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RESEARCH ARTICLE

Effect of grape seed extract on β-catenin gene expression and hyperglycemia in rats induced by streptozotocin

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Üzüm çekirdeği ekstresinin streptozotosin ile indüklenen sıçanlarda β-katenin gen ekspresyonu ve hiperglisemi üzerine etkisi

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Öz

Amaç: Tip 2 Diabetes Mellitus, insülin direnci ve/veya insülin eksikliği veya yetersizliği durumunda yüksek kan şekeri ile karakterize metabolik bir hastalıktır. Wnt/β-katenin sinyal yolu, tüm fizyolojik süreçlerde rol oynar. Yoldaki herhangi bir kusur diyabetin gelişmesine neden olur. Bu çalışmanın amacı, deneysel Tip 2 diyabetik sıçanlarda üzüm çekirdeği ekstresinin (GSE) vücut ağırlığı, kan şekeri ve β-katenin geni ekspresyonu üzerindeki etkisini belirlemektir.

Gereç ve Yöntem: Sıçanlar Kontrol, Diyabetik Kontrol ve Tedavi grupları (100, 200, 400 mg/kg GSE) olmak üzere beş gruba ayrıldı. Diyabet ve tedavi gruplarındaki ratlara yüksek yağlı diyet ve düşük doz streptozotosin (35 mg/kg) verildi. Deney süresince sıçanların vücut ağırlıkları haftalık olarak kaydedildi. Suda çözünen ekstraktlar dört hafta boyunca gavaj yoluyla uygulandı ve çalışmada tedavi sonunda hayvanlardan alınan karaciğer, pankreas ve kan örnekleri kullanıldı.

Bulgular: GSE tedavisinin diyabete bağlı genel kilo kaybı üzerine etkisinin minimal olduğu, 100 ve 400 mg/kg GSE dozlarının kan şekerini düşürdüğü gözlendi ($p<0,05$). β-katenin gen ekspresyonu sonuçlarına göre karaciğer dokusu ($p>0,05$) ve pankreas dokusu ($p<0,05$) açısından gruplar arasında istatistiksel olarak anlamlı fark yoktu.

Öneri: Elde edilen bulgulara göre üzüm çekirdeği diyabetin tedavisinde umut verici olabilir.

Anahtar kelimeler: β-katenin, tip 2 diabetes mellitus, gen ifadesi, streptozotosin, üzüm çekirdeği ekstresi

Abstract

Aim: Type 2 Diabetes Mellitus is a metabolic disorder characterized by high blood sugar in case of insulin resistance and/or insulin deficiency or insufficiency. The Wnt/β-catenin signaling pathway plays a role in in all physiological processes. Any defect in the pathway causes diabetes to develop. The aim of this study was to determine the effect of grape seed extract (GSE) on body weight, blood glucose and expression of β-catenin gene in experimental Type 2 diabetic rats.

Materials and Methods: Rats were divided into five groups as Control, Diabetic Control and Treatment groups (100, 200, 400 mg/kg GSE). The rats in the diabetes and treatment groups were fed with high-fat diet and were administered low dose of streptozotocin (35 mg/kg). Body weights of the rats were recorded weekly during the experiment. The water-soluble extracts were administered by gavage for four weeks and liver, pancreas and blood samples taken from animals at the end of treatment were used in the study.

Results: It was observed that the effect of GSE treatment on overall weight loss due to diabetes was minimal, and 100 and 400 mg/kg GSE doses lowered blood sugar ($p<0.05$). According to the results of β-catenin gene expression, there was no statistically significant difference between the groups in liver tissue ($p>0.05$) and pancreatic tissue ($p<0.05$).

Conclusion: According to the findings, grape seed may be promising in the treatment of diabetes.

Keywords: β-catenin, type 2 diabetes mellitus, gene expression, streptozotocin, grape seed extract

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a common metabolic disease caused by impaired insulin secretion, insulin resistance, or a combination of both. And, This disease is characterized by dysregulation of carbohydrate, lipid and protein metabolism (DeFronzo et al 2015). Therefore, this metabolic disease is associated with disorders such as cardiovascular disease, osteoporosis and impaired bone healing, stroke, peripheral arterial disease, retinopathy, nephropathy, possibly neuropathy (Yılmaz 2003, Dominguez 2004, Janghorbani et al 2007, Ayaz 2015, Qian et al 2015).

Due to recent changes in human behavior and lifestyle, as well as rapid economic development and urbanization, the incidence of diabetes has increased in many parts of the World (Khushk et al 2010). Statistical estimates using a model based on 1990-2017 data showed that the global prevalence of Type 2 diabetes could rise to as high as 7079 per 100,000 by 2030 (Khan et al 2020). Controlling diabetes is a global problem and a successful treatment has yet to be discovered (Gupta and Sharma 2012).

Compound scalled proanthocyanidin in Grape Seed Extract (GSE) are highly effective substances that are involved in protecting human health. In addition to helping weight loss, these substances also increase the effect of insulin in the body. More important than these effects is the reduction or prevention of arteriosclerosis, which is mostly observed in T2DM patients. In this way, vascular and heart diseases can be prevented by creating a strong protective effect. In addition, it protects against rapid aging caused by the effect of high blood sugar observed in T2DM patients, and in some cases, it is a strong protector against the development of many diseases such as Alzheimer's, Parkinson's, stroke or cancer development with old age (Gabetta et al 2000, Nakamura et al 2003, El-Awdan et al 2013, Pandey and Rizvi 2014, Amin 2018).

The Wnt signaling pathway is one of the main pathways involved in the activities of many physiological and pathophysiological diseases. This pathway functions in lipid metabolism and glucose homeostasis (Jin 2008). Recently, there has been a rapid increase in research on the Wnt/β-catenin signaling pathway because it plays a crucial role in regulating cell growth, cell development, and differentiation of normal stem cells (Yao et al 2011). Recent data suggest that the Wnt signaling pathway has an important role in regulating islet function, insulin production and secretion, as well as pancreatic development (Fujino et al 2003, Rulifson et al 2007, Liu and Habener 2008, Palsgaard 2012). In addition, it has been reported in recent studies that this pathway is involved in almost every field of liver function, from liver development to metabolism and regeneration (Gonzalez 2006, Thompsonet al 2007, Russell and Monga 2018).

Three Wnt signaling pathways have been identified, namely canonical pathway, Wnt/planar cell polarity (PCP) pathway, and Wnt/Ca2+ signaling pathway (Durak-Kozica et al 2019). The canonical pathway is highly conserved and plays a critical role in regulating cellular processes both during development and in adult tissue homeostasis (Wild 2020). This pathway is related to a protein called β-catenin and modulates β-catenin activity by regulating its levels and cellular localization (Elghazi et al 2012, Centelles 2019). When this Wnt/β-signaling pathway is inactive, which means the absence of Wnt, a component of this pathway, the monomeric concentration of β-catenin is controlled and phosphorylated by the β-catenin degradation complex [APC (adenomatous polyposis coli), adenomatous polyposis coli tumour suppressor (Axin2), Glycogen Synthase Kinase-3β (GSK3β), and Casein Kinase 1 (CK1)]. Phosphorylated β-catenin is recognized by the protein (β-Trcp) containing β-transducin repeats, which causes its degradation. Since β-catenin cannot be released in the cytoplasm, it will not enter the nucleus and thus transcription of target genes such as TCF/LEF will not ocur (Centelles 2019, Chen et al 2021). For example, TCF7L2 is a member of the TCF/LEF family and is involved in insulin synthesis and secretion and pancreatic β-cell proliferation. The expression product of this gene acts as a transcription factor. In recent studies, it has been reported that TCF7L2, as a risk gene for T2DM, affects β-cell function and is the closest susceptibility gene responsible for the pathogenesis of T2DM (Musso et al 2010, Schäfer et al 2011).

In this study, GSE prepared in different doses (100mg/kg, 200mg/kg, 400mg/kg) was administered to streptozotocin (STZ)-induced rats, and the possible curative effects on pancreatic β cell proliferation, survival and disease were investigated. The expression levels of the β-catenin gene, which is one of the important components of the Wnt pathway, were determined in liver and pancreas tissue. It was also aimed to investigate the effect of GSE treatment on the body weight and blood glucose levels of diabetic rats.

In this study, it was aimed to investigate the effect of GSE on body weight, blood glucose and β-catenin gene expression in pancreas and liver tissue in streptozotocin (STZ) induced rats. For this purpose, different doses of GSE (100mg/kg, 200mg/kg, 400mg/kg) were administered to Streptozotocin (STZ)-induced rats and its possible curative effects on pancreatic β-cell proliferation, survival and disease were revealed. It is anticipated that the data obtained from this study will form the basis for the functional uses of the GSE.

Material and Methods

The study protocol was approved by Necmettin Erbakan University Experimental Medicine Research and Application Center, Animal Experiment Ethics Committee (2013-005).

Preparation of grape seed extract

In this study, the seeds of the Red Globe type grape *(Vitis vinifera L.)* cultivated in Denizli Çal district were used (Figure 3.1). Grape grains, which constitute the plant material of the study, were separated from the bunch and their seeds were removed. It was then washed and dried on blotter paper at room temperature. The dried grape seeds were ground with the aid of a grinder, turned into powder and extracted by using the method of Downey et al (Downey et al 2007). The obtained crude extract was weighed in order to calculate the extraction efficiency. It was then lyophilized. Lyophilized GSE was dissolved at the concentration of 100 mg/kg, 200 mg/kg and 400 mg/kg in distilled water.

Animals

Twenty five Female Wistar-Albino rats, aged 8-12 weeks were allocated to metabolic cages individually in an automatic ambient humidity (50 ± 5%), temperature (22 ± 2 ◦ C), and light-dark (12:12) controlled room. Animals were obtained from Konya Necmettin Erbakan University Experimental Medicine Research and Application Center, Turkey. Each healthy group and other experimental groups had five rats. Commercially available rat normal pellet diet and water were given ad libitum to all animals prior to dietary manipulation.

Induction of diabetes

All type 2 diabetic groups had high fat diet which 58% of the metabolic energy is provided from animal fat. The composition of the high-fat diet formed 365 g/kg powdered normal pellet diet, 310 g/kg lard, 250 g/kg casein, 10 g/kg cholesterol, 60 g/kg vitamin and mineral mix, 03 g/kg dl-methionine, 1 g/kg yeast powder, 1g /kg sodium chloride. Nutritional substances of normal pellet diet were dry matter 89%, crude protein 21%, metabolic energy 2850 kcal/kg, crude fiber 5%, methionine and cystein 0.75%, calcium 1.0–2.0%, phosphor 0.5–1.0%, and sodium 0.5% (Optima Feeds, Turkey). After 2 weeks feeding of high fat diet, diabetes (for four groups) was induced in fasted animals by a single intraperitoneal injection of STZ (35 mg/kg bw) (Cat. No: S0130-5G) dissolved in citrate buffer (pH 4.5) (CAS No:18996-35-5) (Srinivasan 2005). Chemicals were obtained from Sigma (MO, USA). One week after STZ injection, non-fasting blood samples were taken from tail vein of all rats. Blood glucose were measured using autoanalyser (Biotecnica Instruments, BT3000 Plus, Italy). Rats with ≥ 300 mg/dl non-fasting blood glucose level were considered to be type 2 diabetic. The control group animals received only citrate buffer.

Experimental groups and treatment

Animals were randomly divided into five groups each of which included 5 rats. Body weight changes of each animal were noted weekly and the experiment lasted nine weeks in total. Animal groups are planed as 2 control groups and 3 treaatment groups and diet content are as follows; Control: healthy control rats fed on standard rat pellet diet, Diabetic Control: Type 2 diabetic rats fed with a high-fat diet and treated with STZ, 100 mg/kg GSE: Type 2 diabetic rats fed with a high-fat diet, administered with STZ and treated with 100 mg/kg/day GSE, 200 mg/kg GSE: Type 2 diabetic rats fed on a high-fat diet, administered with STZ and treated with 200 mg/kg/day GSE, 400 mg/kg GSE: Type 2 diabetic rats fed on a high fat diet, administered with STZ and treated with 400 mg/kg GSE. They received the aforementioned doses by gavage once daily for 28 days starting from the induction of diabetes until the end of the experiment. At the end of this period, blood was taken from their hearts and euthanized by cervical dislocation. Non-fasting blood glucose level was determined from the blood samples obtained using autoanalyser (Biotecnica Instruments, BT3000 Plus, Italy).

RNA extraction and real-time quantitative polymerase chain reaction analysis

Total RNA was isolated from liver and pancreas tissues (30 mg) with the GFTR-100 RNA Isolation Kit (Cat. No: GF-TR-100, Vivantis, Malaysia) as per manufacturer's instructions. The RNA was quantified using Nanodrop spectrophotometer ND-2000 (Thermo Scientific). In addition, the usability of RNAs was determined by running a 1% agarose gel and observing specific 18S and 28S bands. 25 ng RNA per sample was reverse transcribed using Vivantis-RTPL12 (Cat. No. RTPL12, Selangor Darul Ehsan, Malaysia) kit to create cDNA. The obtained cDNA was used for quantitative real-time PCR amplification of targeted genes in an ESCO Swift Spectrum 48 Real Time Thermal Cycler. Primer sets used for expression analysis of target genes at the mRNA level are specific for each transcription analysis, and reference articles were used for β-catenin (target gene) (Shuhong 2013) and β-actin (reference gene) (Marçal-Pessoa 2005). The determined primers were synthesized in the manufacturing company (Biomers).

Samples were amplified in a volume of 20 µl reaction mix, with a concentration of 10 pmol/µl of forward and reverse primers, 10 µl of Maxima SYBR Green qPCR Master Mix (2X) (Cat. No: K0251, Thermo Scientific), 1 µl cDNA, nuclease-free water to 20 µl. All PCR was performed using the following parameters. After initial denaturation for 10 min at 95°C, followed by 40 cycles of 95°C for 20 s, annealing at 60°C for 30 s, extension at 72°C for 30 s. Each sample was tested in triplicate, and results were normalized using amplification

of the same cDNAs with rat reference genes β-actin using 2-ΔΔCt calculations.

Statistical analysis

Results were statistically analyzed with IBM SPSS Statistics 22.0 (IBM Corp., Armonk, New York, USA). Arithmetic means and standard deviations (mean±SD) of all parameters were calculated. In order to determine the homogeneity of the data, the "Shapiro-Wilk" test was performed, and it was determined that the data showed normal distribution. One-way analysisof variance (ANOVA) test was used to determine the differences between the groups, and the Duncan test which is one of the post-hoc tests, was used to determine in which group the difference originated. The difference at the $P<0.05$ level was considered significant. For all statistical tests, a value of $p<0.05$ was considered statistically significant.

Results

The weights of the experimental animals were reported weekly during the experiment and the mean weight changes are summarized in Figure 1. Animals were fed a fatty diet for three weeks. STZ was then administered to the animals, and GSE treatment was started two weeks later. GSE was given by gavage for 28 days.

There was not much difference in the mean weight gain of the animals in the control group, but a significant increase was found in the weight of the other groups until the STZ administration. It was found that their weight decreased significantly in the week after STZ application. However, the weight loss of rats treated with GSE 400 mg/kg was slower than other treatment groups ($p<0.05$).

Blood glucose levels of both diabetic control group and treated diabetic groups (100 mg/kg GSE, 200 mg/kg GSE and 400 mg/kg GSE) were significantly higher than the control group animals (Figure 2). At the end of GSE treatment, it was found that the blood values of the animals that were administered 100 mg/kg GSE and 400 mg/kg GSE doses were also significantly below the blood values of the diabetic control group animals ($p<0.05$).

Changes in mRNA expression levels of the β-catenin gene in liver and pancreatic tissues induced by type 2 diabetes and by GSE treatment in this metabolic disorder were evaluated (Figure 3). In pancreatic tissue, the β-catenin mRNA expression in the STZ-induced diabetic rats was significantly upregulated compared with the control group ($p<0.05$). But, gene expression in pancreatic tissue showed no difference between diabetic groups. Gene expression level in liver tissue did not show statistically significant results ($p>0.05$) (Figure 3).

Discussion

TIn this study, the effects of different doses of GSE treatment on body weight, blood glucose level and β-catenin gene expression were investigated in STZ-induced diabetes-induced rats. After STZ injection, blood glucose levels of both the diabetic control group and treatment groups were higher than the control group animals. This has shown that high fat diet and low dose STZ administration are sufficient in establishing an animal model of type 2 diabetes, by looking at the increase in blood glucose levels.

The results obtained in this study show that a significant weight loss occurred in STZ-induced diabetic rats and that weight loss was maintained during GSE treatment. However, rats treated with 400 mg/kg GSE were found to have slower weight loss than the other treatment groups. It is known that weight loss is among the less common symptoms in diabetes. It has even been suggested that weight loss can be observed in addition to cardinal symptoms such as dry mouth, polydipsia, and polyuria (Dinççağ 2011). Akyol et al (2013) reported that a patient lost excessive weight due to type 2 diabetes in as little as one month. Weight loss which is rare finding of diabetes is explained by the inability to use glucose due to insulin deficiency or lack of insulin effect, and secondary increased glycogenolysis, lipolysis and gluconeogenesis, loss of muscle and fat tissue, and excessive glucose excretion in the urine in hyperglycemia, dehydration as a result of frequent urination. Therefore, in the present study, weight loss can be explained by the rare finding of diabetes.

GSE has attracted considerable clinical attention due to its high content of polyphenolic compounds such as resveratrol and has been used in many studies (Şahin et al 2007, İrak et al 2018). In addition to causing a decrease in blood glucose level by increasing insülin level and secretion, GSE has been shown to have antioxidative properties, protect cells, reduce cell damage, and control cell death (Sano et al 2007, Irina et al 2009, Rostamian et al 2011, El-Awdan et al 2013, Amin et al 2018, Söğütlü 2021). In addition to grape seed, grape leaf extract, which is known to show antidiabetic and antioxidant activity, has also been reported to reduce blood glucose levels (Şendoğdu et al 2006). El-Awdan et al (2013) reported that GSE lowered blood sugar in a dose-dependent manner in their study on rats that became diabetic with STZ. They reported that the reason for this is likely to be that GSE lowers serum glucose levels due to both its effect on insulin secretion and its insulin sensitizing effect. Our findings in accordance with these studies showed that GSE effectively lowered a dose-dependent blood sugar levels.

Many signaling pathways associated with T2DM and other metabolic disorders have been identified. Recent studies have shown that the components of the Wnt signaling

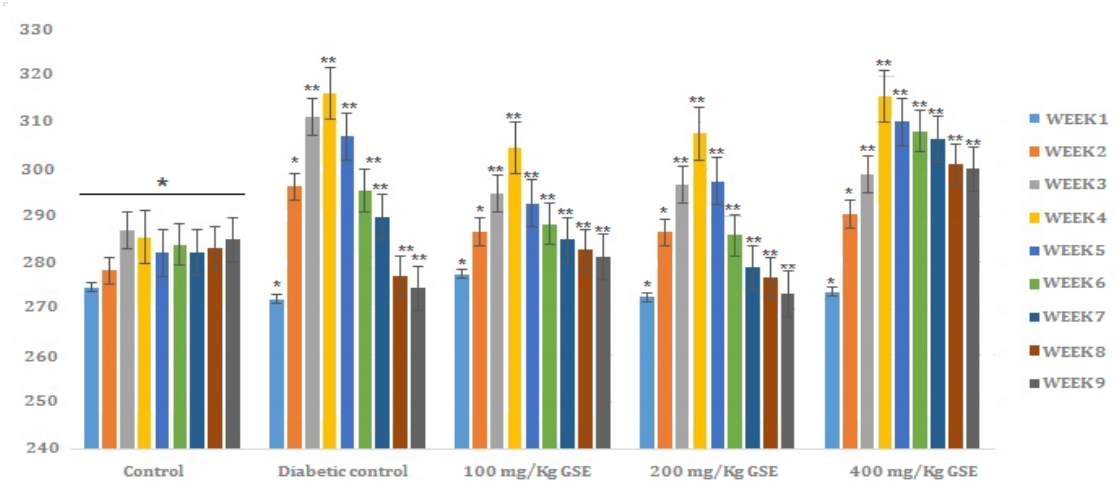

Figure 1. Mean weight change of experimental animals measured in five groups. (* p> 0.05 compared to all other groups; ** p <0.05 compared to all other groups.)

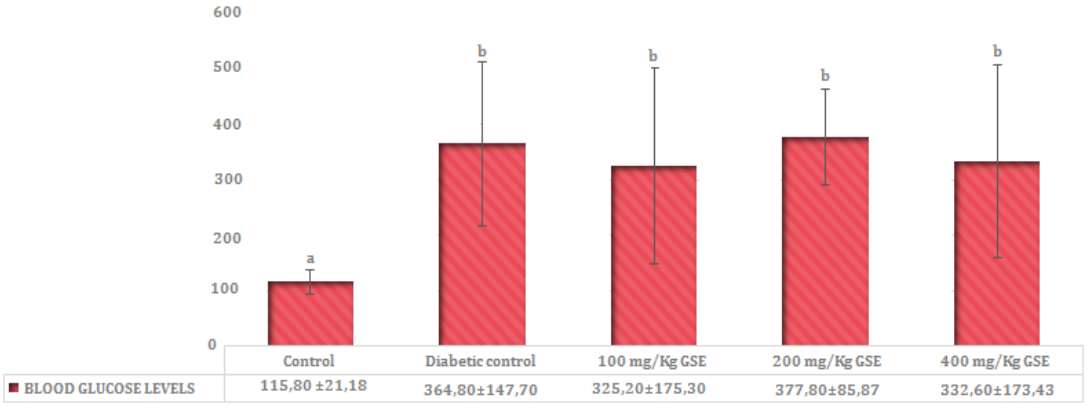

Figure 2. Mean blood glucose and standard deviation graph of experimental animals in five groups (Different letters indicate statistical differences among the groups; p<0.05).

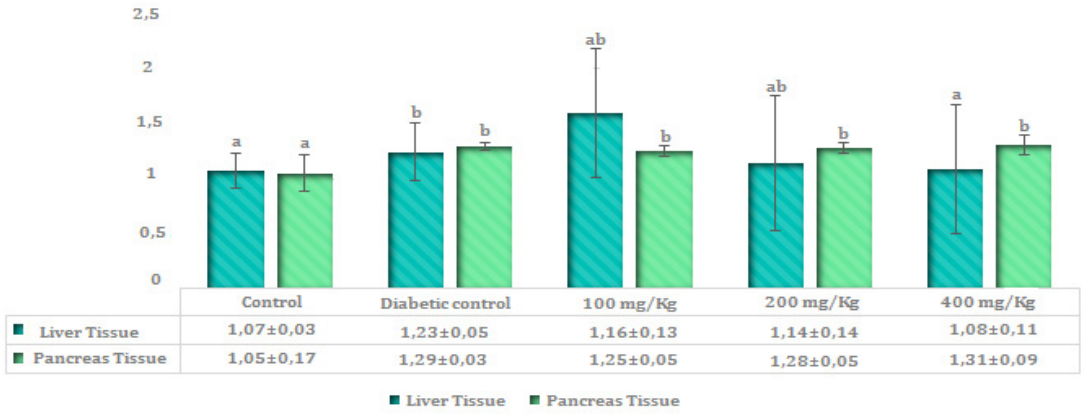

Figure 3. Effects of GSE treatment on the β-catenin gene expression in liver and pancreatic tissues (Different letters indicate statistical differences among the groups, liver tissues; p>0.05 and pancreatic tissues; p<0.05).

pathway are associated with T2DM and other metabolic diseases, and that this pathway plays a critical role in maintaining normal metabolism (Chen et al 2021).

The Wnt/β-catenin signaling pathway plays an important role in pancreatic β-cell proliferation, lipid metabolism and glucose-induced insulin secretion (Ng et al 2019). Researchers have confirmed that high glucose levels activate the Wnt/β-catenin signaling pathway (García-Jiménez et al 2013). However, it is still controversial whether the activation of this pathway occurs as a result of an injury or as a protective response to injury (Liu et al 2015). Phosphorylation of GSK-3β, the key enzyme of the Wnt/β-catenin signaling pathway, is mediated by insulin-activated phosphatidylinositol 3 kinase. As a result, GSK-3β is inhibited and β-catenin cannot be phosphorylated and enters the nucleus (Chong et al 2007, Yi et al 2008). Therefore, the importance of these proteins in high glucose levels is quite large. In addition, it was found that the pancreatic Wnt signaling pathway regulated β-cell proliferation and it was confirmed that Wnt signaling pathway played a role in the regulation of pancreatic growth and differentiation by comparing the data with other studies (Fujino et al 2013). Another group of researchers investigated the expression of molecules involved in the Wnt/β-catenin signaling pathway by administering STZ to pancreatic cells using an immunohistochemical method. The results show that the-catenin gene regulates the gene expression of molecules in this pathway, is involved in this regulation as a core gene, and is overexpressed to increase the regeneration of damaged pancreatic islet cells (Yang et al 2011). Supporting these findings, this study indicates that the expression of β-catenin gene increased in pancreatic tissue when compared to the control group. The liver plays a central role in the control of energy homeostasis, as it contributes to the maintenance of glycemia in changing nutritional conditions through regulation of glucose and lipid metabolism (Saltiel and Kahn 20015, Riu 2014). In recent years, the role of the Wnt/B-catenin signaling pathway (Wnt/β-signaling pathway) in liver biology has come to the fore. In particular, studies have been carried out to explain this signaling pathway in basic physiological processes such as liver development, growth, regeneration, metabolism and oxidative stress (Thompson et al 2007, Rusell and Monga 2018). It is also thought to be an important player during liver homeostasis and repair, with recent studies supporting its role in proliferation of the liver parenchyma (Wild et al 2020). This is a study showing the expression of the β-catenin gene, a member of the Wnt/β-catenin signaling pathway involved in the regulation of glucose metabolism, in the liver and pancreatic tissues of type 2 diabetic rats. β-catenin gene expression levels in liver tissue were not statistically significant ($p>0.05$). In a study, it was reported that β-catenin is necessary but not sufficient for the development of diet-induced fatty liver (Behari et al 2014). On the other hand, Boj et al. (2012) reported that TCF7L2, an important component of the Wnt pathway, plays a key role in regulating the metabolic activity of hepatocytes. When evaluated together with these studies presented above, the evaluation of β-catenin gene expression in rats fed a high-fat diet with diabetes was not significant in our study. Because not only the β-catenin gene expression, but also the determination of the expression levels of genes such as TCF7L2, which are involved in this pathway, may contribute to the formation of more meaningful results.

In a study investigating the effect of 8 weeks of high-intensity exercise and Black Grape Seed Extract supplementation on Wnt and B-catenin gene expression in pancreatic tissue in male rats with type 2 diabetes, it was found that exercise significantly increased the mean expression of Wnt and β-catenin genes, but exercise with the extract in the pancreas of diabetic rats. It has been reported that there is no significant effect on the expression of Wnt genes (Fatemeh et al 2020). Consistent with this study, there was no difference between β-catenin levels in pancreatic tissue of all groups with T2DM after GSE application.

The gene expression studies of β-catenin were mostly performed in the embryonic development of diabetic rats, gastroenteric tumors such as colon cancer, diabetes, diabetic cutaneous ulcers, and regeneration of epidermal stem cells in wound healing (Wang et al 2006, Zhong et al 2011, Zhang et al 2018). Significant reduction of epidermal stem cells and β-catenin protein expressions in diabetic rats proves that T2DM causes cell damage.

Conclusion

The treatment of T2DM, which is the metabolic disease, has had a significant impact worldwide with the improvement of living standards. Many scientists have conducted studies to explore the pathogenesis of this metabolic syndrome. It is clear that the Wnt signaling pathway affects pancreatic beta cell function by regulating insulin secretion. The Wnt/β-catenin signaling pathway can be activated by a network of protein interactions at high glucose levels. However, the relationship between the pathway and T2DM is still uncertain and many future questions need to be answered in uncovering the complex protein interaction network. Studies to identify proteins involved in the active Wnt/β-catenin signaling pathway at high glucose levels untilnow have been relatively concentrated in in vitro animal experiments. These experimental methods have been used to regulate gene expressions and determine mRNA expressions by increasing or decreasing proteins involved in this pathway. It shows that the GSE used in this study may be likely to regulate the expression of the β-catenin gene (especially in improving and maintaining the function of pancreatic beta cells) through oral consumption.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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References

Akyol L, Çelikbilek A, Çelikbilek M, Sarıkaya S, et al., 2013. A Case of type-2 diabetes mellitus presenting with excessive weight loss. Bozok Med J, 3(3): 59-61.

Amin KA, Ahmed RR, Hozayen WG, Mahmoud SS et al., 2018. Renoprotective effect of grape seed extract in diabetic nephropathy by attenuating hyperglycemia-facilitated oxidative stress. Int J Clin Exp Med, 11(11): 12285-12293.

Ayaz M, Baba F, Akgun N, Bas AL, et al., 2015. Protective effect of distillated Nerium oleander on heart of type 2 diabetic rats. Bratisl Med J, 116 (7): 451-456.

Behari J, Li H, Liu S, Stefanovic-Racic M, et al., 2014. β-catenin links hepatic metabolic zonation with lipid metabolism and diet-ınduced obesity in mice. Am J Clin Pathol, 184 (12): 3284-3298.

Boj S-F, van Es J-H, Huch M, Li V-S-W, et al., 2012. Diabetes Risk Gene and Wnt Effector Tcf7l2/TCF4 Controls Hepatic Response to Perinatal and Adult Metabolic Demand. Cell, 151(7): 1595-1607.

Centelles JJ, 2019. Wnt Pathways are related to type 2 diabetes. Pharmacol Toxicol, 2(1): 117.

Chen J, Ning C, Mu J, Li D, Ma Y, Meng X, 2021. Role of Wnt signaling pathways in type 2 diabetes mellitus. Mol Cell Biochem, 1-14.

Chong ZZ, Shang YC, Maiese K, 2007. Vascular injury during elevated glucose can be mitigated by erythropoietin and Wnt signaling. Curr Neurovasc Res, 4(3): 194-204.

DeFronzo RA, Ferrannini E, Groop L, Henry RR, et al., 2015. Type 2 diabetes mellitus. Nat Rev Dis Primers, 1: 15019.

Dinççağ N, 2011. Up to date diagnosis and treatment of diabetes mellitus. İç Hastalıkları Dergisi, 18: 181-223.

Dominguez LJ, Muratore M, Quarta E, Zagone G, Barbagallo M, 2004. Osteoporosis and diabetes. Reumatismo, 56: 235–241.

Downey MO, Mazza M, Krstic MP, 2007. Development of a stable extract for anthocyanins and flavonols from grape skin. Am J Enol Vitic, 58 (3): 358-364.

Durak-Kozica M, Paszek E, Stępień El, 2019. Role of the Wnt signalling pathway in the development of endothelial disorders in response to hyperglycaemia. Expert Rev Mol Med, 21.

El-Awdan SA, Jaleel GAA, Saleh DO, 2013. Grape seed extract attenuates hyperglycaemia-induced in rats by streptozotocin. Bull Fac Pharm, 51(2): 203-20.

Elghazi L, Gould AP, Weiss AJ et al., 2012. Importance of β-catenin in glucose and energy homeostasis. Scientific reports, 2(1): 1-12.

Fatemeh S-M, Mahsa M, Foad F, 2020. The effect of 8 weeks of HIIT training and supplementation of black grape seed extract on wnt and β-catenin gene expression in pancreatic tissue in male rats wıth type 2 diabetes. J Diabetes Metab Disord, 19(2): 61-70.

Fujino T, Asaba H, Kang MJ et al., 2003. Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion. Proc Natl Acad Sci, 100: 229-234.

Gabetta B, Fuzzati N, Griffini A, Lolla E, et al., 2000. Characterization of proanthocyanidins from grape seeds. Fitoterapia, 712: 162-175.

Ganjali Z, Javadian F, Estakhr J, Heidari A,2012. The effect of hydro-alcoholic extract of grape seed (Vitis vinifera) on sugar and lipids in serum of diabetic rats. J Anim Vet Adv, 6(10): 547-549.

García-Jiménez C, García-Martínez JM, Chocarro-Calvo A, De la Vieja A, 2013. A new link between diabetes and cancer: enhanced WNT/β-catenin signaling by high glucose. J Mol Endocrinol, 52(1): R51-66.

Gonzalez FJ, 2006. Role of beta-catenin in the adult liver. Hepatology, 43(4): 650-653.

Gupta R, Sharma SB, 2012. Effect of germinated glycine max seeds on glycemic control in stz+ nad induced type 2 diabetic models: a preliminary study. J Exp Integr Med, 22: 155-160.

Irina CC, Ungureanu MI, Marton A, Simedrea R, et al., 2009. Antioxidant effects of a grape seed extract in a rat model of diabetes mellitus. Diab Vasc Dis Res 6 (3): 200-204.

İrak K, Mert N, Mert H, Ayşin N, 2018. The effects of grape seed extract on the some enzymes and metabolites in diabetic rats. Van Vet J, 29 (3): 147-152.

Janghorbani M, Van dam RM, Willett WC, Hu FB, 2007. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol, 166: 495–505.

Jin T, 2008. The WNT signalling pathway and diabetes mellitus. Diabetologia ,51(10): 1771-1780.

Khan MAB, Hashim MJ, King JK, Govender RD, et al., 2020. Epidemiology of type 2 diabetes-global burden of disease and forecasted trends. J Epidemiol Glob Health, 1(1):107-111.

Khushk I, Dahot M, Baloach S, Bhutto M 2010. The evaluation of soybean extracts in alloxan-induced diabetic rabbits.

World Appl Sci J 8: 22-25.

Liu W, Liang X, Yang D,2015. The effects of chinese medicine on activation of wnt/-catenin signal pathway under high glucose condition. Evid Based Complement Alternat Med, 2015: 295135.

Liu Z, Habener JF,2008. Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta cell proliferation. J Biol Chem, 283: 8723-8735.

Marçal-Pessoa AF, Bassi-Branco CL, Salvatierra CdSB et al., 2015. A low-protein diet during pregnancy prevents modifications in intercellular communication proteins in rat islets, Biol Res, 48(1): 1.

Musso G, Gambino R, Pacini G, Pagano G, et al., 2010. Transcription factor 7-like 2 polymorphism modulates glucose and lipid homeostasis, adipokine profile, and hepatocyte apoptosis in NASH. Hepatology, 492: 426-435.

Nakamura Y, Tsuji S, Tonogai Y, 2003. Analysis of proanthocyanidins in grape seed extracts health foods and grape seed oils. J Health Sci, 49 (1): 45-54.

Ng LF, Kaur P, Bunnag N, Suresh J, et al., 2019. WNT signaling in disease. Cells, 8(8): 826.

Palsgaard J, Emanuelli B, Winnay JN, Sumara G, et al., 2012. Cross-talk between insulin and Wnt signaling in preadipocytes: role of wnt co-receptor low density lipoprotein receptor-related protein-5 (LRP5). J Biol Chem, 287: 12016-12026.

Pandey KB, Rizvi SI, 2014. Role of red grape polyphenols as antidiabetic agents. Integr Med Res, 3(3): 119-125.

Qian C, Zhu C, Yu W, Jiang X, Zhang F, 2015.High-fat diet/low-dose streptozotocin-ınduced type 2 diabetes in rats ımpacts osteogenesis and wnt signaling in bone marrow stromal cells. PLoS ONE, 10(8): e0136390.

Riu L, 2014. Energy metabolism in the liver. Compr Physiol, 4: 177-197.

Rostamian V, Shakeri F, Estakhr J, 2011. The effect of hydro-alcoholic extract of grape seed (Vitis vinifera) on sugar and lipids in serum of diabetic rats. Res J Biol Sci, 6(10): 547-549.

Rulifson IC, Karnik SK, Heiser PW, Ten Berge D, et al.,2007. Wnt signaling regulates pancreatic β cell proliferation. Proc Natl Acad Sci, 104(15): 6247-6252.

Russell JO, Monga SP, 2018. Wnt/β-catenin signaling in liver development, homeostasis, and pathobiology. Annu Rev Pathol,13: 351-378.

Saltiel AR, Kahn CR, 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature, 414(6865): 799-806.

Sano A, Uchida R, Saito M, Shioya N, et al., 2007. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. J Nutr Sci Vitaminol, 532: 174-182.

Schäfer SA, Machicao F, Fritsche A, Häring H-U, et al., 2011. New type 2 diabetes risk genes provide new insights in insulin secretion mechanisms. Diabetes Res Clin Pract, 93: 9-24.

Shuhong Z, Hongjun L, Bo C, Li X, Bingyin S, 2013. The effect of dantonic pill on β-catenin expression in a rat model of streptozotocin-ınduced early stage of diabetic nephropathy. J Diabetes Res, 2013: 848679.

Söğütlü İ, Mert N, Mert H, Mis L, et al., 2021. The effects of grape seed extract on ınsulin, adiponectin and resistin levels in diabetic rats. TURJAF, 9(4): 709-713.

Srinivasan K, Viswanad B, Asrat L, Kaul C, et al., 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res, 52 (4): 313-320.

Şahin F, Avcı ÇB, Avcu F et al., 2007. Red grape seed extract and its compound resveratrol exert cytotoxic effect to various human cancer lines. Turk J Hematol, 24: 102-109.

Şendoğdu N, Aslan M, Deliorman OD, Ergun F, et al., 2006. Antidiabetic and antioxidant effects of Vitis vinifera L. leaves in streptozotocin-diabetic rats. Turkish J Pharm Sci, 3(1): 7-18.

Thompson MD, Monga Satdarshan PS, 2007. WNT/β-catenin signaling in liver health and disease. Hepatology, 45(5): 1298-1305.

Wang Q-M, Zhang Y, Yang K-M, Zhou H-Y, et al., 2006. Wnt/beta-catenin signaling pathway is active in pancreatic development of rat embryo. World J Gastroenterol, 12(16): 2615-2619.

Wild SL, Elghaji A, Grimaldos Rodriguez C, Weston SD et al., 2020. The canonical wnt pathway as a key regulator in liver development, differentiation and homeostatic renewal. Genes, 11(10): 1163.

Yang K, Hu X, Yang H, Li A, et al., 2011. The changes of Wnt/β-catenin pathway in diabetic rat pancrease and its role in regulating the differentiation and proliferation of pancreatic stem cells. Chin J Diabetes Mellit, 19 (2): 149-151.

Yao H, Ashihara E, Maekawa T, 2011. Targeting the Wnt/β-catenin signaling pathway in human cancers. Expert Opin Ther Targets, 15(7): 873-887.

Yılmaz S, 2003. Comparison of some kinetic properties of pyruvate kinase in liver tissue of normal and diabetic rats. Turkish J Vet Anim Sci 27(4): 991-997.

Yi F, Sun J, Lim G, Fantus I, Brubaker P, et al., 2008. Cross talk between the insulin and wnt signaling pathways: evidence from intestinal endocrine L cells. Endocrinology, 149(5): 2341-2351.

Zhang H, Nie X, Shi X, Zhao J, et al., 2018. Regulatory Mechanisms of the Wnt/β-Catenin Pathway in Diabetic Cutaneous Ulcers. Front Pharmacol, 9:(1)114.

Zhong Q-L, Liu F-R, Liu D-W, Peng Y, et al., 2011. Expression of β-catenin and cyclin D1 in epidermal stem cells of diabetic rats. Mol Med Rep, 4 (2): 377-381.

Author Contributions

Motivation / Concept: EA, HA; Design: EA, HA, EGM; Control/ Supervision: EA, HA; Data Collection and / or Processing: EGM; Analysis and / or Interpretation: EGM; Literature

Review: EGM; Writing the Article: EGM, Critical Review: EGM, EA.

Ethical Approval

Research meets ethical guidelines is a required field. The study protocol was approved by Konya Necmettin Erbakan University Experimental Medicine Research and Application Center Laboratory Animals Ethics Board (No: 2013-005).