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Original article

Improvement of Blood Glucose, Serum Antioxidant Status and Liver Enzyme Changes by Hydroalcoholic Extract of *Solanum melongena* Green Cap in Diabetic Rats

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Abstract

Background and objectives: Diabetes is associated with a wide range of disorders including oxidative stress. *Solanum melongena* L. possesses antioxidant compounds and was introduced as an antidiabetic herb in Asian traditional texts. The aim of the present study was to investigate the effect of hydroalcoholic extract of *S. melongena* green cap on blood glucose and antioxidant status of liver in diabetic rats. **Methods:** In this experimental study, the extract was prepared by maceration method and 42 male Wistar rats (190-220 g) were divided into 6 groups (n=7/groups) including: 1. Control; 2. diabetic groups receiving 1 mL distilled water daily; groups 3 and 4 of normal rats receiving 100 and 200 mg/kg extracts, respectively, and diabetic treated groups 5 and 6 with 100 and 200 mg/kg extract, respectively. Diabetes was induced by intraperitoneal injection of streptozotocin (55 mg/kg). At the end of study, serum levels of fasting blood glucose (FBG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, nitric oxide (NO) level and total antioxidant capacity (FRAP) were measured. Histological changes of liver were also assessed using hematoxylin and eosin staining. **Results:** Treatment of diabetic animals with the extract of *S. melongena* cap (100 and 200 mg/kg) significantly reduced serum levels of FBG, ALT, ALP, NO ($p<0.001$) and AST ($p=0.017$), while total protein ($p<0.001$) and FRAP ($p=0.007$) levels increased significantly. The extract also improved the liver tissue changes induced by diabetes. **Conclusion:** *Solanum melongena* cap is effective in improving liver complications and serum antioxidant status in diabetic rats.

Keywords: anti-diabetic agents; antioxidants; liver; nitric oxide; *Solanum melongena*

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Introduction

Diabetes is one of the most common problems in today's world that leads to impaired metabolism of carbohydrates, fats and proteins [1]. Liver is a key organ of the body's metabolism and maintaining blood-glucose balance. Chronic hyperglycemia leads to oxidation/reduction imbalance in hepatocytes through the increase level of advanced glycation end products (AGEs) and impairs production of superoxide dismutase, glutathione peroxidase and catalase, and results in free radical production [2].

In cases of hepatic tissue damage, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline

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phosphatases enzymes are released into the bloodstream. These enzymes are used as standard tests to evaluate hepatocytes function [3]. They increase in diabetic patients, which is a sign of liver damage [4]. On the other hand, diabetes decreases the activity of antioxidant enzymes and the imbalance between antioxidant capacity and the production of cellular free radicals leads to oxidative stress (OS). Free radicals of the biological system are often nitrogen-derived there are also exist [5].

Nitric oxide (NO) is a small lipophilic molecule with a short half-life that is involved in many biological systems. This molecule is an intra- and inter-cellular messenger in the control of physiological processes, and plays a key role in maintaining homeostasis. On the other hand, NO is capable of reacting with the oxygen molecule and producing N_2O_3, which in turn produces free oxygen species that can cause tissue damage [6]. Superoxide dismutase is a metalloprotein that acts as the first and most important defense against the superoxide radicals produced in the cell [7]. Increased glucose in diabetes inactivates antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Glycosylation of these proteins causes oxidative stress and lipid peroxidation [8]. Cells in the body are constantly exposed to damage caused by free radicals and reactive oxygen species (ROS) that are produced during normal body metabolism or external damage [9].

Although current and effective treatments for diabetes consist of hypoglycemic agents and insulin, these compounds show undesirable long-term effects. Therefore, there is a need to find more effective compounds with fewer side effects in the treatment of diabetes [1]. Traditional treatments of diabetes are used worldwide with natural herbs or compounds. Medicinal herbs are important in the treatment of metabolic diseases due to fewer side effects [10]. The identification of novel antioxidant compounds that have broad biological effects is warranted [11].

Solanum melongena L. (eggplant) is a herb belonging to the Solanaceae family. In traditional medicine of some countries like India, its beneficial effects in the treatment of anemia and inflammation, prevention of bleeding and myocardial infarction, hypotension and antioxidant effects have been reported [12]. *Solanum melongena* is a rich source of flavonoids with high antioxidant activity and it has shown favorable antimicrobial effect on all three strains of *Streptococcus mutans*, *S. sobrinus* and S. *sanguis* [13]. Anthocyanins are one of the important eggplant pigments that inhibit lipid peroxidation. *Solanum melongena* is characterized by high amounts of water and low-calories [14] and its extract is recommended for treatment of arthritis, asthma, bronchitis and diabetes in Asian traditional medicine. Studies have shown that its extract reduces blood sugar and cholesterol level in humans and mice [15]. There is no scientific report on the effect of eggplant green cap on liver enzyme changes and antioxidant status induced by diabetes. The aim of the present study was to investigate the effect of hydroalcoholic extract of *S. melongena* green cap on FBG, liver enzyme changes, serum antioxidant status and liver histology in diabetic rats.

Materials and Methods

Ethical considerations

The animal procedures were carried out according to the local Laboratory Ethics Committee of Kermanshah University of Medical Sciences (ethic code: KUMS.REC.1397.983) and the ARRIVE guidelines for reporting animal research.

Plant extraction

Solanum melongena fruit was collected at the end of spring (2019) from local farm and was identified by our academic staff of Traditional Medicine Department and their green cap was dried in the shade and powdered. The powder was minced and macerated in 70% ethanol for 48 h in dark at room temperature with stirring. The final solution was filtered, and after evaporation of alcohol, was kept in the refrigerator [16].

Animals

Male Wistar rats (190-220 g) were used in standard conditions (12 h light and 12 h dark at 22±2 °C) with free access to standard food and tap water ad libitum.

Experimental design

Intraperitoneal (i.p.) injection of streptozotocin (STZ) (55 mg/kg) was used to induce diabetes and after 72 h, the animals with FBG above 250 mg/dL were considered diabetic. Rats were randomly divided into 6 groups (n=7), and each

group was treated with i.p. injection once daily for 2 weeks, as follows:
1. Control; 2. diabetic groups receiving 1 mL distilled water daily; 3 and 4: groups of normal rats receiving 100 or 200 mg/kg extracts of *S. melongena* cap; 5 and 6: diabetic groups treated with 100 or 200 mg/kg extract. Doses of extract and study periods were selected through a pilot study. Finally, the FBG of the animals was measured. The rats were then weighed and anesthetized using ketamine (80 mg/kg) and xylazine (5 mg/kg) and heart blood was collected and centrifuged for 15 min at 2500 rpm. Then sera were separated and used to measure levels of AST, ALT, ALP, nitric oxide (NO), and total antioxidant capacity (FRAP). The liver was removed and washed with saline solution and fixed in 10% formalin.

NO Assay
Griess colorimetric method was used to measure NO levels. Because of instability of NO, its direct measurement is difficult and nitrite and nitrate levels of various liquids are calculated as NO concentration. Serum samples were deproteinized using zinc sulfate (6 mg) and centrifuged for 12 min at 12,000 rpm. One hundred µL of the supernatant was mixed with 100 µL of vanadium chloride solution + 50 µL of sulfanilamide and 50 µL of N-(1-naphthyl) ethylenediamine dihydrochloride and incubated for 30 min. The absorbance rate was recorded at 540 and 630 nm using ELISA reader (STAT Fax 100, USA) and were compared with the standard solution (0, 12.5, 25, 50, 100 and 200 µM of sodium nitrate) [17] and the standard curve was plotted.

Total antioxidant capacity
Total antioxidant capacity of serum was determined by ferric reducing ability of plasma (FRAP) method, which is based on the potency of serum antioxidants in the reduction of Fe^{3+} to Fe^{2+} ions in presence of tri-pyridyl triazine (TPTZ). The reducing power of each sample was measured by a spectrophotometer (Unico, 2800P UV/VIS, China) at 593 nm. The standard was different concentrations (125, 250, 500, 1000 mM) of $FeSO_4$ [18] and standard curve was plot.

Histological examination
Liver specimens were prepared by routine processing methods including: dehydration, clearing, impregnation, and embedding steps. Serial sections were stained with standard H&E method. Each slide was captured using an optical microscope equipped with a Motic camera and software (Moticam 2000, spain). Two researchers reported liver tissue changes separately [18].

Statistical analysis
Normal distribution of data was determined by Kolmogorov-Smirnov method and data was analyzed by one-way ANOVA and Tukey post hoc tests, and expressed as mean± SD. P values less than 0.05 were considered significant.

Results and Discussion
Induction of diabetes with STZ significantly increased FBG ($p<0.001$); treatment of diabetic rats with 100 or 200 mg/kg doses of *S. melongena* extracts significantly decreased blood glucose. The extract did not change FBG in normal rats (figure 1A). Body weight significantly reduced in diabetes animals ($p<0.001$); treating animals with different doses of the extract significantly improved body weight (figure 1B).

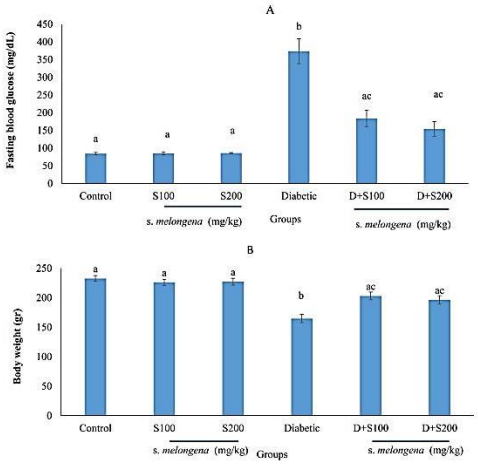

Figure 1. Fasting blood glucose (A) and body weight (B) in different groups receiving *Solanum melongena* extract. Groups (columns) with different superscript letters are statistically significant (one-way analysis of variance, Tukey's post hoc: $p<0.05$)

Diabetes increased the liver enzymes (ALP, ALT and AST), the extract significantly reduced AST ($p=0.017$), ALT and ALP ($p <0.001$) (figures 2A, B, C). Improvement of liver enzymes by the extract was dose dependent and in normal rats, the extract did not show a significant effect on liver enzymes.

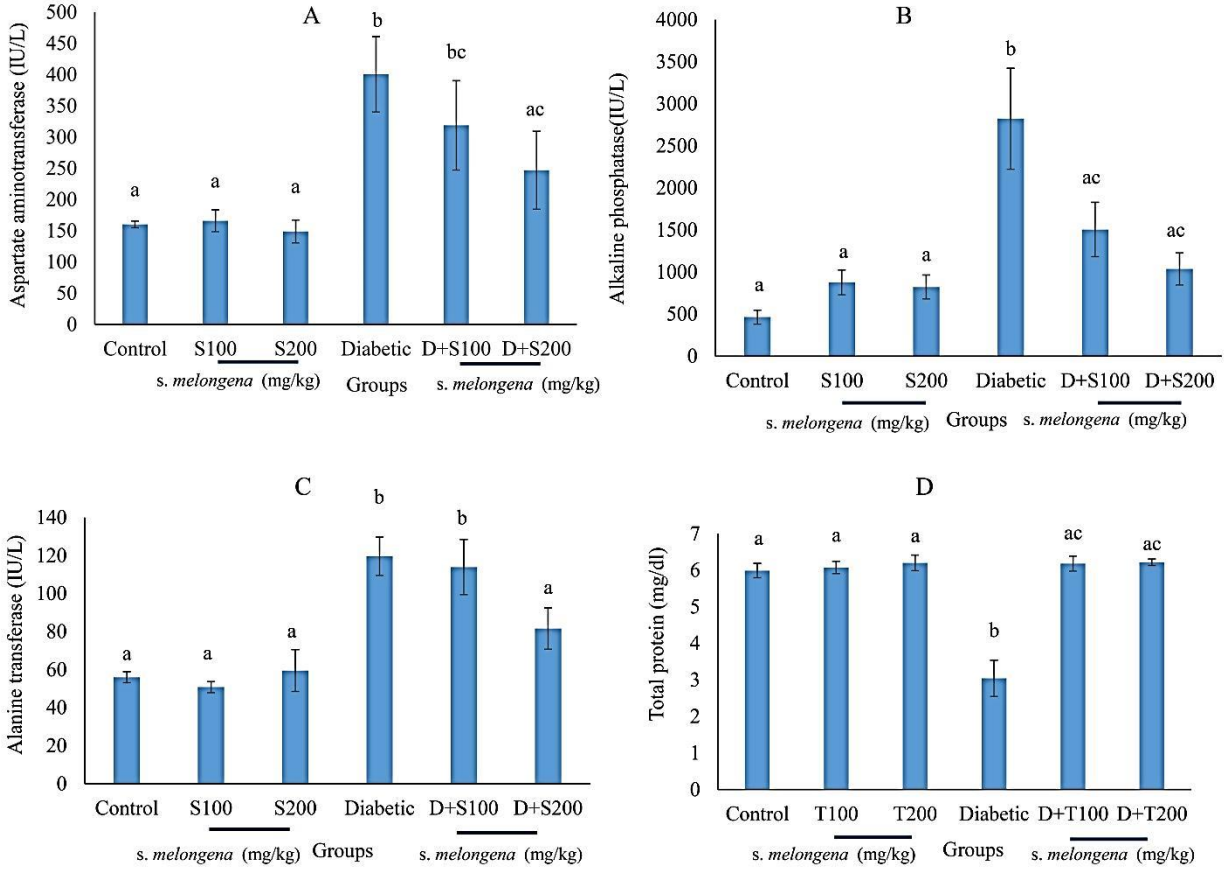

Figure 2. Changes in liver enzymes in groups receiving *Solanum melongena* extract; aspartate aminotransferase (A); alkaline phosphatase (B); alanine aminotransferase (C); total protein (D); Groups (columns) with different superscript letters are statistically significant (one-way analysis of variance, Tukey's post hoc: $p<0.05$)

Serum total protein decreased significantly in diabetic rats and different doses of extract (100, 200 mg/kg) increased this factor significantly ($p<0.001$). There were no significant differences between two doses in normal rats (figure 2D).Serum total antioxidant capacity (TAC) in diabetic rats significantly decreased, while extract treatment significantly ($p=0.007$) increased TAC dose-dependently. There was no significant difference between normal rats receiving the extracts with control groups (figure 3A). Serum level of NO was significantly higher in the diabetic group; whereas, the extract treatment significantly reduced NO level. On the other hand, significant difference was not observed between the normal rats receiving the extracts and control groups (figure 3B). Microscopic examination of liver tissue in control groups (figure 4A) and extract (figure 4B) revealed normal structure of lobules, sinusoids, portal spaces, and hepatocytes (nucleus and cytoplasm).

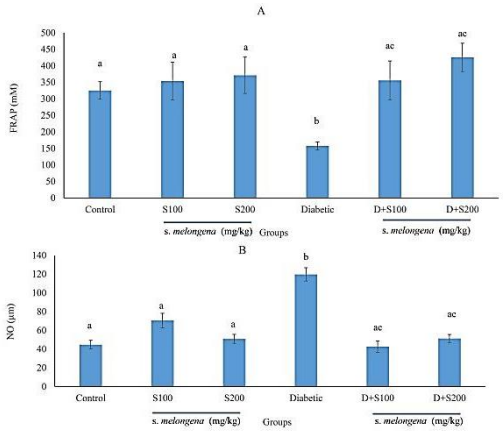

Figure 3. Changes of FRAP (A) and NO (B) values in different test groups receiving *Solanum melongena* extract; Groups (columns) with different superscript letters are statistically significant (one-way analysis of variance, Tukey's post hoc: $p<0.05$)

In the diabetic groups, dilatation of the venous system, compressed hepatocytes with dark nuclei next to the central vein, and local lymphocytic infiltration were observed (figure 4C). However, in the treated diabetic groups, there were not these hisopathological changes (figure 4D). Diabetes increased blood glucose and induced widespread changes in liver enzymes, NO and TAC levels. *Solanum melongena* significantly reduced FBG, improved changes of liver enzymes and histopathology, TAC and total protein; however, the extract did not cause any significant change in non-diabetic rats. Chronic hyperglycemia is one of the leading causes of adverse events in diabetic patients and control of blood glucose reduces the incidence of these complications [19]. Hypoglycemic effect of *S. melongena* is probably due to flavonoids and glycoside compounds [1,12,20]; they are able to lower blood glucose with their antioxidant effects [20]. At the same time, part of the hypoglycemic effects of the extract can be attributed to decreased cellular resistance to insulin, stimulation of peripheral tissues for glucose uptake and concomitant reduction or inhibition of intestinal glucose uptake [21].

STZ enters the beta cell via glucose transporters and damages the DNA chain, induces glucose oxidation, and destroys pancreatic beta cells, which reduces insulin biosynthesis and secretion and increases NO and free radicals [22]. Diabetes is one of the OS- associated diseases that show devastating effects on various tissues of the body including the liver. In the present study, diabetes led to increase in NO and decrease in TAC which is consistent with previous studies [23].

Increased free radicals damage the cell membrane and cause lipid peroxidation and cell necrosis. Liver cell damage causes ALT, AST, and ALP release into the bloodstream and increases their serum levels [24]. In the present study, diabetes increased ALT, AST and ALP. The return of these serum enzymes to normal levels using *S. melongena* extracts may be due to the inhibition of hepatocytes destruction and the maintenance of cell membranes and cell regeneration. Hamzah et al. showed that eggplant antioxidant compounds reduced carbon tetrachloride-induced liver injury in rats, which supports the reduction of AST, ALT, and ALP levels [25].

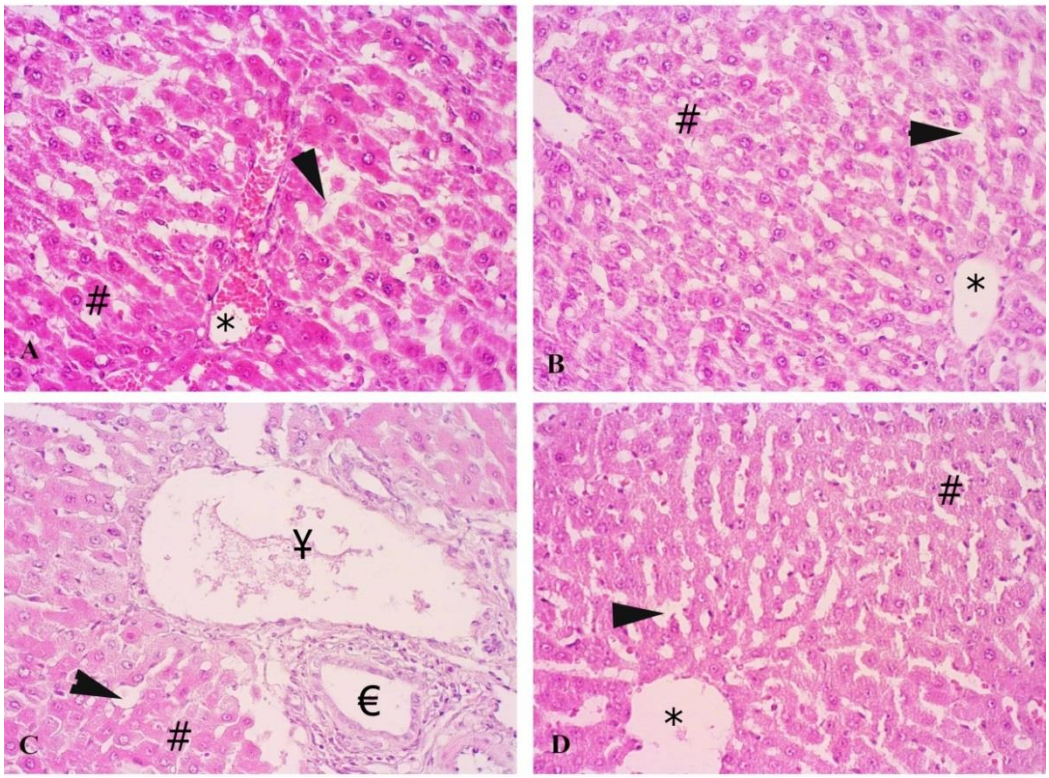

Figure 4. Liver tissue changes in different groups; Control (A); 200 mg/ kg *Solanum melongena* extract (B); diabetic (C); diabetic treated with 200 mg/ kg extract (D); #: Hepatocytes; *: Central vein; Arrowhead: Sinusoids; ¥: dilated portal vein; €: bile duct

Other studies have shown that *S. melongena* extract is effective in the treatment of a number of diseases, including cancer, hypertension and liver disease due to the presence of anthocyanins and strychnine [26].

Solanum melongena cap has high antioxidant content and capacity, and by collecting free radicals prevent the destruction of various cells, and it shows cellular protective effects against damages caused by toxins and free radicals [27]. On the other hand, *S. melongena* reduced NO and increased total antioxidant capacity. Serum levels of albumin and total protein are correlated with liver cell function, and the increased levels indicate improved liver activity and are one of the main indicators of liver treatment [2]. In the present study, the extract improved the total proteins in diabetic rats and by enhancing its total antioxidant capacity prevented the deleterious effect on liver tissue thereby reduced serum levels of ALT, AST and ALP enzymes.

Diabetes causes destructive changes in liver tissue, including necrosis and inflammation [28], which are consistent with our results. Examination of liver tissue in extract-treated groups showed that diabetes-induced hepatic impairments such as venous system dilation, compacted cells with the dark nucleus, and focal lymphocyte infiltration were reduced. The concordance of biochemical results with the histological findings of liver confirmed the effect of *S. melongena* extracts on decreasing the adverse effects of diabetes which resulted in reduced enzyme leakage from hepatocytes. These effects are probably due to the presence of antioxidant compounds such as flavonoids, anthocyanins, saponins and glucosides.

Solanum melongena demonstrated a hypoglycemic effect and improved serum changes of hepatic enzymes, nitric oxide, and total antioxidant capacity in diabetic animals. It may be considered as a beneficial supplement in diabetic patients.

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Author contributions

Mozafar Khazaei contributed to the design of the study and prepared and edited the manuscript; Fatemeh Khazaei, Elham Ghanbari and Ali Rezvani performed the plan and the biochemical analysis; Somayeh Ghanbari and Mozafar Khazaei prepared the plant extract and performed the statistical analysis; Elham Ghanbari and Somayeh Ghanbari contributed to the data collection and histological testing. Elham Ghanbari and Fatemeh Khazaei were involved in animal handling and treatments. All authors approved and read the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

FBG: fasting blood glucose; TP: total protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; NO: nitric oxide; FRAP: ferric reducing antioxidant power; AGEs: advanced glycation end products; OS: oxidative stress; ROS: reactive oxygen species; IP: intraperitoneal; STZ: streptozotocin; TPTZ: tri-pyridyl triazine; TAC: total antioxidant capacity