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Antihyperglycemic and Antihyperlipidemic effects of Cornus mas extract in diabetic rats compared with glibenclamide

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ABSTRACT

Background: Diabetes mellitus is important risk factors for some disorders such as nephropathy, retinopathy, neuropathy and cardiovascular diseases that its prevalence is increasing in human society. Herbal medicine is the oldest kind of diseases cure has recognized. However, rational prescription of effective medicinal plants for diabetes cure requires precise information of action mechanism of these plants. Purpose of our project is investigation of *Cornus mas* effects on blood biochemical parameters in alloxan-induced diabetic rats.

Methods: In present study, 40 male rats divided in 4 groups. Group 1(normal group) was treated by saline. Group 2(diabetic control) was treated by alloxan monohydrate (120 mg/kg, i.p). Group 3(extract group) diabetic rats that received hydro-alcoholic extract of *C.mas*(100mg /kg, i.p). Group 4(glibenclamide group) diabetic rats that received glibenclamide(500mcg /kg, i.p). After 72 hours, the animals were anesthetized and the blood was collected into a tube, then, levels of serum glucose, lipoproteins (HDL, LDL, and VLDL), triglycerides, and total cholesterol were evaluated by enzymatic kits.

Results: The results showed significant reduction of glucose, triglyceride, VLDL and LDL levels in group 3 in comparison with group 2($P<0.001$). Glucose and LDL reduction by *C.mas* are similar to glibenclamide and TG, VLDL reduction by *C.mas* are more than glibenclamide. *C.mas* also could increase HDL levels significantly in comparison with group 2($P<0.001$) that this effect is similar to glibenclamide effect on HDL.

Conclusion: According to the results, *C.mas* can be use in diabetics in order to glucose and lipid reduction, nevertheless, requires more researches.

Introduction

Diabetes mellitus is the most prevalent endocrine disease and afflict more than 100million people in the world every year. This disease is the 7th death cause [1]. A lot of diabetics are unknowing of their disease and they understand it when it has progressed and the body can't control the blood glucose. Insulin relative shortage or decrease results in acute and chronic metabolic disorders in diabetes [2, 3]. Though, primary and effective cure for diabetes is insulin and hypoglycemic drugs usage, but these compositions have some undesirable side effects. Medicinal plants and their derivatives have been used to cure diabetes since past years but scientific investigations are necessary to prover of their effects [4].

Cornus mas of *Cornaceae* family use in traditional medicine to cure of squirt, intestinal bulge, malaria, fever sedation, kidney stone, kidney and bladder infection treatment. *Cornus mas* fruit has a little glucose and sacharose and a lot of calcium, folic acid, C, B1, B2 and E vitamins, anthocyanins, flavonoides and mucilage [5, 6]. *Cornus mas* also has antioxidant materials and high capability to cancer suppression [7, 8].

Anthocyanins can relief degenerative diseases such as atherosclerosis, cardiovascular diseases, cancer and diabetes. Researches show *C.mas* has plenty of anthocyanins such as cyanidin-3-galactoside, delphinidin-3-galactoside and plargonidin-3-galactoside [9, 10], therefore it's one of the main antidiabetic drugs in Asia and use traditionally to cure diabetes depended diseases [11].

In present study, we induced diabetes in rats. After diabetes verification, we evaluated effect of *C.mas* hydroalcoholic extract on blood glucose.

Materials and methods

Plant materials and extraction. Fresh fruit of *C.mas* were bought from Ardabil Department for Natural Resources (1 kg), and authenticated by expert. The fruits were cleaned and powder was prepared with mill, and ethanol 96% was added to cover the surface of the powder. Then it was positioned on the shaker. After 24 hours the solution was filtered through filter paper (Whatman qualitative grade 1), and again ethanol 75% was added to the remained waste, and was positioned on the shaker for 12 hours. Finally, the combined filtrate was then concentrated in a rotary evaporator (35–40 °C), to a thick, dark green colored crude extract up to $^1/_3$ the primitive volume. For proteins isolation and material refining, after the filtered solution decantation 3 times by chloroform, was positioned in incubator at 50^0 C. After a few days, the powder was ready and included net and effective material of the plant. A crude residue (32g) was obtained giving a yield of 4 %. The powder was dissolved in normal saline for experiments, and dilutions were made fresh on the day of experiment.

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Animals: The experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996). Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC). Male rats (*Rattus Norvegicus Allivias*) used in the study (200-230 g) were housed in the animal house of the Ardabil Payame Noor University. Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature (23-25^{0}C), relative humidity (45-55%) and 12 hrs dark/light cycles were maintained in the quarantine. All the animals were fed with rodent pellet diet and water was allowed *ad-libitum* under strict hygienic conditions. After the adaptation period, each group of rats was weighted and marked, and then treated by the specified dose of materials.

Diabetes induction. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative [12]. Glucose and alloxan structural similarity causes alloxan connects and enters beta cells. Alloxan degenerates specially beta cells thus uses as a suitable material to induce diabetes in animals. Meanwhile alloxan causes Reactive Oxygen Species production only in Langerhauns islets [13]. Alloxan injection causes diabetes induction in rats which it's similar to human type 1 diabetes. In this study, criterion for diabetes induction was blood glucose more than 300mg/dl [14]. After 72 hours of alloxan injection, the diabetic rats were separated and used for the study. Animals were assigned to 4 groups having the following characteristics:

1) Normal group: was treated by saline (2 ml/kg, i.p.)
2) Diabetic control group: was treated by alloxan monohydrate (120mg/kg, i.p.) for 3 days alternately. Then, blood glucose was evaluated by blood glucose test meter (Glutest PRO R; Sanwa-kagaku, Nagoya, Japan).
3) Extract group: was treated by alloxan monohydrate for 3 days alternately and, after blood glucose evaluation and diabetes verification, animals received hydro-alcoholic extract of *C.mas* (100 mg/kg, i.p.) for 10 days alternately.
4) Glibenclamide group: was treated by alloxan monohydrate for 3 days alternately and diabetes verificated after blood glucose evaluation, and after 48 hours, received also glibenclamide (500mcg/kg/i.p) for 10 days alternately.

72 hours after extract administration, the animals were anesthetized and blood samples were collected from heart of each rat and were analyzed for glucose and lipid content by enzymatic kits.

Statistical analysis All the experiments were repeated at least 3 times with appropriate controls. Data are presented as the Mean±SD and P<0.05 was considered statistically significant. Statistical analysis was performed using a one-way ANOVA and the relevant figures were drawn with Excel.

Results

The results of glucose, triglyceride and lipoproteins biochemical experiments mentioned table1.

Glucose mean difference of *extract* group with *normal* and *control* groups is significant (p<0.05)
Triglyceride mean difference of *extract* group with *control* group is significant (p<0.05)
There is not significant mean difference between the groups for **cholesterol** due to regulator mechanisms of plasma cholesterol concentrations.
LDL mean difference of *extract* group with *control* group is significant (p<0.05)
HDL mean difference of *extract* group with *control* group is significant (p<0.05)
VLDL mean difference of *extract* group with *control* group is significant (p<0.05)
Extract group and glibenclamide group didn't have any significant differences in studied factors
Each column illustrates mean±SD

Discussion

In present study hypoglycemic effect of *C.mas* hydroalcoholic extract was evaluated in diabetes-induced rats and results compared with glibenclamide effect. Glibenclamide is one of the sulfonylurea antidiabetic drugs which increase insulin secretion of beta cells. In addition to, this drug has insulin-like effects on glucose metabolism, as decreases glycogenesis and gluconeogenesis, thus by reduction of two mentioned mechanisms, blood glucose is reduced [15, 16].

Blood sugar reduction by hydro-alcoholic extract of *C.mas* is similar to Ataie et al finding [17].One of the extract effects probably is reducing of hepatic glucose-6-phosphatase activity which increased in diabetes mellitus [18]. Other hypoglycemic effect of the extract is presumably liver phosphorylase inhibition, which inhibits glycogen storage breakdown in hepatic cells and increases the enzyme activity that result in glycogen synthesis improvement. Meanwhile recent studies have shown plants flavonoides, alkaloids and pectin have hypoglycemic and hypolipidemic effects in diabetics [19].

Following alloxan injection, and blood sugar increasing, triglyceride increased too, demonstrates insulin role in lipids metabolism adjustment [20]. As alloxan induced diabetes mellitus in rats includes clear undesirable changes in plasma lipids and lipoproteins as in alloxan or streptozotocin-induced diabetic rats, increase triglycerides and cholesterol level [21, 22]. On the other hand, in alloxan-induced diabetic rats, glucose increment result in cholesterol, triglyceride, LDL and VLDL increment and HDL reduction[23] which it's partly justifier of undesirable changes in plasma lipids in the diabetic rats of this study.

Following glibenclamide injection, triglyceride decreased, In addition too, LDL decreased and HDL increased too, it's similar to *Bruner, Wasbort, Regitz and Tuval* findings [24]. The extract reduced triglyceride, cholesterol, LDL and VLDL levels and increased HDL too. Considering to occurs stress oxidative

Table1- Effect of *C.mas* extract on glucose, cholesterol, triglyceride and lipoproteins levels in rats

	experimental groups (n=10)			
Index	normal	control	extract	glibenclamide
Glucose (mg/dl)	124.61±15.75	767.82±117.44	274.01±45.25	221.83±13.11
Triglyceride(mg/dl)	106.30±14.42	226.31±15.35	130.19±29.82	156.29±12.13
Cholesterol (mg/dl)	93.79±12.32	110.92±10.54	104.25±10.85	110.37±12.20
LDL (mg/dl)	23.37±6.88	39.04±12.09	23.98±9.89	27.25±9.82
HDL (mg/dl)	49.16±7.34	26.58±6.15	54.23±12.18	51.86±9.22
VLDL (mg/dl)	21.25±2.88	45.29±3.10	26.03±5.96	31.25±2.42

intensification in diabetes mellitus and result in blood biochemical changes in diabetes type1[25], and *C.mas* decreases stress oxidative due to anti-oxidant materials, thus result in desirable changes on glucose and triglyceride levels in rats. In addition to effective materials of the extract such as anthocyanins induce desirable metabolic changes in hepatic enzymes and improve undesirable changes of blood glucose and lipids [26,27]. Considering to stress oxidative intensification in diabetes mellitus that result in biochemical changes specially in diabetes type 1[21], thus *C.mas* antioxidant materials such as anthocyanins can reduce stress oxidative and decrease blood glucose and triglyceride[28].

By triglyceride reduction by the extract, VLDL level reduced significantly too. About this event we can say: intercellular triglyceride increasing causes VLDL synthesis increasing. Because triglyceride level was reduced significantly by the extract, it is safe to expect VLDL synthesis to reduce. Meanwhile, 90% blood VLDL made in liver and liver cells triglycerides enter VLDL structure, thus each factors reduce triglycerides, can decrease blood VLDL too[28]. Considering VLDL involves LDL particle generation indirectly, thus by significant reduction of VLDL by the extract, we can expect LDL levels to decrease too[29,30].

Considering plasma HDL concentration has inverse association with plasma triglyceride concentration, and recalling that *C.mas* could reduce triglyceride level, and then by decrease of triglyceride level, increase of HDL level should be expected [29].

Conclusions

According to the results, defines *C.mas* has hypoglycemic effect in diabetes mellitus experience model in rat and it causes useful changes on blood lipids. We suggest more investigations to clear the extract mechanism on blood biochemical parameters in both normal and diabetic treatments.

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