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Research Article

Effect of *Ziziphus vulgaris* L. (Jujube) Powder and Extract on Liver Damage in STZ-Induced Diabetic Rats

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Received: February 16, 2021; **Published:** February 20, 2021

Abstract

Purpose: Diabetes mellitus (DM) is a metabolic disorder arises as a result of deficient in production and secretion of insulin. *Ziziphus vulgaris* L. (jujube) is a medicinal plant used for the treatment of DM and liver diseases traditionally. The aim of the present study was to investigate the protective effects of jujube fruit on the liver changes due to DM.

Methods: Intra-peritoneal injection of 60 mg/kg bw streptozotocin (STZ) was using to DM induction in rats. Jujube fruit powder (1 g/ kg bw) and extract (1 g/kg bw) were administered for 2 weeks prior and 3 weeks after STZ injection. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined.

Results: Serum AST levels were found to remain unaltered compared to pre-diabetic state following treatment with jujube powder but not jujube extract and powder showed a preventing effect on AST elevation. Whilst treatment with jujube powder or extract had no significant reduction on ALT and ALP levels. Also, histopathological examinations revealed a significant reduction in hepatic injury with both jujube preparations.

Conclusion: The present findings suggest a hepatoprotective role of jujube supplementation in particular in the powdered form against DM-induced liver damage.

***Keywords**: Ziziphus vulgaris; Diabetes Mellitus; Streptozotocin; Liver Enzymes*

Introduction

Diabetes mellitus (DM) is an endocrine disease associated with tremendous morbidity and mortality worldwide [1]. Chronic hyperglycemia is associated with dysfunction of several body organs [2]. Involvement of liver is one of the notable causes of death in DM [3]. The liver is an insulin-dependent tissue that plays a vital role in glucose and lipid homeostasis [4]. The efficacy of currently available anti-diabetic medications is maintaining blood glucose within the normal range and controlling the metabolic and hepatic complications is limited. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as important biomarkers of hepatic function are often elevated in diabetic patients [5]. Medicinal plants represent a viable therapeutic option for DM owing to their multifunctional activities, rich content of bioactive phytochemicals and potential safety. It has been estimated that over 1200 species of plants with anti-diabetic properties are used in the traditional medicine [6]. *Ziziphus vulgaris L.* (jujube) is a medicinal plant belonging to the Rhamnaceae family and has been used for the treatment of several diseases including DM [7]. This plant grows in

tropical and subtropical regions of Asia and America [8]. The genus of *Ziziphus* is known for its medicinal properties such as hypoglycemic, anti-inflammatory, antioxidant and hepatoprotective effects [9]. They are commonly used in folklore medicine for the treatment of various diseases such as liver dysfunction, obesity, fever and bronchitis [10]. Phytochemical studies on the *Ziziphus* species led to the isolation of several compounds such as cyclopeptide alkaloids, flavonoids, sterols, tannins and triterpenoid saponins [11]. Jujube fruit also contains a significant level of antioxidants [12].

Aim of the Study

The present study aimed to investigate the hepatoprotective effects of jujube in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Plant preparation

Fruits of *Ziziphus vulgaris* were purchased from a local herbal grocery from the Khorasan-Razavi province. The plants were identified and authenticated at the Department of Isfahan University (voucher no.5352). Dried fruits were powdered and extracted with ethanol (70%) and water with a 1:1 ratio. Following solvent removal in a vacuum distillation set (Heidolph/Germany), the extract was dried in shadow at room temperature (20 - 22˚C).

Phytochemical analyses

The content of kaempferol, as a major flavonoid of jujube, was determined in the plant extract using a validated high-performance thin-layer chromatography (HPTLC) method. Kaempferol content of samples was quantified from a standard calibration curve of standard kaempferol (Sigma Aldrich, USA) in the concentration range of 10 to 200 µg/mL. The relationship between the concentration and peak-height was measured using the least square method [13].

Experimental design

The study was performed on forty adult male Wistar rats weighing 200 - 250g. Animals were housed in cages and maintained in normal temperature (24˚C) with a 12-h light-dark cycle. The experimental protocols were approved by the Ethics Committee of the Isfahan University of Medical Sciences (Isfahan, Iran) and the animal care was according to the approved standards for laboratory animal care.

They were fed a standard pelleted chow diet (Pars Dam Co, Tehran, Iran) and water ad libitum. The rats were randomly divided into four equal groups (n = 10) as follows: non-diabetic control rats treated with 0.5 mL distilled water; STZ-induced diabetic control rats treated with 0.5 mL distilled water; STZ-induced diabetic rats receiving jujube powder at a dose of 1 g/kg body weight (bw); and STZ-induced diabetic rats receiving jujube hydroalcoholic extract at a dose of 1 g/kg bw [14]. Each dose of powder and extract was dissolved in 0.5 mL distilled water and animals in all groups were treated for 14 days. Experimental diabetes was induced following a 16-h fasting by an intra-peritoneal injection of STZ (sigma Aldrich, USA) at a dose of 60 mg/kg bw [15]. Then, the animals were treated for another three weeks with the same diet as that received prior to STZ injection (Figure 1).

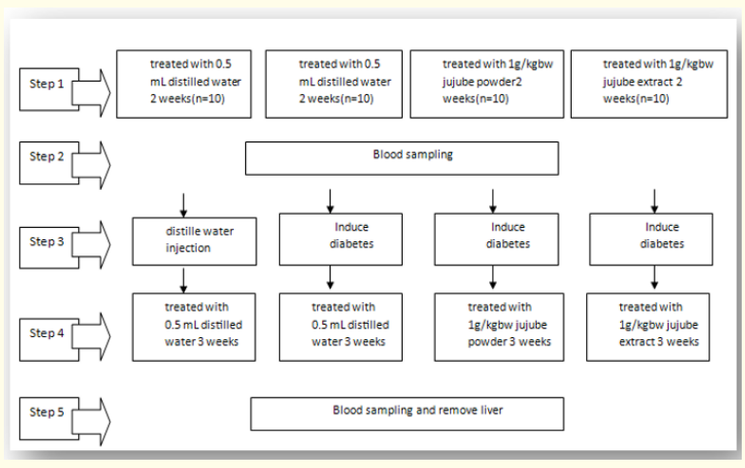

Figure 1: *The chart of experimental design.*

Biochemical analyses

Blood sampling was done prior to diabetes induction and at the end of study, both after a 16-h fast. Serum concentrations of liver enzymes including AST, ALT and ALP were measured colorimetrically on an autoanalyzer (Hitachi Co., Tokyo), using commercial kits (Pars Azmoon, Iran). For histopathological examinations, the whole liver was removed after sacrificing the animals and was fixed in 10% formalin. Microtomic sections were cut and stained by hematoxylin and eosin (H&E). Histological assessments were performed according to the Ishak scoring system [16].

Statistical analysis

All values were expressed as mean ± standard deviation (SD). Significant differences among the groups were determined by Repeated-Measures Analysis of Variance (RM-ANOVA) using the SPSS software version 13.0. A two-tailed *p*-value of < 0.05 was considered to be statistically significant.

Result

The kaempferol content of *Ziziphus vulgaris* extract was found to be 13 ± 0.5% mg/g. STZ treatment caused a significant elevation on liver enzymes (ALP, AST and ALT) in diabetic control rats compared with non-diabetic group. Jujube supplementation in both forms, powdered and extracted, had no preventing effect on liver enzymes elevation include ALT and ALP. Serum levels of AST remained statistically unchanged compared with baseline values in the diabetic rats that receiving jujube powder, whilst there were significant increases in diabetic rats receiving jujube in extract form (Table 1).

Biochemical factors	Group	Time 1	Time 2	p-value
AST (u/l)	Diabetic	214.1 ± 17.8	627.6 ± 200 [a €]	0.018
	Non diabetic	221.6 ± 30.3	207.9 ± 42.5 [b]	0.333
	Treated with powder	214.4 ± 33.7	225.6 ± 264.1 [a]	0.093
	Treated with extract	213.7 ± 39.3	327.9 ± 146.2 [a €]	0.017
	p-value	0.74	0.064	
ALT (u/l)	Diabetic	70.8 ± 9.1	187.2 ± 125.7 [a €]	0.028
	Non diabetic	55.3 ± 9.7	69.5 ± 13.2 [b]	0.057
	Treated with powder	78 ± 11.9	248.6 ± 105 [a €]	0.012
	Treated with extract	61.7 ± 14.6	179.8 ± 111.9 [a €]	0.036
	p-value	0.051	0.001	
ALP (u/l)	Diabetic	438.6 ± 101.6	2195.7 ± 949.7 [a]	0.009
	Non diabetic	648 ± 144	562.7 ± 85.4 [b]	0.593
	Treated with powder	450 ± 97.6	1574.5 ± 825.3 [a €]	0.028
	Treated with extract	445.1 ± 174.9	1738.5 ± 723.9 [a €]	0.028
	p-value	0.189	0.004	

Table 1: *Levels of liver enzymes in non-diabetic control, diabetic control, diabetic treated rats with jujube powder and diabetic treated rats with extract of jujube. (Means ± SE) (p < 0.05).*
Time 1, two weeks after the start of the experiment and before induce diabetes, time 2, at the end of the study.
(€): Means differ significantly between time 2 and time 1 (p < 0.05), similar letter that are front of means show no differ significantly between group.

Histopathological examination of the liver tissue from diabetic control group revealed congestion of portal vessels and sinusoids with mild centrilobular hepatocyte degeneration, sinusoidal clutter, hepatic cord disorganization and binuclear hepatocytes. There was not any sign of fat deposition or lipid droplets in the diabetic rat liver, nor was any degeneration of glycogen or necrosis. There was also no lymphoplasmacytic infiltration and tissue inflammation in the portal tracts (Figure 2A).

Hepatocytes of the non-diabetic control group showed a normal lobular shape. Its sinusoids were quite clear and no congestion was seen in this group. There were more concentric arrangement of hepatocytes and portal vessels and the sinusoids were congested (Figure 2B).

Reduced disorganization of hepatic cords and sinusoids was observed in the liver of rats treated with both forms of jujube. In addition, hyperemia and congestion were clearly reduced in jujube-fed groups, with a greater effect observed with jujube powder (Figure 2C and 2D).

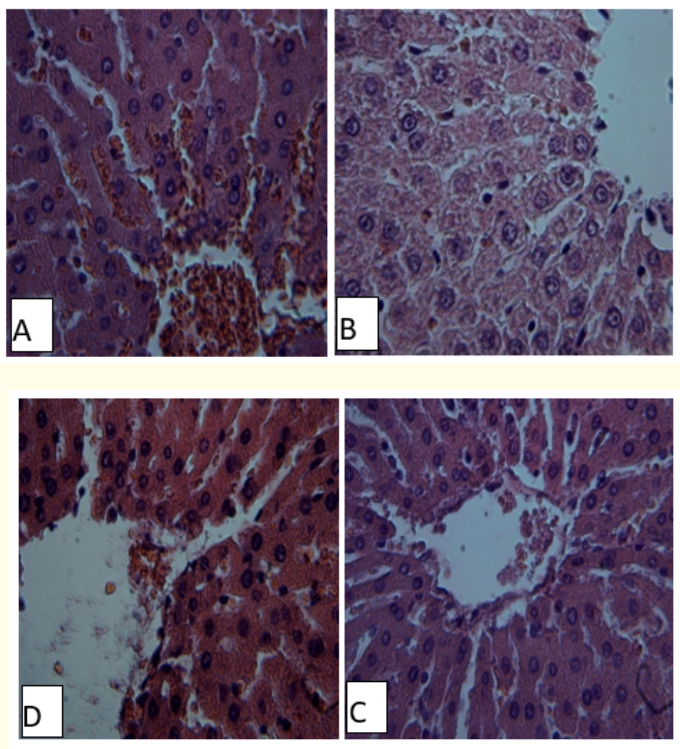

Figure 2: *A: Section of liver in diabetic control group, B: Non-diabetic control group, C: Treated group with powder of jujube, D: Treated group with extract of jujube. H & E Staining (40X).*

Discussion

Scientists use the extract or powder of plants in medicinal herbal studies usually. In the present study, hepatoprotective effects of both forms of jujube fruit, powdered and extracted forms, were investigated in STZ-diabetic rats. Previous studies not determined the jujube powder effects on liver injury while it was more effective than its extract form in this study.

In the present study, although none of the jujube preparations had protective effect on ALT and ALP elevation. Whilst, the serum AST was significantly reduced in the group receiving powdered form of jujube fruit. Unlike the biochemical findings, some of positive effect was observed in histological examinations of liver in both jujube-treated groups.

STZ is a standard model agent for experimental diabetes induction and it is causes multiorgan damage owing to its diabetogenic, hepatotoxic, nephrotoxic and gastrotoxic properties [17]. Diabetes causes liver malfunction and alterations in the circulating concentrations of liver enzymes. The liver enzymes play a critical role in the maintenance of carbohydrate homeostasis. Abnormal levels of serum AST, ALT and ALP are of toxicological importance, being indicative of tissue damage by toxicants or disease conditions [18]. Among them, the ALP determine is more important in bone disease diagnosis and has a lesser diagnostic value versus aminotransferases enzymes in liver disease. ALT and AST assay is more sensitive test in acute liver diseases and chronic liver disorder respectively and the AST activity is three times that of ALT [19]. According to this information, the preventing effect of powdered form of jujube on AST is a notable result in the present study.

In view of the Indian and Chinese systems of medicine, jujube has hepatoprotective role and is used for the treatment of hepatic diseases [20]. Dietary supplementation with *Ziziphus* species has been shown to limit liver injury in previous studies [20-23]. Other studies indicated that jujube extract administration decreases ALT, ALP and AST, attenuates histopathology of hepatic injury, reduced hepatic necrosis and portal inflammation, and improves the oxidative stress in rats [24-26].

Most of the protective effects of jujube could be attributed to the anti-inflammatory and antioxidant properties of this plant. The antioxidant capacity of jujube has proven in previous studies [12,22,27]. Antioxidant component such as flavonoids, phenols, carotenes, fatty acids, alpha tocopherol and other vitamins that are present in jujube have both anti-inflammatory and antioxidant properties and thus can account for the observed properties [23,28]. Studies suggested that the jujube fruits play a protective role against experimental acute and chronic inflammatory reactions in rat [29]. Saponins and tannins are from the strongest antioxidants with proven hepatoprotective and anti-inflammatory and hepatoprotective effects reported by several previous studies [30-32].

Lack of any remarkable effect of jujube on ALT and ALP in the present study could be due to the short period of supplementation. Another short-term (14 days) study by Solati and Soleimani indicated that the use of jujube extract does not decrease the liver enzymes in these times [15]. hepatoprotective effects in other studies that *Ziziphus* species supplementation was in longer periods are proven. For example, Dahiru., *et al*. reported that pretreatment of rats with *Ziziphus mauritiana* fruit extract for six weeks effectively protects against hepatotoxicity as evidenced by decreased serum ALT, AST and ALP levels [22]. This data is consistent with the results of another study showing that 8-week consumption of *Zizyphus spina-christi* significantly reduces the activities of serum ALT and AST [33].

Aside from duration of supplementation, another possibility is that higher doses of jujube may cause stronger hepatoproptective effects on liver enzymes. Hence, future studies are recommended to undertake multiple dose investigation of hepatoprotective effects of jujube in diabetic models and also explore the effect of jujube on indices of hepatic insulin resistance.

In this study, although the jujube supplementation didn't have the liver enzymes reduction effects, it could able to maintain serum AST Level unaltered in powder treated rat compared to pre-diabetic state. Hepatoprotective effects of jujube are better supported with histopathological part of this study. DM-induced liver damages were improved in jujube treated rats in compare with diabetic.

Conclusion

The present findings suggest a hepatoprotective role of jujube supplementation in particular in the powdered form against DM-induced liver damage.

Acknowledgement

This study was conducted with financial support that was provided by the Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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