Original Article

Effect of Ethyl Acetate and Aqueous Fractions of *Ziziphus jujuba* Extract on Biochemical and Hematological Parameters in Rat

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Abstract

Introduction: Despite the numerous therapeutic effects of the *Ziziphus jujuba* (ZJ), its effect on biochemical and hematological parameters are unknown. Therefore, in this study, the effect of ethyl acetate (EA) and aqueous (AQ) fractions of the ZJ fruit extract on biochemical and hematological parameters were explored. **Material and Methods:** Thirty-six male Wistar rats randomly were divided into six groups as follows: (1) Control, (2) ZJ extract (200 mg/kg), (3, 4) EA fraction of ZJ (EA150 and EA300 mg/kg), and (5, 6) AQ fraction of ZJ (AQ150 and AQ300 mg/kg). Saline, ZJ extract, and its fractions were gavage once a day for 4 weeks. At the day of the experiment (28th day), one blood sample was collected in citrated tube for the counting of the complete blood cell count calculation. The serum was used for biochemical parameters (cholesterol, triglyceride, and glucose concentrations) evaluation. **Results:** The results showed that the blood glucose, cholesterol, and triglyceride levels in all treated groups, especially in AQ groups, significantly decreased compared to the control ($P < 0.01$ to $P < 0.001$) group. Furthermore, the number of red blood cell and white blood cell in treating rats with ZJ extract and its AQ fraction significantly increased ($P < 0.05$), whereas the number of platelets significantly decreased ($P < 0.05$) compared to the control. **Conclusion:** In conclusion, both AQ and EA fractions of ZJ have beneficial effects on biochemical and hematological parameters. Since the effect of AQ fraction is higher than EA, it is suggested that these effects of ZJ mostly mediated by compounds of ZJ that soluble in the polar solvent.

Keywords: *Biochemistry, ethyl acetate, fraction, hematology, Ziziphus jujuba*

Introduction

Medicinal plants have a wide historical background and are still used in the treatment and improvement of various diseases in traditional and complementary medicine. The World Health Organization (WHO) has estimated that 90% of people in developing countries use medicinal plants for primary health-care needs. The WHO also announced 21,000 plants are used for medicinal propose in the world.[1] One of the well-known medicinal plants is *Ziziphus jujuba* (ZJ) that belongs to the *Rhamnaceae* family. This plant is native to Asia and Southern Europe. The local name of this plant varies from country to country. In Iran, it is commonly known as "Annab," and in China named a red date.[2] Fruit, seed, and leaves of this plant were used as a remedy, flavoring, and edible food.[3] ZJ fruit has several pharmacological properties, including antifungal,[4] antiulcer,[5] anti-inflammatory,[6] anti-oxidant,[7] and cardio tonic effects.[8] The important biological components of ZJ are polysaccharides, phenols, flavonoids, mucilage, iron, ascorbic acid, riboflavin, pectin, and different chemical components such as jujubosides A, B, and C, zizyberenalic acid, and betulinic acid.[8-11] In a previous study, the effect of ZJ seed on the biochemical parameters has been investigated,[12] but effects of the ZJ fruit and its fractions on biochemical and hematological parameters are not yet investigating. The aim of this study was to explore the effect of the ZJ fruit extract and its two fractions (aqueous [AQ] and ethyl acetate [EA]) on these parameters.

Materials and Methods

Extract and fraction preparation

After the collection of ZJ fruits (Birjand, South Khorasan, Iran), it is identified by a botanist in the herbarium of Ferdowsi University of Mashhad. Then, 100 g of the ZJ fruit was powdered and then

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soaked in 500 mL ethanol (70%) and shacked for 72 h in the standard temperature (37°C). Next, the mixture was passed through the filter papers with different filter sizes. The solvent (ethanol) was evaporated by an oven at 40°C.[9,13] At the stage of fraction preparation, 25 g of ZJ extract was dissolved in 100 mL of distilled water. After that, the solution was poured into the decanter; the soluble ingredients were isolated three times by adding 25 ml of EA. The remaining solution was AQ fraction. AQ and EA fractions were placed in the oven to remove the solvent (40°C).[14]

Animals

Thirty-six Wistar male rats were kept in standard laboratory condition in the animal room (12:12 light-dark cycle, temperature 20°C–22°C, and the humidity 25%–35%). Animals were randomly divided into six groups as follows ($n = 6$):

- 1. Control; received saline
- 2. ZJ extract; received extract (200 mg/kg; ip)
- 3, 4. Ethyl acetate (EA) fraction of ZJ (150 and 300 mg/kg)
- 5, 6. Aqueous (AQ) fraction of ZJ (150 and 300 mg/kg)

Saline, ZJ extract, and its fractions were gavage once a day for 4 weeks. All procedures were approved by the Animal Research Ethics Committee of Mashhad University of Medical Sciences (Code number: IR. MUMS.fm. REC.1396.589).

Experimental protocol

After treatment (4 weeks), the rats were deeply anesthetized with urethane (1.4 g/kg, IP).[15] Then the chest cavities of animals were opened, and the fresh blood samples were collected from the heart directly. One blood sample was collected in the citrated tube for the counting of the complete blood count calculation. Another blood sample was collected and its serums were separated by centrifuging at 3000 rpm in 10 min for analyzing the serum biochemical parameters (cholesterol, triglyceride, and glucose concentrations). These parameters were measured by related kits purchased from Pars Azmoon Co, Iran.

Data analysis

Data were explained as the mean ± standard error of the mean. After normalization survey, the data analysis was performed using one-way ANOVA followed by least significant difference *post hoc* comparison test. The statistical significance value was $P < 0.05$.

Results

The results showed that the glucose level same as the previous study was 183.3 mg/dL in normal rat and treated with total extract and both fractions significantly decreased compared to the control ($P < 0.01$ to $P < 0.001$). The glucose level in lower doses of both fractions was significant than the ZJ extract group [$P < 0.01$, Figure 1]. The cholesterol level in all treated rats except ZJ extract group significantly decreased in comparison with the control ($P < 0.05$ to $P < 0.01$). This parameter in rats treated with two doses of EA and a higher dose of AQ fractions significantly reduced compared to the ZJ extract group ($P < 0.05$) [Figure 2]. The level of triglyceride in rats treated with higher doses of both fractions (AQ300 and EA300 mg/kg) significantly decreased compared to the control, and two doses of both fractions are also significantly lower than the ZJ extract group ($P < 0.05$ to $P < 0.01$) [Figure 3].

As shown in Table 1, the levels of white blood cell (WBC) and red blood cell (RBC) in treating rats with ZJ extract and its AQ fraction significantly increased compared to the control ($P < 0.05$) while the platelet level significantly decreased ($P < 0.05$) and this effect in higher doses of AQ extract was statistically significant in comparison with the ZJ extract group ($P < 0.05$).

Table 1: The comparative effects of hematological parameters in all treated groups

CBC parameters	Groups					
	Control	ZJ extract	A150	A300	EA150	EA300
RBC (×1000/µl)	9.1±0.6	9.5±0.0*	9.6±0.1*	9.8±0.1*	9.2±0.1	8.9±0.5
WBC (×1000/µl)	16.6±0.5	17.7±0.5*	17.6±0.9*	17.5±2.1*	16.3±1.0	16.9±1.8
HB (g/dl)	15.1±0.3	15.4±0.1	15.2±0.1	15.1±0.2	15.6±0.1	15.6±0.2
HCT (%)	46.1±1.1	47.5±0.2	46.0±0.2	44.6±0.9	47.5±0.5	47.9±1.2
MCH (pg)	16.5±0.8	16.0±0.2	15.7±0.1	15.4±0.4	17.5±0.9	17.3±0.9
MCHC (g/dl)	32.6±0.6	32.3±0.2	32.0±0.2	33.9±0.3	32.4±0.3	32.7±0.6
MCV (fl)	49.2±1.3	49.6±1.0	47.2±0.1	48.4±0.5	50.2±1.0	53.7±1.0
PLT (×1000/µl)	1000±90.6	817±68.8*	801±53.9*	746.5±30.9*#	900.2±50.6	944.5±49.4

The data presented as mean±SEM by one-way ANOVA test with *post-hoc* LSD. *P<0.05 compared to the control, #P<0.05 compared to the ZJ extract group. A150/300: Aqueous fraction of the ZJ extract; EA150/300: Ethyl acetate fraction of the ZJ extract; CBC: Complete blood cell; RBC: Red blood cell; WBC: White blood cell; HB: Hemoglobin; HCT: Hematocrit; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; PLT: Platelet; ZJ: *Ziziphus jujuba*; SEM: Standard error of mean; LSD: Least significant difference

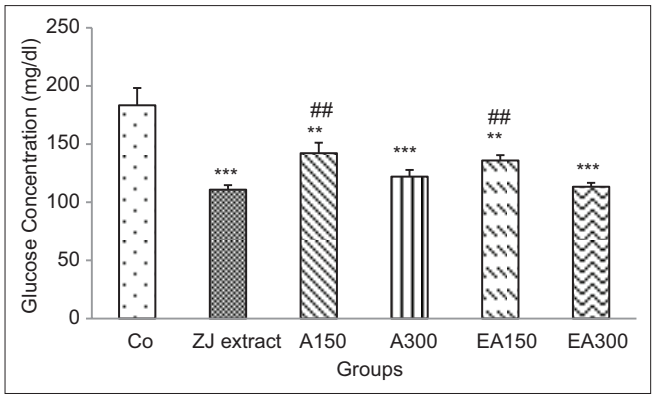

Figure 1: The comparative effects of glucose concentration in all treated groups. The data presented as mean ± standard error of the mean by one-way ANOVA test with *post hoc* least significant difference. ***P* < 0.01, ****P* < 0.001 compared to the control, ##*P* < 0.01 compared to the *Ziziphus jujuba* extract group. A150/300: Aqueous fractions of the *Ziziphus jujuba* extract, EA150/300: Ethyl acetate fraction of the *Ziziphus jujuba* extract

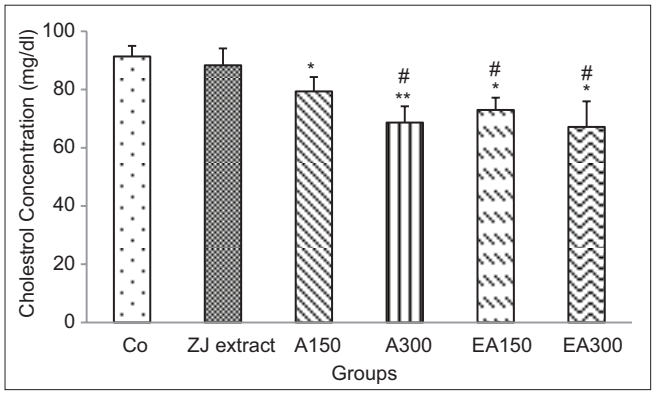

Figure 2: The comparative effects of cholesterol concentration in all treated groups. The data presented as mean ± standard error of the mean by one-way ANOVA test with *post hoc* least significant difference. **P* < 0.05, ***P* < 0.01 compared to the control, #*P* < 0.05 compared to the *Ziziphus jujuba* extract group. A150/300: Aqueous fractions of the *Ziziphus jujuba* extract, EA150/300: Ethyl acetate fraction of the *Ziziphus jujuba* extract

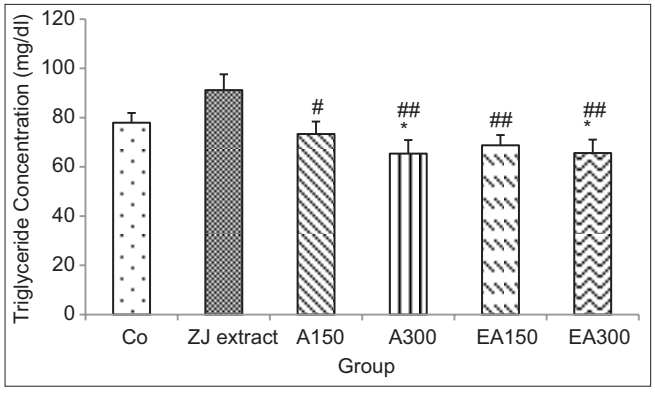

Figure 3: The comparative effects of triglyceride concentration in all treated groups. The data presented as mean ± standard error of the mean by one-way ANOVA test with *post hoc* least significant difference. **P* < 0.05 compared to the control, #*P* < 0.05, ##*P* < 0.01 compared to the *Ziziphus jujuba* extract group. A150/300: Aqueous fractions of the *Ziziphus jujuba* extract, EA150/300: Ethyl acetate fraction of the *Ziziphus jujuba* extract

Discussion

The results indicate that total extract and two fractions of ZJ significantly decreased the glucose level. Serum levels of cholesterol and triglyceride decreased by both higher doses of fractions, but a dose of 200 mg/kg of total extract has not significant effect on these parameters. Total extract and fractions of ZJ also have effect on hematologic parameters and increased RBC and WBS while decreasing platelet amount.

The ZJ fruit is a rich source of the various vitamins, flavonoids, polyphenolic compounds, pectin, alkaloids, glycosides, and terpenoids.[16] In this study, we used polar (AQ) and semi-polar fractions (EA) of the ZJ extract to determine the effect of polar and semi-polar ZJ ingredients on the biochemical and hematological parameters in the normal rats. In a previous study, Davarinejad *et al*. have been shown that the highest components of ZJ extract are polyphenols and flavonoids.[17] The polyphenols and flavonoids are polar compounds, and in this study, the effect of the AQ fraction (polar) of the ZJ extract on the biochemical and hematological parameters was better than the EA fraction that it is conceivable that due to more polarity of the AQ than EA solvent. The numerous studies have been shown that polyphenols and flavonoids isolated of plants have hypoglycemic and hypolipidemic as well as antioxidant effects.[18-20] Therefore, a better effect of AQ fraction in this study on biochemical and hematological parameters attributed the components of this fraction.

In a study, Liu *et al*. have been shown that anthocyanin, one of the derivatives of flavonoids, also has a hypolipidemic effect in humans treated with anthocyanin-rich supplementation. The possible mechanisms of this effect are increasing the fecal excretion of total cholesterol as well as down-regulation of 3-hydroxy-3-methyl-glutaryl-cholesterol O-acyltransferase reductase (HMG-CoA reductase) gene expression in the liver specially. Furthermore, anthocyanin decreases the apo B/C-containing triglyceride-rich particles.[21] In another study also has been indicated that polyphenols such as flavonoids inhibit both acyl CoA and HMG-CoA reductase, modulate adipokines, increasing of the GLUT-4 gene expression, reduction of the oxidative stress, and activate AMP-activated protein kinase.[22] One of the other ingredients in the ZJ extract is pectin that modulates the HMG-CoA reductase activity and increases the hepatic pool of the cholesterol results in a reduction of blood lipid profile.[23] Saponins, glycosidic compounds, have hypoglycemic and hypolipidemic effects.[24,25] Jujubosides are also in the saponins group structurally that isolated from ZJ extract. Therefore, part of the hypoglycemic and hypolipidemic properties of the ZJ attributes to the presence of jujubosides.[26]

About hematological parameters, some studies have been revealed that flavonoids and polyphenols bind to the RBC/WBC membrane and lead to increasing of membrane integrity via reduction of lipid peroxidation.

Therefore, flavonoids by its antioxidant properties result in decrease of hemolysis and increase the RBC/WBC count.[27,28] In addition, flavonoids have an inhibitory effect on the platelet function and aggregation via its anti-inflammatory effects and also flavonoids effect on the secretory process, mitogenesis, and cell–cell interaction.[29,30] These are probably the reasons for reducing platelet count. Furthermore, several studies have been reported that ZJ extract induces the expression and production of the erythropoietin via activation of the hypoxia-inducible factor 1α and other signaling factors that result in the hematopoiesis process enhancement.[31] Briefly, both fractions had better effects than whole extract on biochemical parameters, which is due to the use of relatively high doses of extract fractions.

Conclusion

According to the recent results, AQ and EA fractions of ZJ have beneficial effects on biochemical and hematological parameters. Since these fractions are polar, it is suggested that these effects of ZJ mostly mediated by compounds of ZJ that soluble in polar solvents.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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