problem with

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multiple etiology mainly characterized by hyperglycemia resulting from insulin secretion and/or function abnormalities [1]. Diabetes is also associated with major defects in lipid and protein metabolisms, thereby leading to many severe and life threatening complications including neuropathy, nephropathy, retinopathy, vasculopathy and hepatopathy [2]. Fibrosis, abnormal fat and glycogen deposition, cirrhosis and increased hepatic enzyme activities are some of liver abnormalities associated with diabetes [3]. Moreover, alterations in hepatic cell growth and cell number alter the liver size during diabetes. Increasing data suggests that free radicals generation resulted by glucose oxidation and protein glycation as well as defects in antioxidant defense systems play a critical role in pathogenesis of both types of diabetes [4, 5]. It has been proposed that the most important cause of liver damage in diabetic patients is hyperglycemia-induced oxidative stress and subsequent disturbance in carbohydrate, protein and lipid metabolisms [6]. These events, in turn, lead to further oxidative stress and activation of inflammatory cascades [6]. Nowadays, reduction of hyperglycemia using edible drugs and injection of insulin are the most common strategies for treatment of diabetes. However, gradual resistance to these agents, in addition to their various adverse effects, has increased the need for finding alternative therapies with less or even no side effects for diabetic patients. In recent years, the use of herbal medications has developed to minimize hyperglycemia and other diabetes associated complications. In various investigations, the protective action of herbal antioxidants has been reported in scavenging free radicals and improving liver dysfunction in diabetic experimental models [7-9].

Genus *Artemisia* is one of the most important and largest genera in the family Asteraceae (Compositae). This genus comprises about 500 species, most of which are medicinal plants and have been traditionally used all over the world [10]. *Artemisia turanica* (*A. turanica*) commonly known as "Dermane Ghermez" grows mainly in the northeast of Iran [11]. There are different compounds in the essential oil of *A. turanica* including 1,8-cineol, chrysanthenone, davanone, cis-verbanyl, camphor and oxygen containing monoterpenoids [12]. It has been reported that *A. turanica* possesses different biological activities including antimalarial, anti-fungal, cytotoxic and apoptotic properties [13, 14]. The current study was undertaken to determine whether *Artemisia turanica* aqueous-ethanolic extract could improve the liver dysfunction and oxidative stress in STZ-induced diabetic rats.

2. Materials and Methods

2.1. Extract preparation

Aerial parts of the plant were collected from Mazrae Sheikh, a village in Khaf City, (Razavi Khorasan Province, Iran) and were identified by a botanist in the Herbarium of School of Pharmacy, the Mashhad University of Medical Sciences, Mashhad, Iran (specimen number 12572). The plant was washed, dried, powdered and macerated with 70% ethanol with occasional shaking and stirring.

Then, the filtered mixture was kept at 45°C for ethanol evaporation, yielding a blackish-brown concentrate. The prepared extract was kept at 4°C prior to use.

2.2. Drugs and kits

Metformin (Met) powder was purchased from Samisaz Company (Mashhad, Iran). STZ was obtained from Sigma-Aldrich (St Louis, MO, USA). Moreover, Glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) kits were provided from Pars Azmoon Company (Tehran, Iran).

2.3. Animals

Fifty male Wistar rats (12 weeks old, weighed 200 ± 20 g) were obtained from the Animal House of the School of Medicine, the Mashhad University of Medical Sciences, Mashhad, Iran. All the animals were maintained at $22 \pm 2^\circ\text{C}$ and 12 h light/dark cycle. All the experiments were performed under the authority of the Mashhad University of Medical Sciences and the norms of international animal ethics were followed.

2.4. Experimental Design

The animals were randomly divided into five groups (n = 10) as follows:

Control: free access to food and water with an intraperitoneal (ip) injection of saline at the first of the study

Diabetic: single dose ip injection of STZ (55 mg/kg) for induction of diabetes

Diabetic + Met: oral administration (by gavage) of metformin (300 mg/kg) three days after STZ injection for four consecutive weeks

Diabetic + Ext: oral administration (by gavage) of *Artemisia turanica* extract (70 mg/kg) three days after STZ injection for four consecutive weeks.

Diabetic + Ext + Met: oral administration (by gavage) of *Artemisia turanica* extract (70 mg/kg) and metformin (300 mg/kg) three days after STZ injection for four consecutive weeks.

At the end of the experiment, serum samples were collected from the orbital sinus and kept at -20°C for serum glucose and hepatic enzymes assessment. Then, all the animals were humanely killed and the liver tissue was quickly removed and conserved for oxidative stress assessment.

2.5. Assessment of serum biochemical parameters

Serum glucose concentration as well as AST and ALT activities were detected by the commercial kits based on the manufacturer's instructions.

2.6. Assessment of oxidative stress markers

2.6.1. Malondialdehyde (MDA) measurement

MDA as an index of lipid peroxidation reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance to produce a red colored complex. It has a peak absorbance at 535 nm. In this study, 15 g TCA, 0.375 g TBA and 2 ml HCl were mixed, 2 ml of this mixture was added to 1 ml of serum or kidney homogenate in a centrifuge tube, and the mixture was heated for 50 min in a boiling water bath. After cooling, the mixture centrifuged at 1000 rpm for 10 min. The absorbance (A) of the colored layer was read at 535 nm. MDA concentration was calculated from the following equation [15].

$$C (M) = A / 1.56 \times 105$$

2.6.2. Total thiol content measurement

Total-SH groups were measured using DTNB as the reagent. The reagent reacted with the SH groups to produce a yellow colored complex which had a peak absorbance at 412 nm. Afterwards, 1 ml Tris-EDTA buffer was added to 50 μ l of serum or kidney homogenate and absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20 μ l DTNB reagents (10 mM in methanol) were added to the mixture and after 10 min, the sample absorbance was read again (A2). The absorbance of the DTNB reagents was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation [16]:

$$\text{Total thiol concentration (mM)} = (A2 - A1 - B) \times 1.07 / 0.05 \times 13.6$$

2.6.3. Assessment of antioxidant enzymes activities

Superoxide dismutase (SOD) activity was determined in the stomach tissue by the method of Madesh [17]. In a colorimetric assay, the SOD activity was measured at 570 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the rate of MTT reduction by 50%. The results were shown as unit per milligram protein. Catalase activity was measured according to the method of Aebi with hydrogen peroxide (30 mM) as the substrate. One unit of catalase activity was determined as the micromoles of the hydrogen peroxide consumed per milligram of protein sample.

2.7. Statistical analysis

The data were expressed as mean \pm SEM. One-way ANOVA was run followed by a post hoc LSD comparison test. Statistical significance was considered as $p < 0.05$.

3. Result

3.1. The effect of *A. turanica* extract and metformin on serum biochemical parameters

Serum glucose concentration in the diabetic group significantly increased compared with the control group ($p < 0.001$). However, treatment of diabetic rats with metformin and *A. turanica* extract separately caused a significant decrease in blood glucose compared to the diabetic rats ($p < 0.01$ & $p < 0.001$, respectively) (Fig. 1). As shown in Table 1, serum AST and ALT activities showed significant increase in the diabetic group compared to those of the control group ($p < 0.05$) (Table 1). In the diabetic + Ext and diabetic + Ext + Met groups, serum AST activity was significantly lower compared to the diabetic group ($p < 0.05$) (Table 1). Interestingly, compared with the control group, serum ALT activity significantly decreased ($p < 0.001$) in the diabetic + Ext and diabetic + Ext + Met groups, but showed no significant change in the diabetic + Met group. Serum ALT activity in all the *A. turanica* extract and metformin treated groups significantly decreased compared with the diabetic rats ($p < 0.05$, $p < 0.01$ & $p < 0.001$ respectively) (Table 1).

3.2. The effect of *A. turanica* extract and metformin on hepatic oxidative stress biomarkers liver MDA concentration

MDA concentration was significantly higher in the diabetic rats than in the control animals ($p < 0.001$) (Fig. 2). However, the separate and concomitant treatment of the diabetic rats with *A. turanica* extract and metformin significantly decreased the liver tissue level of MDA as compared with the diabetic group ($p < 0.001$) (Fig. 2). In these groups, MDA concentration showed no significant change when compared with the control group.

Table 1 The effect of *A. turanica* on serum AST and ALT activities in all experimental groups.

| | Control | Diabetic | Diabetic + Met | Diabetic + Ext | Diabetic + Ext + Met |
|-----------|-------------------|--------------------|-------------------------------|-----------------------------------|--------------------------------|
| AST (U/L) | 179.6 \pm 20.99 | 365.8 \pm 30.4* | 313.5 \pm 98.79 | 221.16 \pm 81.77 [#] | 217.5 \pm 54.69 [#] |
| ALT (U/L) | 101 \pm 20.98 | 201.8 \pm 47.05* | 103.8 \pm 40.2 [#] | 37.8 \pm 15.49 ^{#####} | 31 \pm 8.5 ^{#####} |

Met: metformin, Ext: Artemisia turanica extract, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

All data were expressed as mean \pm SEM (n = 10 in each group).

* $p < 0.05$, *** $p < 0.001$ compared to control group.

[#] $p < 0.05$, ##### $p < 0.001$ compared to diabetic group.

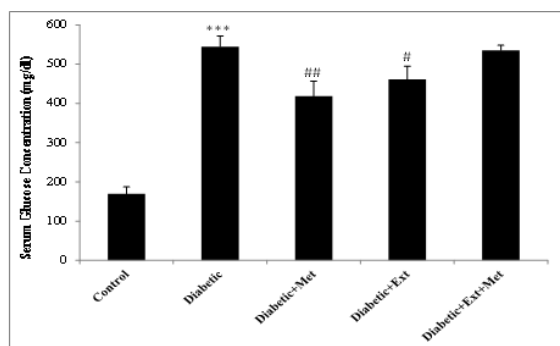


Figure 1 Glucose concentration in all experimental groups. Values are the Mean±SEM. The data were analyzed using one-way ANOVA & post hoc LSD.

*** p<0.001 compared to control group.

p<0.05 and ## p<0.01 compared to diabetic group

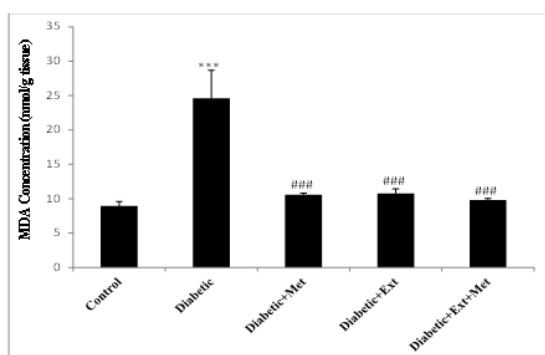


Figure 2 Liver MDA concentration in all experimental groups. Values are the Mean±SEM. The data were analyzed using one-way ANOVA & post hoc LSD.

*** p<0.001 compared to control group.

p<0.001 compared to diabetic group.

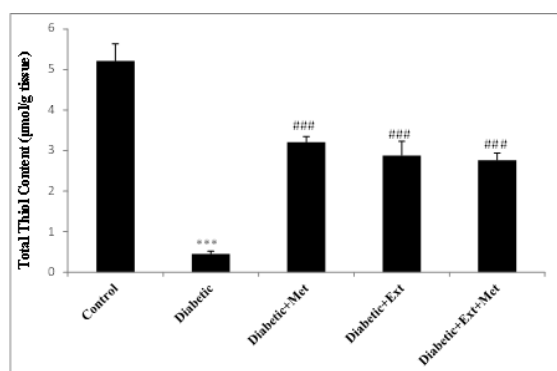


Figure 3 Liver total thiol concentration in all experimental groups. Values are the Mean±SEM. The data were analyzed using one-way ANOVA & post hoc LSD.

*** p<0.001 compared to control group

p<0.01 compared to diabetic group

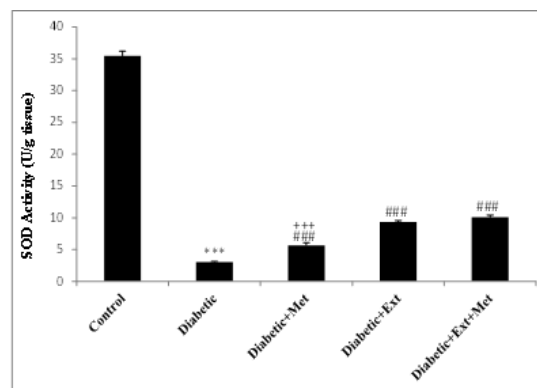


Figure 4 Liver superoxide dismutase (SOD) activity in all experimental groups. Values are the Mean±SEM. The data were analyzed using one-way ANOVA & post hoc LSD.

*** p<0.001 compared to control group.

p<0.001 compared to diabetic group.

+++ p<0.001 compared to Diabetic+Ext+Met group.

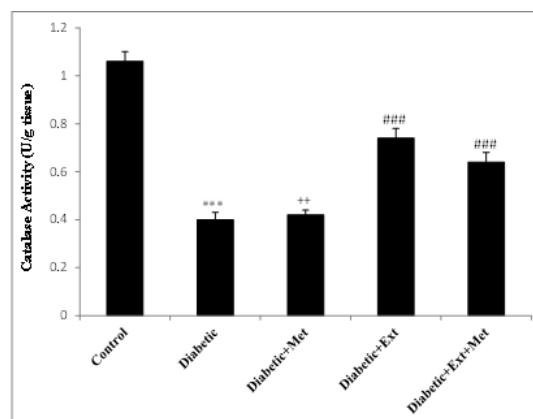


Figure 5 Liver catalase activity in all experimental groups. Values are the Mean±SEM. The data were analyzed using one-way ANOVA & post hoc LSD.

*** p<0.001 compared to control group.

p<0.001 compared to diabetic group.

++ p<0.01 compared to Diabetic+Ext+Met group.

3.3. Liver total thiol content

As shown in Fig. 3, total thiol content in the diabetic rats was significantly lower than control animals ($p < 0.001$) (Fig. 3). In all the metformin and *A. turanica* extract treated groups, total thiol content showed a significant increase compared with the diabetic group ($p < 0.001$) (Fig. 3).

3.4. Liver antioxidant enzymes activities

Figures 4 and 5 demonstrate the activities of SOD and catalase enzymes in the liver tissue in all the experimental groups. The results showed that SOD and catalase activities significantly decreased in the diabetic group compared to the control group ($p < 0.001$). Separate and

concomitant treatment of the diabetic rats with *A. turanica* extract and metformin significantly increased the SOD activity as compared with the diabetic group ($p < 0.001$) (Fig. 4). However, catalase activity showed a significant increase in the diabetic + Ext and diabetic + Ext + Met groups ($p < 0.001$) (Fig. 5). Compared to the diabetic + Ext + Met group, SOD and catalase activities were significantly lower than those of the diabetic + Ext and diabetic + Met groups ($p < 0.001$ & $p < 0.01$, respectively) (Figs. 4 & 5).

4. Discussion

In the current study, administration of *A. turanica* extract separately or concomitant with metformin significantly improved serum alterations in hepatic enzymes and liver tissue oxidative damage induced by diabetes. In the present investigation, in agreement with previous studies, we showed that STZ was significantly able to increase serum glucose concentration, which confirmed the accuracy of the present study in induction of diabetes. STZ-induced diabetes was also associated with significant alterations in liver dysfunction and oxidative stress markers. Hyperglycemia is the most common feature in both types of diabetes. Previous studies suggest that free radicals generation and simultaneous defect in cells antioxidant capacity are the main mechanisms for diabetes complications [18]. Different endogenous organs are highly affected by diabetes with liver being one of the most important organs [19]. It has been reported that hyperglycemia-induced oxidative stress and subsequent disturbance in carbohydrate, protein and lipid metabolisms are the most important causes of liver damage in diabetic patients [6]. Based on our results, treatment of the STZ-induced diabetic rats separately with *A. turanica* extract and metformin significantly decreased blood glucose level as compared to the untreated diabetic rats. In the same context, Ghazanfar et al. (2014) reported significant decrease in blood level of glucose in STZ-induced diabetic animals receiving *Artemisia amygdalina* extract [20]. The antidiabetic activities of *Artemisia judaica*, *Artemisia annua* and *Artemisia herba-alba* have also been reported [21-23]. The hypoglycemic effect of *A. turanica* extract in our study might be due to stimulation of insulin secretion from β cells and reduction of tissue resistance to insulin, mainly by its terpenoid compounds [24, 25].

However, the exact mechanism for the lack of hypoglycemic action of metformin and *A. turanica* co-administration in the diabetic rats is not clear; however, these two factors appear to counteract each other in a way that is not elucidated. In the current study, in all the metformin and *A. turanica* extract treated groups, MDA concentration significantly decreased but the content of the total thiol groups and SOD activity significantly increased compared with the diabetic untreated rats. However, treatment of the diabetic rats with metformin separately could not change the catalase activity when compared with the diabetic group. These results are in agreement with previous studies describing the protective role of different species of *Artemisia* on liver oxidative damage induced by diabetes [26-28]. Therefore, the beneficial action of *A. turanica* on liver tissue oxidative damage in the STZ-induced di-

abetic rats might be due to its antioxidant effects mainly by terpenoid compounds [29]. As observed in our results, treatment of the diabetic animals only with *A. turanica* led to more protection, approximately 25%, against antioxidant enzymes dysfunction, as compared to the co-administration of metformin and *A. turanica* extract. In the present work, diabetes was also associated with a significant increase in serum ALT and AST activities. AST and ALT are two well-known hepatic enzymes that cause liver cell damage in case of having elevated serum activities, as observed in the diabetic rats. We observed that oral administration of *A. turanica* extract alone and in combination with metformin significantly attenuated the elevated activities of AST and ALT.

This may be due to the protective action of the extract possibly via its antioxidant effect in reversing liver damage induced by diabetes. Similar findings were also reported by Taofik et al. (2013) using *Artemisia afra aqueous* extract in diabetic rats [30].

5. Conclusion

In conclusion, the findings of the present study revealed that *A. turanica* extract could improve the liver oxidative damage and dysfunction in the STZ-treated diabetic rats. However, our results demonstrated that separate administration of *A. turanica* exerted more protection against liver injury induced by diabetes than the co-administration of metformin and *A. turanica*. Further investigations are required to elucidate mechanisms for beneficial actions of melatonin during diabetes.

Conflict of interest

The authors declare that there is no conflict of financial interest.

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