Antidiabetic Activity of Aqueous Fruit Extract of *Ribes biebersteinii* in Streptozotocin Induced Diabetic Rats

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Abstract

Background: ***Ribes biebersteinii (Rb)*** **is one of pharmaceutical plants which was used in traditional medicine for decreasing blood sugar.**

Objective: In this study, effect of aqueous fruit extract of *Rb* on blood sugar of rats was investigated.

Methods: In this research 60 male Wistar rats were categorized randomly in 10 equal groups with two timeframes (7 & 14 days). Extraction of Rb was performed with 50 g in 500 ml of distilled water for 18 hours by using a soxhlet extractor. For inducing diabetes, streptozotocin 7 mg/0.5 ml in normal saline was injected intramuscularly per 100 gram of rat body weight. Aqueous fruit extract of *Rb* was given orally to rats daily (80 mg/0.5 ml in distilled water per 100 gram of rat body weight). Then the blood samples sent to the laboratory to measure the following factors: glucose, lipids; and activity of the enzymes in plasma serum.

Results: *Ribes biebersteinii* compared to diabetic group had significant effect on blood sugar ($P \leq 0.0001$). After 14 days, there is significant difference between weight of treated rats with fruit extract of *Rb* (162.2 ± 1.70) and diabetic group (149.7±2.16) ($P \leq 0.001$). Also fruit extract of *Rb* had no effect on blood fat (Cholesterol, Triglyceride, LDL, HDL) of treated rats.

Conclusion: According to the results concluded that extract of *Ribes biebersteinii* had effect on the blood sugar of rats and led to decrease blood sugar of rats. Because the fruit is rich in anthocyanins, this effect may be due to the its antioxidant effect.

Keywords: ***Ribes biebersteinii*****, Diabetes, Rat, Streptozotocin**

Introduction

Diabetes mellitus (DM) is a metabolic and chronic disease caused hyperglycemia which is due to an insufficiency or insulin function, or both. Diabetes is associated with various disorders in the metabolism of glucose, protein, and fat [1, 2, 3]. According to the International Diabetes Federation (IDF), It is estimated that 8.3% of the world population is affected by this disease [4]. The treatment of DM is based on insulin and oral anti-diabetic drugs. Oral hypoglycemic agents, such as biguanides and sulfonylurea are available for the treatment of non-insulin dependent diabetes mellitus (NIDDM) but they have significant side effects [5, 6]. The researchers conducted over the last several decades have shown that medicinal plants have high potential to treat and control diabetes and its complications [3, 7]. The plants more extensively studied in the control of diabetes mellitus are *Trigonella foenum- graecum*, *Vaccinium myrtilus*, *Allium sativum* and *Securigera securidaca* [3, 8]. The author published antidiabetic effect of *Diospyrus lotus*, *Myrtus communis* and *Teucrium polium* [1, 2, 3, 10]. More than 17 medicinal plants were reported in Iranian traditional text books for managing of diabetes [11].

Ribes biebersteinii Berland. ex DC from Grossulariaceae *family* with Persian names "Ghareghat" *is* one of the medicinal plants used in Persian folk medicine to control diabetes. *Ribes biebersteinii* (*Rb*) is a shrub without thorn, with a length of 1 to 2 meters, fruit berry, spherical with 2 to 4 mm in diameter, red to black-purple, and without hair [12]. This species is widely distributed in temperate regions of the northern hemisphere and often in mountainous regions. It is distributed in Armenia, the Caucasus, the Republic of Azerbaijan and Turkey. This plant grows in Ahar, Calaibar and Arasbaran forests in northwest of Iran [13]. Studies on the fruit of this plant have shown the presence of anthocyanin compounds. Anthocyanosides show vasoprotective properties, antihypertensive effects, reduce platelet aggregation, and prevent coronary artery spasm and, on the other hand, are free of any side effects [13, 14, 15].

This study was performed to access the antidiabetic effect of aqueous fruits extract of *Ribes biebersteinii* on streptozotocin-induced diabetic rats.

Methods and Materials

Plant material

Fruits of *Ribes biebersteinii* was collected on September 2016 from the Arasbaran region (around East Azerbaijan, Iran), and confirmed scientifically. Voucher specimen of the plant in number of E1-273-12 is deposited in the department of pharmacognosy, Faculty of pharmacy, Mazandaran University of Medical Sciences, Sari, Iran. The fruits were air dried and then milled using mechanical grinders.

Preparation of extract

The fruits of *Ribes biebersteinii* were dried in an incubator for two days at 40°C. The fruits were powdered (mesh 350) by using electrical grinder. Extraction was performed with 50 g of the powder in 500 ml of distilled water for 18 hours by using a soxhlet

extractor. The solvent was evaporated by rotary evaporator and then the brown extract was dried in oven at 50 $^{\circ C}$ for 24 h [12, 16, 17].

Selection of animals

The study was conducted on 60 normoglycemic (with glucose level 85 ± 5 mg/dl) matured male Charles-River rats (150-160 g) which were housed in colony cages (six rats per cage). All animals were purchased from the animal house of Mazandaran University of Medical Science. The rats were kept in cages with standard laboratory conditions (temperature 22 ± 2°C, relative humidity 45-55 with a 12/12 h light – dark cycle) and were allowed *ad libitum* access to normal laboratory diet and tap water.

Induction of diabetes mellitus

Diabetes mellitus was induced by single intraperitoneal (IP) injection of 7mg/0.5ml/100g of streptozotocin (Upjohn, USA) (STZ) dissolved in 0.9% fresh cold normal saline in 12 h-fasted rats. The STZ injected animals exhibited hyperglycemia after 24 h [1, 2, 10, 12]. Blood for glucose level determination was taken from the tail artery of the rats. After the injection, they had free access to food and water. Rats with hyperglycemia (that is, with blood glucose level higher than 250 mg/dl) after 2 weeks were chosen for the experiment.

Experimental design

The sixty diabetic rats were divided into ten equal groups as follows [1, 12]:

I. Control group: six normal rats were received intramuscular injection of a normal saline unit at the dose of 0.5 ml/100g body weight for 7 days.

II. Control diabetic group: six normal rats were received a single intraperitoneal (IP) injection of streptozotocin at the dose of 7 mg/0.5ml/100g body weight.

III. Diabetic + insulin group: six diabetic rats were received an intramuscular injection of insulin at the dose of 5 unit/kg body weight per day for 7 days.

IV. Diabetic + Glibenclamide group: six diabetic rats were forcefully fed by gastric tube with glibenclamide (Profarmaco, Italy) at the dose of 10 mg/kg body weight per day for 7 days.

V. Diabetic + *Ribes biebersteinii* extract group: six diabetic rats were forcefully fed by gastric tube with *Ribes biebersteinii* extract at the dose of 80 mg/0.5ml/100g body weight per day for 7 days.

VI. Control group: six normal rats were received intramuscular injection of a normal saline unit at the dose of 0.5 ml/100g body weight for 14 days.

VII. Control diabetic group (this was different from group II): six normal rats were received a single intraperitoneal (IP) injection of streptozotocin at the dose of 7 mg/0.5ml/100g body weight.

VIII. Diabetic + insulin group: six diabetic rats were received an intramuscular injection of insulin at the dose of 5 unit/kg body weight per day for 14 days.

IX. Diabetic + Glibenclamide group [1]: six diabetic rats were forcefully fed by gastric tube with glibenclamide (Profarmaco, Italy) at the dose of 10 mg/kg body weight per day for 14 days.

X. Diabetic + *Ribes biebersteinii* extract group [11, 12]: six diabetic rats were forcefully fed by gastric tube with *Ribes biebersteinii* extract at the dose of 80 mg/0.5ml/100g body weight per day for 14 days.

Selection of the 80 mg/0.5ml/100g dose for *Ribes biebersteinii* extract was derived from some dose response.

Before giving the extract, the level of primary blood glucose was measured in all groups. Regarding the treatment of seven and fourteen days in this study, the fasting blood glucose level of all groups was measured after 7 days and 14 days. In this way, the animal was anesthetized with the help of the ether, then blood samples were taken from the tail vein and the results were expressed in mg/dL. In order to test the serum lipids and biochemical activity of the enzymes after the 14th day, the blood samples were taken from all groups and sent to the laboratory to measure the following factors (by using photometric method): quantitative analysis of cholesterol, triglycerides, HDL, LDL; and detection of biochemical activity of the enzymes of AST (aspartate aminotransferase), ALT (alanine transaminase) and ALP (alkaline phosphatase) in plasma serum.

Statistical analysis

Statistical analysis of data was performed using the one-way analysis of variance and student's t-tests. The data were analyzed in Graphpad Prism-6. The differences between the means were considered significant at the probability level $P < 0.05$.

Results

Blood Glucose Comparison in Streptozocin and Controlled Diabetic Rats

The results obtained from the statistical analysis indicate that the mean fasting blood glucose of the rat was normal (85.33 ± 1.93). As shown in Figure 1, fasting blood glucose significantly increased after 24 hours streptozotocin injection compared to control group (363.30 ± 10.64). In fact, t-test results show that there is a significant difference between blood sugar control and diabetic group (P-value≤0.0001).

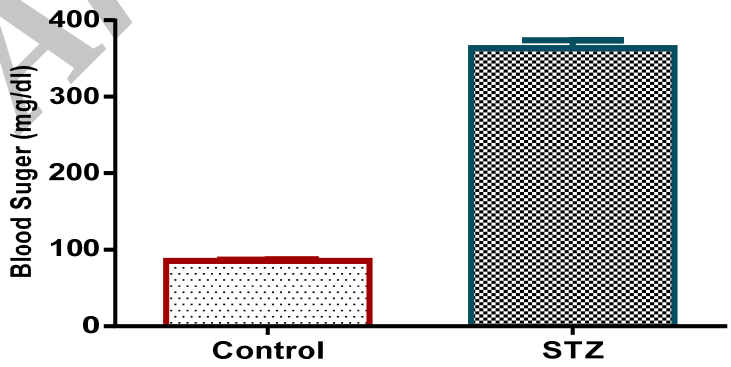

Figure 1- Blood glucose comparison in male rats in the streptococcal and diabetic group

Comparison of blood glucose in the studied groups after seven and fourteen days

One-way ANOVA was used to compare the blood glucose of the studied groups. There was a significant difference between the blood glucose levels of the studied groups compared to the diabetic group after the time of seven and fourteen days (P-value≤0.0001). As shown in figure 2, there is no significant difference between the blood glucose group and the extract group (95.7 ± 1.77) and the control group (85.17 ± 2.47). In other words, Rb extract has been significantly able to reduce blood glucose levels in diabetic rats after seven and fourteen days (P-value≤0.0001).

Generally, the results show that blood glucose in the studied groups is almost the same after 7 and 14 days, and there is no significant difference in these groups. In other words, it can be concluded that insulin (89.83 ± 3.83), Rb extract (1.77 ± 95.00), and glibenclamide (2.77 ± 100.30) have been able to reduce the blood glucose in diabetic rats by approximately the same amount (Figure 2).

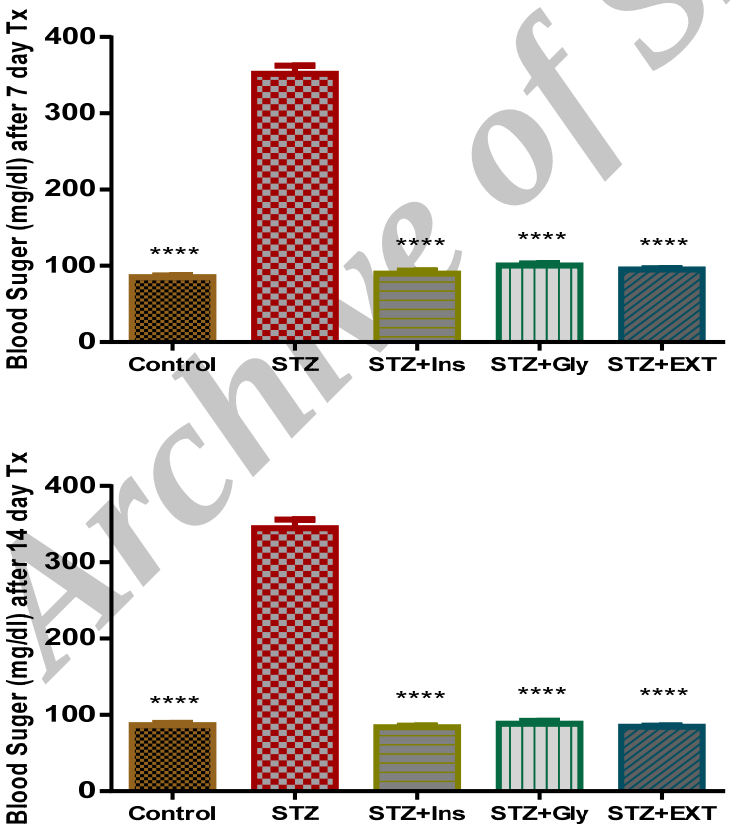

Figure 2- Comparison of blood glucose in the studied groups at intervals of seven to fourteen days, **** P-value≤0.0001

Comparison of the weight of the rats in the studied groups after seven and fourteen days

The results of statistical analysis of one-way ANOVA show that there is a significant difference between the different groups except the insulin group and the control group after the time of seven days (P-value≤0.01). The weight of rats in different groups has decreased in comparison with the control group. Also, the results show that after fourteen days, there is a significant difference between the weight of the control groups and the insulin group with the diabetic group. The weight of the rats in all groups except the diabetic group increased so that the weight gain of the control group was higher than the other groups (Figure 3).

The results show that, after fourteen days, the average weight in group fed by extracts (159.8 ± 1.76) was not significantly different from the diabetic group (155.8 ± 2.21) (Figure 3).

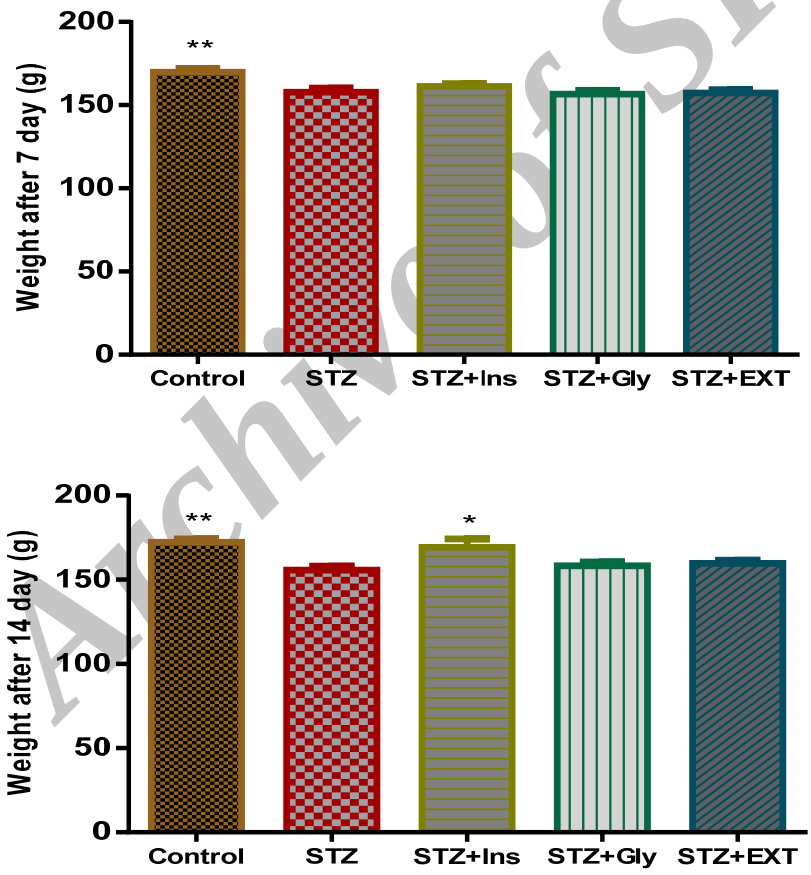

Figure 3- Comparison of the weight of the mice in the studied groups at intervals of seven to fourteen days
**** P-value≤0.01, * P-value≤0.05**

Investigation of blood lipids and liver enzymes in the studied groups

- Comparison of blood cholesterol levels in the studied groups

The results of statistical analysis of one-way ANOVA showed that there is no significant difference between the levels of blood cholesterol in different groups (Figure 4). The results of the study show that after fourteen days, the mean blood cholesterol level in diabetic extract rats (11.46 ± 115.3) was not significantly different in comparison with the diabetic group (112.2 ± 11.82). Also, the mean blood cholesterol level in the rats was significantly lower than the control group (127.3 ± 10.75). However, this decline was not significant (Figure 4).

Comparison of blood triglyceride levels in the studied groups

The results of the statistical analysis of one-way ANOVA showed that there is a significant difference between the level of triglyceride in the control group and the other groups (P-value≤0.0001) (Figure 5). In other words, as Figure 5 shows, the amount of triglyceride in the control group is significantly reduced compared to other groups. Also, the levels of triglyceride in other groups were not significantly different.

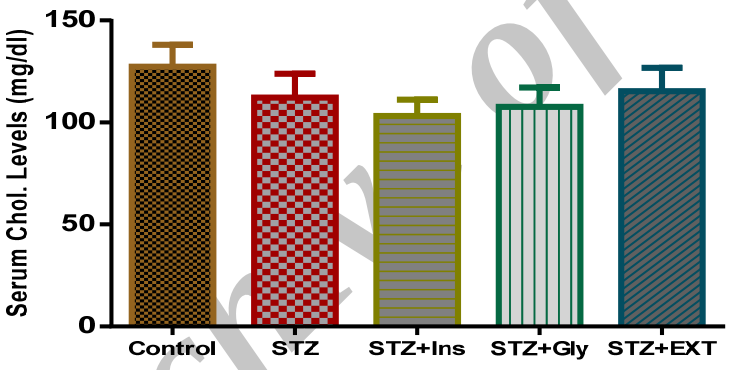

Figure 4- Comparison of blood cholesterol in the studied groups

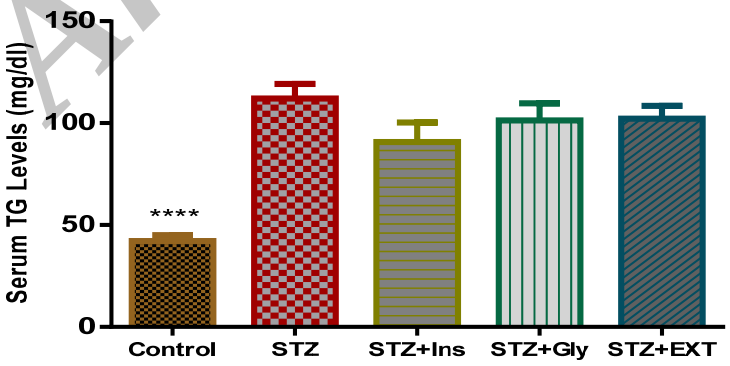

Figure 5- Comparison of blood triglyceride in the studied groups, **** P-value≤0.0001

- Comparison of HDL blood levels in the studied groups

The results of statistical analysis of one-way ANOVA showed that there was no significant difference between HDL in different groups. There is only a significant difference between control groups and glibenclamide (Figure 6). In other words, as shown in figure 6, HDL levels in the control group are slightly increased compared to other groups, but this increase is significant only in the glibenclamide group and is not meaningful in other groups (Figure 6).

- Comparison of LDL blood levels in the studied groups

The results of the statistical analysis of one-way ANOVA showed that there was no significant difference between LDL in different groups. In other words, as shown in figure 7, the control and diabetic groups have the highest levels of LDL in the blood, respectively. Generally, it can be argued that LDL levels in the studied groups are relatively similar (Figure 7).

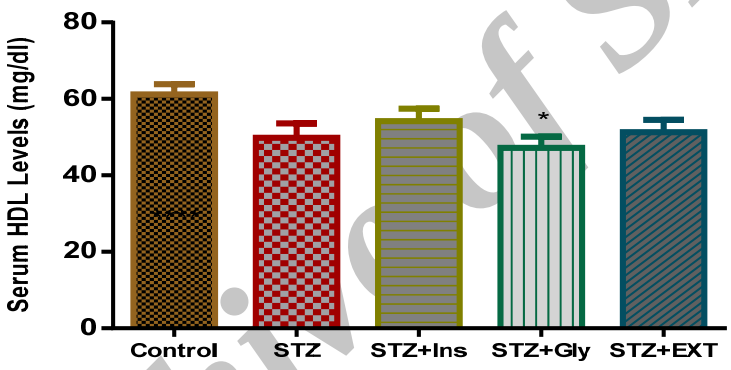

Figure 6- Comparison of blood HDL in studied groups, * P-value≤0.05

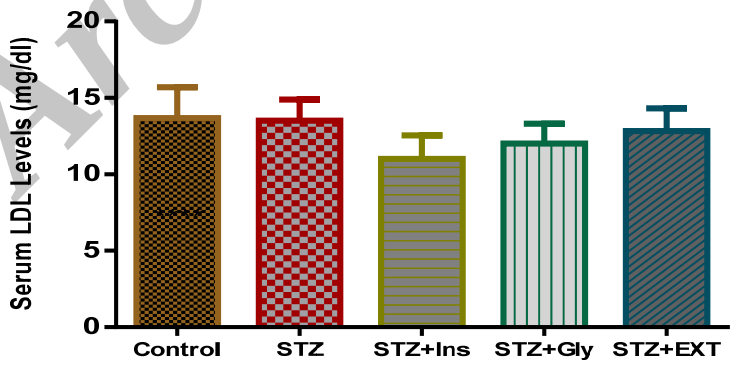

Figure 7- Comparison of LDL in the studied groups

- Comparison of AST blood levels in the studied groups

The results show that glibenclamide and diabetic groups have the highest and lowest AST levels, respectively. Statistical analysis of one-way ANOVA showed that the AST level of the control and glibenclamide groups showed a significant difference with diabetic and insulin groups (P-value≤0.01). Also, the extract group has only a significant difference with the glibenclamide group (Figure 8). In other words, as Figure 8 shows, there is no significant difference between diabetic and insulin groups.

- Comparison of ALT levels in the studied groups

The results showed that the control and diabetic groups had the lowest and highest ALT, respectively. Statistical analysis of one-way ANOVA showed that ALT level of the control group was significantly different with other groups (P-value≤0.0001). In other words, the level of ALT in the control group was significantly lower than other groups. (Figure 9).

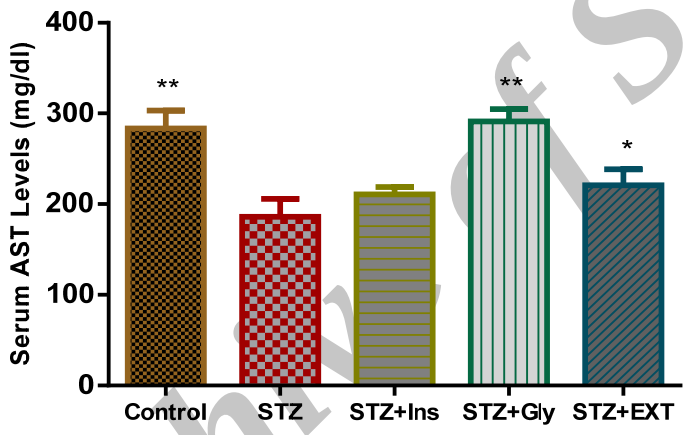

Figure 8- Comparison of blood AST in the studied groups, ** P-value≤0.01, * P-value≤0.05

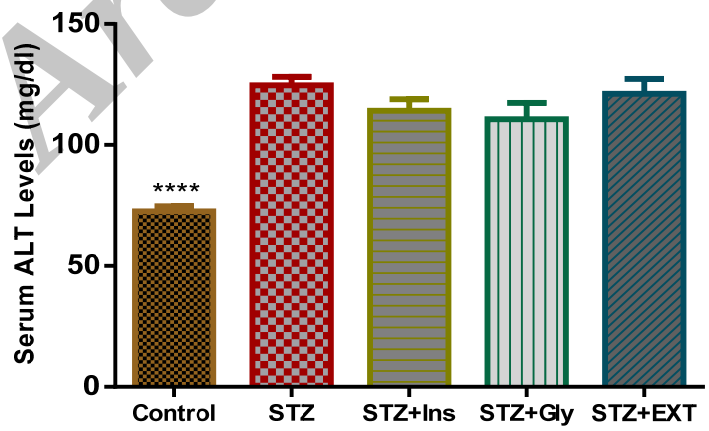

Figure 9- Comparison of blood ALT in the studied groups, **** P-value≤0.0001

- Comparison of ALP levels in the studied groups

The results show that the control and diabetic groups have the lowest and highest ALP levels, respectively. The result of statistical analysis of one-way ANOVA showed that there is a significant difference between the ALP values of the studied groups in the diabetic group (P-value≤0.0001). In other words, ALP levels in the diabetic group have increased more than other groups. (Figure 10).

The results of blood glucose analysis showed that there was a significant difference between the blood glucose levels of Rb aqueous extract group compared to diabetic group, so it can be concluded that Rb extract can significantly increase after 7 days Blood glucose lowering in male rats compared to diabetic group. It should be noted that after two weeks (14 days) blood glucose reduction in the Rb extract group was not significant in comparison with seven days.

Discussion

In the study by Babaei and colleagues in 2009, Rb extract induced relaxation in rat aortic rings pre-contracted with phenylephrine or PGF2α dose–dependently in both intact and endothelium-denuded aortic rings. The differences between the relaxant response of intact and endothelium–denuded rings were not significant (P>0.05) [18].

In the study by Mirfeizi and colleagues in 2012, as a randomized trial on 75 patients with type II diabetes (30 people in intervention and 45 people in control group) in Karaj-Iran. *Vaccinium arctostaphylos* (another Ghareghat in Iran) extract (capsule 500 mg, twice a day after breakfast and lunch) was administered in intervention group. Vein blood samples were taken at the beginning and end of study to measure fasting blood glucose, glycosylated hemoglobin (HbA1c), insulin serum, Triglyceride, total Cholesterol and its contents. Using HOMA Score, insulin resistance was also measured. After 90 days of the

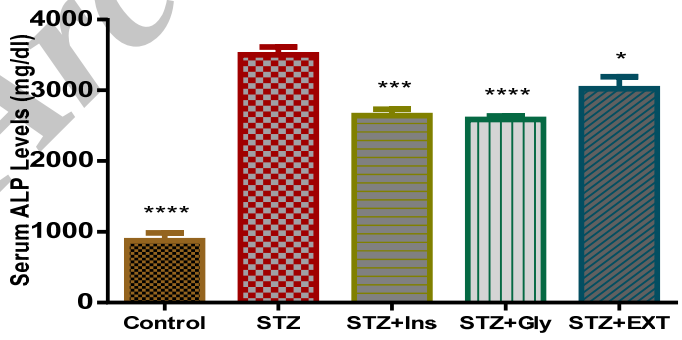

Figure 10- Comparison of blood ALP in the studied groups, **** P-value≤0.0001, *** P-value≤0.001, * P-value≤0.05

intervention, there was a significant difference in FBS, two-hour blood sugar and HOMA Score between the drug and control group. Also in the drug group, in all glucose control indexes, there were significant differences After 90 days of the intervention [19].

In the study by Delazar and colleagues in 2010, Reversed-phase preparative HPLC analyses of the methanol extract of the leaves of Rb afforded four flavonol glycosides, quercetin 3-Osophoroside, quercetin 3-O-sambubioside, kaempferol 3-O-sophoroside and kaempferol 3,5-di-O-βD-glucopyranoside. The free-radical-scavenging properties of the n-hexane, DCM and MeOH the extracts of Rb, as well as the isolated compounds had antioxidant activity [20].

So far no studies have been done to prove the effect of glucose reduction on *Ribes biebersteinii*, and the use of this plant for this purpose is based only on traditional beliefs.

Comparison of weight of different groups showed that after 7 days there was no significant difference between the weight of the rats in different groups except the control group. However, after 14 days, the Rb group was able to weigh the rat more than the diabetic group. In other words, the Rb extract has an effect on the weight gain of the rat. This result is consistent with the previous finding in this study that the Rb extract has an effect on blood glucose lowering, which means that by reducing blood glucose in rat, its weight increased after 14 days of Rb gavage extract.

The results of the blood cholesterol test showed that there was no significant difference in blood cholesterol in the studied groups. In other words, blood cholesterol in different groups was normal and not significantly different. Based on the results obtained, it can be concluded the levels of cholesterol in the diabetic group and the Rb group were similar, so Rb extract had no effect on cholesterol in the studied rats.

Regarding the triglyceride factor, the values indicated that the amount of this factor in the Rb aqueous extract group was lower than the diabetic group, but this difference was not significant. Triglyceride values obtained from other groups were also normal.

Concerning the HDL factor, the value of this factor was normal in all studied groups. In the Rb extract group, it was 51.33 which compared with the diabetic group with a significant reduction of 49.83. There is no significant difference between these two meanings.

The values obtained from the LDL assay in the studied groups indicate that the value of this factor in all groups is within the normal range. LDL was found in the Rb extract group of 12.83, which was compared to LDL diabetic group 13.50, did not significantly decrease.

The AST values in the studied groups showed that there was a significant difference between the AST content in the control groups, diabetic + glibenclamide group and the Rb extract group with diabetic and diabetic + insulin groups. The amount of AST in the diabetic group was 186.17 and in the Rb group of 202.87, which means that there is a significant difference between these two values, that the extract of Rb after 14 days increased this factor as a result of the proposed it will be further investigated in future studies.

Concerning the obtained ALT values, there was no significant difference between the ALT values in all groups except the control group and ALT in the control group was lower than in the other groups. In the group gavage with extract of Rb this amount was 12.21, which was not significantly reduced compared to the diabetic group.

The amount of ALP obtained shows that its value in the diabetic group is 3501 and in the Rb extract group is 3023. This indicates that there is a significant difference between the ALP of the extract group and the diabetic group, that is, the extract of Rb has been able to reduces ALP value.

Regarding the effect of Rb extract on blood lipids and hepatic enzymes, some of the similar results that are consistent with the results of this study are referred to *Vaccinium arctostaphylos* (another Ghareghat in Iran) [19].

Conclusion

The present data indicated that the *Ribes biebersteinii* aqueous fruit extract significantly decreased serum glucose in diabetic rats as compared with control diabetic rats but there was no significant difference in blood cholesterol, triglycerides, LDL and HDL factors in the studied groups. Because the fruit is rich in anthocyanins (as a phenol compounds), decreasing serum glucose in diabetic rats may be due to the its antioxidant effect (20-24).

The *Rb* aqueous extract could increase the weight of the rat relative to the diabetic group. The extract after 14 days increased AST value factor but the ALP value decreased and ALT value has no affected by the extract, so it is suggested AST and ALP values more to be considered in future studies.

Considering the results obtained in this study and the similarities and inconsistencies with other researches, it is suggested that further studies such as on plant fractions and mechanisms of its effect on glucose, lipids and enzymes should be carried out.

Authors' contributions

SMA is pharmacy student, carried out the study and prepared the draft of manuscript, MA is head of study and participated in its design and coordination and prepared the manuscript. NA participated in the taking result and performed the statistical analysis.

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This study was the Pharm. D. thesis of Seyyedeh Marzieh Ahmadi, Ramsar student of pharmacy, Mazandaran University of medical sciences, Ramsar, Iran.

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