Biochemical Study on the Hypoglycemic Effects of Extracted Phenolic Compounds from Grape Seeds in Streptozotocin -Induced Diabetic Rats

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Abstract

Introduction: Many traditional treatments have been recommended in the alternative system of medicine for treatment of diabetes mellitus; however, the mechanism of most of the herbals used has not been defined. **Objective**: The present study was designed to evaluate the potential beneficial effects of grape seed extracts (GSE)in streptozotocin induced diabetic albino rats through assessment of the Blood sugar, serum insulin ,C-peptide , α-amylase , α-Glucosidase and lipase.

Materials & Methods: This experimental study was conducted in 2018 at college of veterinary medicine / Tikrit University. Sixty rats were randomly divided to ten equal groups: non diabetic control, diabetic control, experimental groups received grape seed extract (GSE) at doses100 mg/kg and 300 mg/kg of body weight in a period of 7 weeks . Blood sugar, serum insulin ,C-peptide , alpha amylase , and lipase activity were measured. The data were analyzed by one way analysis of variance, ANOVA, followed by Turkey, s test with SPSS software.

Results: Compared to normal control, the diabetic control rats showed significant increase in serum glucose, α-Glucosidase , and lipase levels and decrease in serum insulin and C-peptide, and α-amylase levels were observed. Concurrently, administration of GSE decreased the serum glucose, α-Glucosidase , and lipase levels meanwhile, significant increase in serum insulin and C-peptide, and α-amylase activities when compared with untreated induced diabetic group.

In conclusion, these results suggest that the GS Extract" may possess anti-hyperglycemic potential in diabetes.

Keyword: *Rat, Phenolic, Hypoglycemic, Grape, Biochemical study*

Introduction

Diabetes mellitus (DM) is a metabolic disorder manifested by elevated levels of glucose in the blood due to derangement in carbohydrate, fat, and protein metabolism that have a severe impact on public health. The pancreatic β-cell destruction or dysfunction occur in type I diabetes which leads to full or almost full deficiency of insulin[(1-4)].Several drugs are used to control diabetes, however, perfect glucose control is rarely achieved. Moreover, plants have been used as an alternative therapy for the diabetes treatment[(5)].

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Grape seed (*Vitisvinifera* Linn.) is one of the most commonly consumed fruits in the world. Grapes can be categorized into grapes with edible seeds, seedless, wine grapes, table grapes, and raisin grapes[6].It has various biological functions, due to its rich vitamin E, linoleic acid and polyphenol ingredients contain including the stilbene resveratrol, the flavanol quercetin, catechins and anthocyanins[7,8]. GSPE have been reported to possess a variety of potent properties, including anti-non enzymatic glycation, antiinflammation, anti-atherosclerosis, anti-tumor, and so on immune function modulator, antithrombotic agent, and LDL oxidation inhibitor[9].

Alpha amylase is a digestive enzyme(α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) It is synthesized in the acinar cells of the salivary glands and stored in secretory granules in these cells[10], it catalyzes the hydrolysis of α-1,4-glycosidic linkages in starch, glycogen, and other oligo- and polysaccharides. Inhibitors of this enzyme could be of use in the treatment of diabetes[11].

Insulin acts by binding to its own receptor on acinar cells, leading to the stimulation and potentiation of amylase secretion through multiple signaling pathways, including regulation of amylase gene transcription and stimulation of the synthesis of the corresponding protein in acinar cell[12].

On that perspective the present study was designed to investigate the therapeutic potentiality of the GS extract of leaves on the blood glucose level, serum amylase, lipase activity, normal and STP- induced diabetic albino Wister rats.

Keywords**:** *Diabetic rats, α-Glucosidase, α-amylase, lipase, biochemical parameters.*

Materials and Methods

Wistar Albino rats weighing 150-200g were used for the research. They were obtained from the animal house of the Department of Pharmacology, college of veterinary medicine / Tikrit University . The rats were kept in properly ventilated cages where bedding was replaced daily, at a room temperature of about 27°C and 12 hours light/dark cycle.

Induction of diabetes: Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (65 mg/kg) dissolved in cold 0.1 M citrate buffer, pH 4.5. Citrate buffer alone was injected in control rats as a vehicle. The blood glucose levels were measured after 2 days, then was started the experiment period [11].

Phenolic extraction of grape seeds (500 g) of the dry powder wall nuts were defatted by washing five times with n-hexane(1L) at (60°C), then it was macerated with (800mL) of acetic acid (2% v/v), the mixture were placed in conical flask volume (2000mL) and put in water bath (60°C) for 8 hrs, then the extraction process done by reflex condenser. The mixture was heated at 50°C (water bath) for 15 min and left to cool. The suspension was filtered by Buchner funnel by What man No.1 filter paper and by the use of vacuum pump. The precipitate was canceled and the filtrate volume was measured. npropanol was added in to filtrate with the same volume of filtrate. Then sodium chloride was added until to become solution super saturated. Then, it was evaporator by using rotary evaporator until drying[14,15].

Experimental design

Five groups of rats six in each groups received the following treatment schedule for 14 days.

Group I - Normal control (normal saline 10 ml/kg, p.o.).

Group II - Streptozocin treated control (65 mg/kg, i.p.) .

Group III - Streptozocin (65 mg/kg, i.p.)+ GSE (100 mg/kg)

Group IV - Streptozocin (25 mg/kg, i.p.)+GSE (300 mg/kg orally three times per week for 7 weeks.

Biochemical Analysis

Blood glucose levels were measured immediately after sampling with a glucose test meter (Glutest Ace; Sanwa Kagaku Kenkyusyo, Nagoya, Japan). Serum insulin levels were determined using a supersensitive rat insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan).C-peptide level was assayed by radioimmuno assay kit, Missouri, USA. Measurement of serum pancreatic amylase was carried out by the method of Landt *et al*[16].

Statistical Analysis

The differences were examined by the one way analysis of variance (ANOVA) followed by the Test of Mann-Whitney using the SPSS 15 program (SPSS Inc., Chicago, IL, USA). A difference at $p < 0.05$ has considered statistically significant.

Results

STZ-induced diabetic rats showed significant increase in the serum levels of glucose, α-Glucosidase, and lipase (340.43±117.67 vs 87.40 ± 4.39 mg/dl), (14. 64 ± 1.648 vs2. 72 ± 0.648 U/mg protein), and (438.33 ± 13.3 vs 160 ± 5.249U/L)respectively, accompanied with marked decrease in α-amylase , Insulin , and C-Peptide (13.6 ± 1.442 vs15.85 ± 1.19 U/dL), (8.42 ± 0.03b vs16.62 ± 0.5 µU/ml), and (1.848 ± 0.51 vs 6. 613 ± 0.99a ng/ml) respectively.

Regarding GSE treated – diabetic rats, the obtained results showed significant reduction in the serum glucose, α-Glucosidase, and lipase concentration (187.57 ± 151.30 mg/dl), (10. 181 ± 1.274U/mg protein), (270.46 ± 5.48) at dose100 mg/kg respectively and (118.50 ± 61.14mg/dl), (14.09 ± 0.699U/mg protein), lipase (241.95 ± 68.8) at dose300 mg/kg, accompanied with significant elevation in serum values of α-amylase , Insulin , and C-Peptide (14.04 ± 0.594U/dL), (12.61 ± 0.12µU/ml), and (3. 580 ± 0.24d ng/ml) respectively at dose100 mg/kg and (14.09 ± 0.699U/dL), (12.61 ± 0.12µU/ml), and (3. 580 ± 0.24 ng/ml) respectively at dose300 mg/kg.

Table (1): Effect of phenolic extract of grape seeds on biochemical parameters in alloxan induced diabetic rats.

	Glucose mg/dl	α-Glucosidase (U/mg protein)	α-amylase (U/dL)	Lipase (U/L)	Insulin (µU/ml)	C-Peptide (ng/ml)
NC	87.40 ± 4.39	2. 72 ± 0.648	15.85 ± 1.19	160 ± 5.249	16.62 ± 0.5	6. 613 ± 0.99a
DC	340.43 ± 117.67**	14. 64 ± 1.648	13.6 ± 1.442	438.33 ± 13.3	8.42 ± 0.03b	1.848 ± 0.51b
D+ GSE (100 mg/kg)	187.57 ± 151.30	10. 181 ± 1.274	14.04 ± 0.594	270.46 ± 5.48	11.52 ± 0.64c	2.208 ± 0.58c
D+GSE (300 mg/kg)	118.50 ± 61.14**	9. 25 ± 1.523	14.09 ± 0.699	241.95 ± 68.8	12.61 ± 0.12d	3. 580 ± 0.24

Data are expressed as means ± S.E. of six rats. ANOVA: P = probability Tukey test: a = significant difference as compared to control group. b = significant difference as compared to STZ-diabetic group after 2 wk.

*Significant different at P< 0.05 level. **Significant different at *P*< 0.01 level

Discussion

Blood glucose concentration is known to depend on the ability of the liver to absorb or produce glucose. The liver performs its glucostatic function owing to its ability to synthesize or degrade glycogen according to the needs of the organism, as well as via gluconeogenesis [17].

Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. C-peptide, initially considered an inactive molecule, has, currently, been shown to be a bioactive molecule when it binds to the surface of several cell types, and activates the calcium-dependent intracellular signaling pathway The measurement of both insulin and C-peptide levels has been reported to be a valuable index of insulin secretion rather than insulin alone[18,19].

As shown in Table 4, STZ caused a significant elevation in the levels of the glucose but serum Insulin and C-peptide levels observed a significant decrease in the DM group when compared to the NC group because the cytotoxic effect of STZ be closely related to free radical generation in pancreatic - cells which interfered with the cellular metabolic oxidative mechanisms [20], due to the destruction of β-cells of pancreas there by inhibiting insulin release[21].This result was previously obtained by Entedhar R. Sarhat[22], Siham A.Wadee[23].

Interestingly, oral administration of GSE decreased BG as well as increased serum insulin and C-peptide levels in the diabetic rats to the near normal levels. The mechanisms underlying GSE anti diabetic action may be attributed to the potentiation of pancreatic secretion of insulin by β -cell of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased levels of serum insulin in diabetic rats treated with plant extract which may stimulate insulin secretion from regenerated β-cells or caused the release of insulin from the residual β-cells.

Alpha-amylase catalyzes the hydrolysis of starch to smaller oligosaccharides consisting of maltose, maltotriose, and a number of α-(1–6)- and α-(1–4)-oligoglucans, which are further degraded by α-glucosidases to glucose. This process may lead to the elevated hyperglycemia occurring in diabetes[24].The analysis of serum α-amylase enzyme was suggested to provide additional informative parameters for the assessment of chronicity and illness as of the response to therapy in diabetes[25].

The results from this research work showed a significant ($P<0.01$) increase in α-amylase level in diabetic rats compared with control rats, due damage of the acini cells through the oxidative stress and inflammatory mediators generated by alloxan which lead to leakage of amylase and lipase into blood circulation[26,27], a condition that were controlled to normal by reversed by oral administration of extracts from leaves of MOE due to its ability to stabilize acini cell membrane, thereby reducing the release of enzymes into circulation. It is possible that its anti-diabetic activity may be related to increased repair of damaged beta cells and acini cells and regeneration of new ones to increase numbers as wells as the functioning of b-islets for the improved synthesis of insulin, that may improve glucose utilization process.

α-Glucosidase (α-d-glucoside glucohydrolase) is an exo-type carbohydrase distributed widely in microorganisms, plants, and animal tissues. **α-glucosidase** anchored in the mucosal brush border of the small intestine catalyzes the end step digestion of starch and sucrose that are abundant carbohydrates in human diet, , which works to facilitate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides[28,30]. Alpha-glucosidase inhibitors act as competitive inhibitors of enzymes needed to digest carbohydrates which delay the rate of carbohydrate digestion, delay the carbohydrate absorption from the digestive tract, and diminish the postprandial blood glucose excursion in diabetic subjects and thus have a lowering effect on postprandial blood glucose and insulin levels [31].

Lipases functions as a lipolytic enzyme that hydrolyzes TGs and phospholipids in circulating plasma lipoproteins[32].During diabetes, enhanced activity of lipase increases lipolysis and releases more fatty acids into the circulation[33].In this study we have observed an increase in lipase in STZ- induced rats. After the treatment the level was reduced, which is another indication of improvement in insulin action since insulin inhibits the activity of pancreatic lipase[34].The inhibition of this enzyme significantly decreases the digestion and uptake of lipids, thereby decreasing the level of blood

glucose in DM[35,36].

These results indicate that GSE decreased serum glucose, lipase and increase serum insulin and C-peptide, and α-amylase were observed in STZ-induced diabetic rats and this effect might exert the anti-diabetic effect of GSE. Of course, further studies for its long term effect for the prevention of diabetes are required.

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