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Original article

Investigating the effects of *Capparis Spinosa* on hepatic gluconeogenesis and lipid content in streptozotocin-induced diabetic rats

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ABSTRACT

The present study aimed to investigate the effects of administration of *Capparis spinosa* (CS) fruit aqueous extract on liver metabolism in streptozotocin (STZ)-induced diabetic rats. The aqueous extract of CS was orally administered at a dose of 20 mg/kg for 28 consecutive days and then its effects on blood glucose, lipid and insulin levels in normal and STZ diabetic rats were comparatively investigated. Furthermore, the effects of CS on the activity and expression of the key enzymes of gluconeogenesis and hepatic lipid content were investigated. The results showed that administration of CS extract in the STZ diabetic rats significantly decreased blood glucose level, while no significant influence on the insulin level. In addition, CS significantly decreased blood and liver triglyceride and cholesterol content in STZ diabetic rats. Furthermore, CS administration significantly reduced the mRNA expression and enzyme activities of glucose-6- phosphatase and phosphoenolpyruvate carboxykinase in liver tissues. Our findings demonstrated the beneficial effects of CS on blood glucose and lipid levels in an insulin- independent manner. This study also showed that CS improved the circulating levels of triglyceride and cholesterol. In addition, direct inhibition of gluconeogenesis in liver may be a probable mechanism of action of this plant. Since CS also decreased liver lipid content, we suggest that CS administration might be a beneficial therapeutic approach for metabolic syndrome and fatty liver.

1. Introduction

Diabetes mellitus (DM) is one of the common metabolic disorders with significant morbidity and mortality [1]. It is a major worldwide problem, characterized by the presence of chronic hyperglycemia due to defective insulin secretion and/or insulin action. Type 2 diabetes or non-insulin-dependent DM is the predominant type of the disease, accounting for 90%–95% of cases in which the body does not produce enough insulin or properly use it [2]. According to the World Health Organization, the diabetic population is likely to increase up to 300 million or more by the year 2025 [3]. Chronic hyperglycemia can lead to long term micro and macro vascular complications [4]. Glycemic control is necessary to prevent diabetes-related complications [5,6]. Many of currently available therapies for diabetes have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation [7]. In different regions of the world, several traditional medicinal plants have been used to treat DM patients [8,9]. Caper (*Capparis Spinosa* (CS)) belongs to family Capparidaceae and widely found in southern regions of Iran and some other countries [10,11]. The Caper fruits and flower buds pickles are traditionally used as food by diabetic patients owing to the belief that they have hypoglycemic and hypolipidemic actions. Hypoglycemic and hypolipidemic effects of aqueous CS extract have been demonstrated in experimental type 1 DM [12–14]. However, the underlying mechanisms of these effects have not been demonstrated.

The liver is the main organ responsible for glucose production. Hepatic glucose production mainly comes from gluconeogenesis [15]. In type 2 DM patients, the liver overproduces glucose because it becomes resistance to the suppressive effects of insulin [16]. The rate of gluconeogenesis is largely determined by 2 rate-limiting enzymes: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [17].Therefore, the present study was aimed to comparatively investigate the effects of CS on blood

Abbreviations: CS, *Capparis spinosa*; STZ, streptozotocin; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase.

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glucose, lipid and insulin levels in normal and STZ diabetic rats. In addition, the effects of CS on the activities and expression of PEPCK and G6Pase were analyzed to determine a probable mechanism of action of this plant. Finally, the effects of CS on hepatic lipid content were analyzed.

2. Materials and methods

2.1. Preparation of the caper aqueous extract

Specimens of *Capparis spinosa* were collected from Shoosh (Khuzestan, Iran) in July 2014.Taxonomic identification was performed and the samples were deposited at the herbarium, faculty of the Paramedical sciences, Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). The aqueous extract was prepared according to the methods described by Eddouks et al. [13]: CS fruits were washed with distilled water, dried at 40 °C and powdered. 100 ml distilled water was added to 10 g powdered fruits and the compound was stirred for 3 h. This mixture was boiled for 10 min and then cooled for 15 min. The aqueous extract was then filtered using a 0.2 μm Millipore filter (Millipore 0.2 mm, St Quentin en Yvelines, France). The resulting filtrate was then freeze-dried and stored at −20 °C for the subsequent use. The aqueous extracts were prepared daily, immediately prior to the administration. The administration was as follows; the freeze-dried extracts were reconstituted in 1.5 ml of distilled water and given orally to different groups at a dose of 20 mg/kg according to the Eddouks et al. [13].

2.2. Experimental animals

Male Wistar rats, weighing 250 to 300 g, were housed under the standard environmental conditions (temperature 25 ± 2 °C, with 55% ± 5% humidity and a 12 light/12 dark cycle). The rats were maintained with free access to standard commercial chow and drinking tap water ad libitum and were handled with humane care according to the guidelines of ethics committee of Ahvaz Jundishapur University of Medical Sciences, which approved the study.

2.3. Diabetes induction

Streptozotocin (sigma, St.Louis, Mo, USA) was dissolved into 0.1 M fresh cold citrate buffer at pH 4.5 before the use. The rats were received a single intra-peritoneal (IP) injection of streptozotocin at a dose of 65 mg/kg. After 3 days, the rats with stable fasting blood glucose levels greater than 200 mg/dl were deemed to be diabetic and included in the further experimental procedures of the study.

2.4. Experimental design

The animal of group 1 (control group) were orally treated daily with distilled water for 28 days, while the group 2 (control + CS group) was received the aqueous extract of CS fruits at a dose of 20 mg/kg body weight. Group 3 and 4 were given single 60.0 mg/kg body weight intra-peritoneal injections of streptozotocin (STZ) dissolved in 0.2 ml of 0.1 M citrate buffer, pH 4.5, after an overnight starvation. Group 3 (diabetic group) was treated with distilled water while group 4 (diabetic + CS group) treated with the aqueous extract of CS fruits at a dose of 20 mg/kg body weight. All the animals were sacrificed at the end of the study and blood samples were collected by cardiac puncture for serum glucose, insulin, triglyceride, and cholesterol analysis. The rat livers were excised, rinsed in normal saline and snap frozen in liquid nitrogen and stored at −180 °C for further analysis of gene expression and hepatic enzymes.

2.5. Serum biochemical parameters

Fasting blood glucose (FBG) tests were done on treatment days 0 and 28. Blood glucose concentrations were analyzed by a portable glucometer (Accu-Chek Aviva, Roche Diagnostics, Mannheim, Germany) after tail pitching. The animals in all treatment groups were starved overnight prior to the FBG tests.

Fasting serum insulin concentrations were determined by ultrasensitive rat insulin enzyme-linked immunoassay kit (DRG Diagnostics, Marburg, Germany) according to the manufacturer's manual.

Triglycerides and cholesterol levels were determined enzymatically by colorimetric specific kits (Randox, UK), respectively. The kits used in this study for substrates analysis were specified for both human and rat blood samples at the same percentage.

2.6. Hepatic enzyme assays

Frozen liver samples were homogenized in buffer containing 50 mM HEPES, 100 mM KCl, 2.5 mM dithiothreitol, 1 mM EDTA, and 5 mM MgCl2. Homogenates were centrifuged at 100,000*g* for 1.0 h at 4 °C to sediment the microsomal fraction. The activity in the microsomal fraction was determined using the procedure described by Lange et al. [18] where glucose-6 phosphate hydrolyze to glucose by tissue microsomal fraction containing G6Pase. The protein content determined by Bradford method [19]. The microsomal fractions were incubated with different concentrations of glucose-6 phosphate (0, 0.5, and 1.0, 2.5, 5 and 10 mM). The reaction was performed at 37 °C and stopped after 30 min with a solution containing acid molybdate, with 2/9 volumes of 10% SDS and 1/9 volume of 10% ascorbic acid. The reaction mixture was then incubated at 45 °C for 20 min, and the absorbance read at 820 nm. A standard curve was generated using different concentrations of free phosphate.

The PEPCK activity was performed using modification to the methods described by Bentle and Lardy [20]. The activity of enzyme was assayed in a final 1 ml volume containing 7 mmol/L sodium HEPES, 1 mmol/L inosine 5′-diphosphate (IDP), 1 mmol/L $MnCl_2$, 1 mmol/L di-thiothreitol, 0.25 mmol/L NADH, 2 mmol/L phosphoenolpyruvate, 50 mmol/L $NaHCO_3$, and 7.2 U of malic dehydrogenase. Total protein in the enzyme sources was determined using the Bradford technique [19]. The enzyme's activity was measured at 25 °C and 340 nm. The enzyme activity was expressed as mmol of oxaloacetate (OAA) formed/min/g of the liver protein.

2.7. Hepatic lipid content

Hepatic concentrations of triglycerides, cholesterol, and glycogen were measured using commercial kits (Bio Vision Inc., CA) according to the manufacturer's instructions.

2.8. Gluconeogenic enzyme expression

Total RNA was isolated from frozen liver tissue with TRIzol reagent (Invitrogen, Grand Island, NY) according to the manufacturer's instructions. The cDNA was produced using the reverse transcriptase system (promega, Madison.WI). Quantitative real-time PCR (RT-PCR) was performed with a SYBR Green PCR kit (Takara Bio, Otsu, Japan) and a prism 700 real-time PCR system (Applied Biosystem, Foster City, CA) according to the manufacturer's instructions. The G6Pase and PEPCK gene expressions were

assessed using the comparative threshold cycle method and then normalized with β-actin.

The primer sequences were as follows: 5′-CCCTGAACCC-TAAGGCCAACCGTGAA AA-3′ and 5′-TCTCCGGAGTCCATCA-CAATGCCTGTG-3′ for β-actin, 5′- CGACTCGCTACCTCCAAGTG -3′ and 5′- TCCCTGGTCCAGTCTCACAG -3′ for G6Pase, and 5′-TGGGTGATGACATTGCCTGG -3′ and 5′- TGGGTGATGACATTGCCTGG -3′ for PEPCK.

2.9. Statistical analysis

Data were expressed as mean± SEM. The statistical significance was evaluated by one-way ANOVA. For all of the statistical analyses the significant level was considered as $p \leq 0.05$.

3. Results

3.1. Effects of caper fruit extract on fasting serum glucose and insulin levels (Fig. 1A, B)

Values of Fasting blood glucose concentration were similar between non-diabetic control and CS-treated non-diabetic control group, but were significantly different between the diabetic group compared to control ($P < 0.001$) (Fig. 1). The treatment with CS significantly ($P < 0.001$) reduced FBG levels in diabetic rats, compared with the diabetic group. Fasting plasma insulin concentrations were similar between CS-treated non-diabetic and control group but were significantly ($P < 0.001$) decreased in diabetic groups compared to the control group. The CS treatment did not significantly increase the fasting serum insulin concentrations in diabetic rats, compared with the on-treated ones.

3.2. Effect of caper fruit extract on fasting serum triglyceride and cholesterol (Fig. 2A, B)

The triglyceride concentrations were similar between CS-treated non-diabetic and control group, whereas were significantly different between the non-treated diabetic group and the control group ($P < 0.001$).The treatment with CS significantly ($P < 0.001$) reduced triglyceride levels in CS-treated diabetic rats, compared with the non-treated diabetic group. The cholesterol concentrations were similar between the CS-treated non-diabetic and control group, whereas were significantly different between the non-treated diabetic group and the control group ($P < 0.001$). The treatment with CS significantly ($P < 0.001$) reduced cholesterol levels in the CS-treated diabetic rats, compared with the non-treated diabetic group.

3.3. Effect of caper fruit extract on liver G6Pase and PEPCK mRNA (Fig. 3A, B)

The PEPCK and G6Pase expressions were similar between the CS-treated non-diabetic and control group, whereas were significantly different between the non-treated diabetic group and the control group ($P < 0.01$). The treatment with CS showed a significant reduction in expression of gluconeogenesis regulatory genes, G6Pase, and PEPCK in the treated diabetic group .

3.4. Effect of fruit extract on liver triglyceride and cholesterol content (Fig. 4A, B)

The liver triglyceride and cholesterol contents were similar between CS-treated

non-diabetic and control group, whereas were significantly different between the

non-treated diabetic group and the control group ($P < 0.01$). Liver triglyceride and cholesterol contents in the diabetic control rats were significantly increased ($P < 0.01$), compared with the non-diabetic controls. The CS treatment for 28 days significantly reduced the liver triglyceride and cholesterol content, compared with the diabetic control rats.

3.5. Effect of fruit extract on liver G6Pase and PEPCK enzymes activities (Fig. 5A, B)

The liver G6Pase and PEPCK enzymes' activities in the diabetic control rats were significantly increased ($P < 0.01$), compared to the non-diabetic controls. However, the CS treatment for 28 days significantly reduced the liver triglyceride and cholesterol contents, compared with the diabetic control rats.

4. Discussion

Type 2 DM is a complex metabolic disorder and accounts for about 90% of all diabetes deaths worldwide which highlighting the urgent need for novel and effective treatment strategies [21,22]. Traditional herbal medicine has recently received a plenty research interests for the treatment human diseases including diabetes [23–25]. The hypoglycemic activity of CS was demonstrated in STZ-induced diabetic rats after a single and repeated oral administration [13]. However, the exact mechanism of actions involved in this effect has not been clearly determined. It is widely known that the liver is a key organ in glucose and lipid metabolism. It has been demonstrated CS root and fruit extracts could improve elevated liver markers [12,26], indicating that CS may have therapeutic

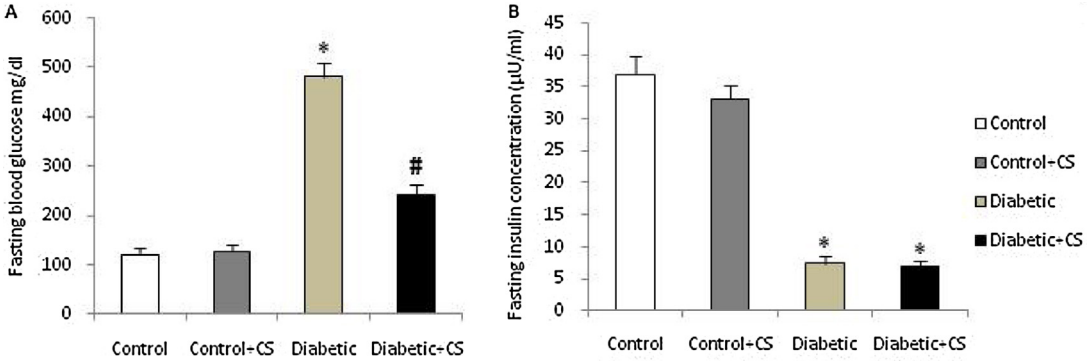

Fig. 1. (A) Fasting serum glucose (mg/dl) and (B) Serum insulin concentrations (μU/ml) after once daily repeated oral administration of *Capparis spinosa* (CS) extract for 28 days in normal (control) and diabetic rats. Data (n = 7) expressed as mean ± SEM. * $P < 0.001$ vs. Control and Control + CS, # $P < 0.001$ vs. Diabetic.

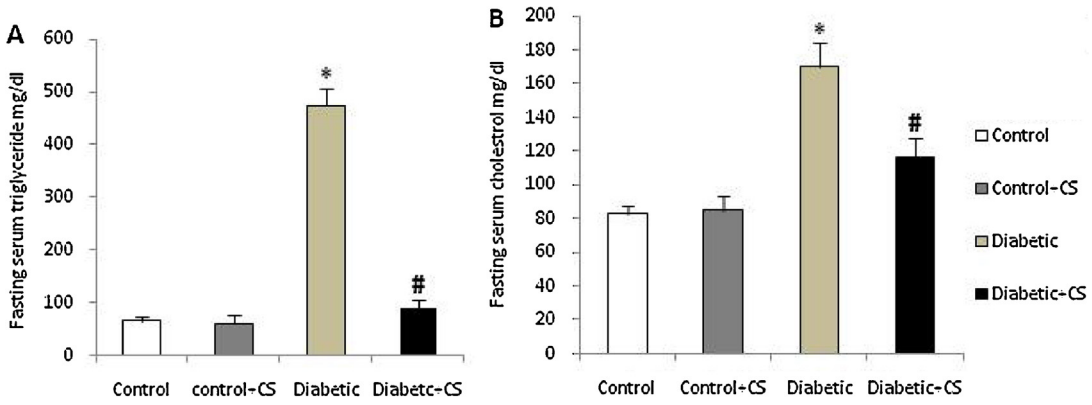

Fig. 2. (A) Fasting serum triglyceride (mg/dl) and (B) fasting serum cholesterol (mg/dl) after once daily repeated oral administration of *Capparis spinosa* extract (CS) for 28 days in normal and diabetic rats. Data (n = 7) expressed as mean ± SEM. * $P < 0.001$ vs. Control and Control + CS, # $P < 0.001$ vs. Diabetic.

effects on acute and chronic liver injury. To elucidate the mechanism of actions of CS on liver, this study has investigated the effects of CS on serum glucose and lipid levels, hepatic gluconeogenesis, and lipid accumulation in the STZ-induced diabetic rats. The result showed that aqueous extract of CS induced significant hypoglycemic effect in STZ-induced diabetic rats and blood glucose levels were approximately normalized in the STZ- induced diabetic rats (Fig. 1A). In addition, we found no significant change in the serum insulin concentration in both normal and diabetic rats (Fig. 2B), indicating that CS extract reduced blood glucose levels without affecting insulin secretion.

Hepatic glucose production (HGP) is abnormally elevated in both type 1 and type 2 diabetes and is a major factor contributing to fasting hyperglycemia. Excessive HGP in type 2 DM primarily results from sustained gluconeogenesis [27–29]. The rate of gluconeogenesis is largely determined by 2 rate-limiting enzymes: PEPCK and G6Pase.PEPCK catalyzes one of the earliest rate-limiting steps in gluconeogenesis through concurrent decarboxlateing and phosphorylating of oxaloacetate into phosphoenolpyruvate. G6Pase catalyzes the last step of gluconeogenesis and works as the final gatekeeper for glucose efflux from the cell. The transcription of these genes is heavily regulated with the involvement of many transcriptional factors [15,30,31]. In the present study, STZ administration significantly increased the mRNA expression in the PEPCK and G6Pase as well as enzyme activity in the STZ induced diabetic rats, compared with the normal rats. These effects were consistent with previous studies that found that insulinopenia in STZ rats was associated with glucose overproduction mainly via hepatic gluconeogenesis. Expression PEPCK and G6Pase are important factors responsible for hepatic gluconeogenesis and have been shown to increase in diabetic rats. Our findings showed that CS significantly reduced PEPCK and G6Pase mRNA expression and enzyme activities in the STZ induced diabetic rats. These findings indicate that CS may exert its hypoglycemic effect, at least partially through inhibiting the expression and activities of these key enzymes.

There are other possibilities in liver gluconeogenesis pathway that may be regulated. Pyruvate carboxylase and fructose 1, 6 bisphosphatase are also the key enzymes in the pathway that in contrast to PEPCK and G6Pase are subject to allosteric regulation. CS may also affect the activity of these enzymes. More controlled studies are needed to clarify further effects of CS on liver gluconeogenesis.

Furthermore, our study showed that triglyceride and cholesterol levels were increased in STZ induced diabetic rats, whereas significantly decreased by CS administration. Researchers have suggested that CS administration decreases plasma triglyceride and cholesterol in STZ diabetic rats [14]. In addition, we showed

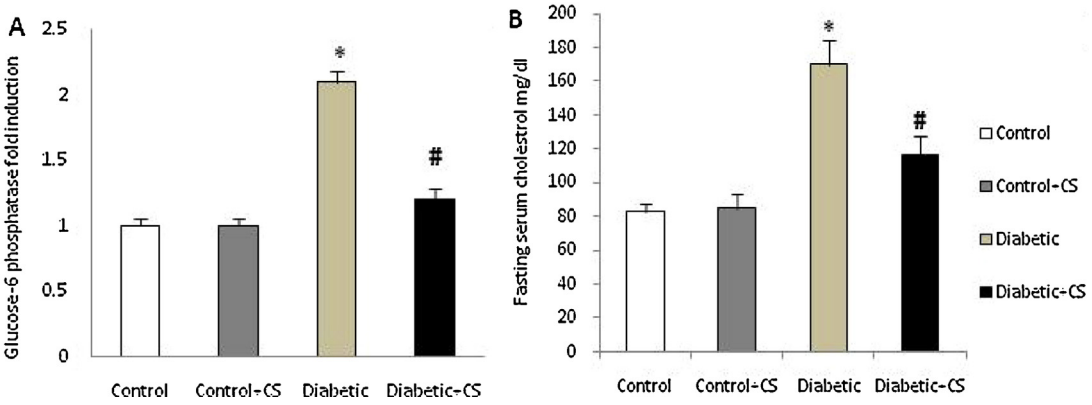

Fig. 3. (A) Effect of *Capparis spinosa* fruit extract on G6Pase and (B) PEPCK mRNA expression after a daily repeated oral administration of CS extract for 28 days in normal and diabetic rats. The mRNA levels were quantified by real time PCR and were normalized to endogenous β actin. The G6Pase and PEPCK gene expressions in the Control + CS, Diabetic, and Diabetic + CS groups against the Control group are shown as fold induction. The G6Pase and PEPCK gene expressions were respectively increased by 2.1 and 3.3 fold in the Diabetic group. CS treatment in Diabetic + CS group reduced the G6Pase and PEPCK expression to 1.2 fold and 1.5 fold respectively. Statistical analysis was performed by the one-way ANOVA. Data (n = 7) expressed as mean ± SEM. * $P < 0.01$ vs. Control and Control+ CS, # $P < 0.01$ vs. Diabetic.

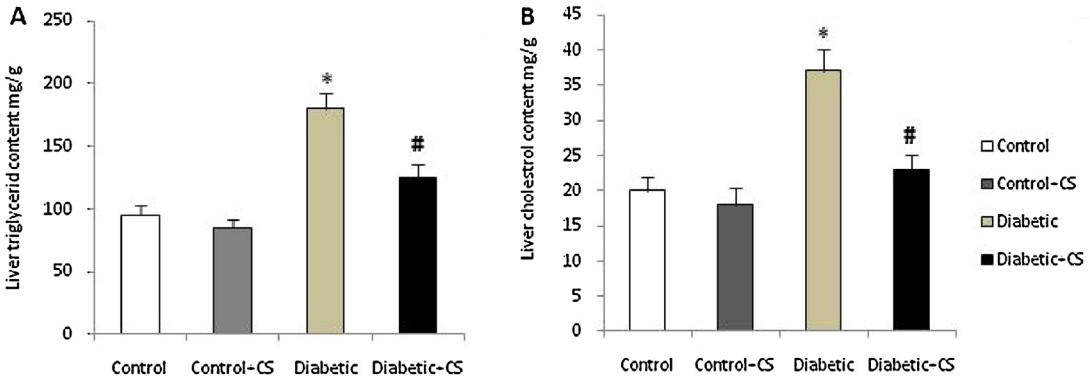

Fig. 4. Liver triglyceride (A) and cholesterol (B) contents (mg/g) after once daily repeated oral administration of *Capparis spinosa* (CS) extract for 28 days in normal (control) and diabetic rats. Data (n = 7) expressed as mean ± SEM. * $P < 0.001$ vs. Control and Control + CS, # $P < 0.001$ vs. Diabetic.

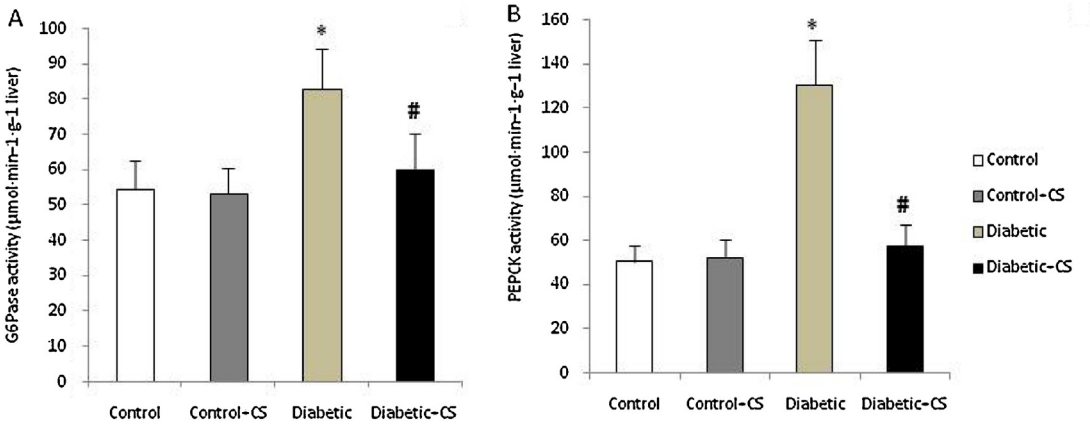

Fig. 5. Liver G6Pase (A) and PEPCK (B) activities (µmol min−1 g−1) after once daily repeated oral administration of *Capparis spinosa* (CS) extract for 28 days in normal (control) and diabetic rats. Data (n = 7) expressed as mean ± SEM. * $P < 0.01$ vs. Control and Control+ CS, # $P < 0.01$ vs. Diabetic.

that CS significantly decreased liver lipid content. In the present study, the liver lipid content was measured directly. Although fatty liver infiltration can be determined by many different methods, direct measurement of hepatic fat is considered the gold standard method. These findings showed that intrahepatic lipid accumulation worsens hepatic glucose metabolism, suggesting that fatty liver in patients with type 2 DM is a therapeutic target [32]. Our findings suggest that CS administration decreases hyperglycemia and liver lipid contents; therefore, it might be a beneficial therapeutic approach for metabolic syndrome and fatty liver.

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