Experimed 2021; 11(1): 27-32

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

DOI: 10.26650/experimed.2021.879204

Protective Effect of Chard Extract on Glycoprotein Compounds and Advanced Oxidation Protein Product Levels in Diabetic Rat Livers

Diyabetik Sıçan Karaciğerinde Pazı Ekstresinin Glikoprotein Bileşikleri ve İleri Oksidasyon Protein Ürün Seviyeleri Üzerindeki Koruyucu Etkisi

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Cite this article as: Ertik O, Sacan O, Kabasakal L, Sener G, Yanardag R. Protective effect of chard extract on glycoprotein compounds and advanced oxidation protein product levels in diabetic rat livers. Experimed 2021; 11(1): 27-32.

ABSTRACT

Objective: Diabetes mellitus is a metabolic disease of global importance. It affects many people by reducing their quality of life and even causing death. Therefore, research on diabetes mellitus maintains its popularity and continues to develop. Chard (*Beta vulgaris* L. var. cicla) is commonly used in diets and is known to have alternative hypoglycemic effects in diabetic individuals. This study investigated the protective effects of chard on glycoproteins (hexose, hexosamine, fucose and sialic acid) and the advanced oxidation protein product levels in diabetic rats' livers.

Materials and Methods: For this experiment, male Sprague–Dawley rats were separated into three groups: the control; Streptozotocin (STZ)-induced diabetic rats; and STZ-induced diabetic + chard extract. Fourteen days after diabetes induction, chard extract (2 g/kg/day, gavage) was administered for 45 days. On day 60, liver samples were collected, and 10% (w/v) homogenate was prepared for the analysis of glycoprotein components and advanced oxidation protein products.

Results: Levels of glycoprotein parameters that include hexose, hexosamine, fucose, and sialic acid, as well as advanced oxidation protein product levels, increased in the diabetic group. Chard extract administration curatively reverted the altered biochemical parameters.

Conclusion: From the results obtained, it can be suggested that chard extract has a protective effect on the diabetic livers of rats.

Keywords: Diabetes, chard, liver, glycoprotein

ÖZ

Amaç: Diyabet, yaşam kalitesini düşüren ve hatta ölüme neden olan, tüm dünyada birçok insanı etkileyen metabolik bir hastalıktır. Bu yüzden diyabet üzerine yapılan araştırmalar popülerliğini korumakta ve gelişimini devam ettirmektedir. Pazı (*Beta vulgaris* L. var. cicla) Türkiye'de şeker hastalarının alternatif hipoglisemik ajan olarak kullandığı bir bitkidir. Bu çalışmada pazı ekstraktının, diyabetik sıçanların karaciğer dokusunda bulunan glikoproteinler (heksoz, heksozamin, fukoz ve siyalik asit) ve ileri oksidasyon protein ürün seviyeleri üzerindeki koruyucu etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Deneyde kullanılan erkek Sprague-Dawley sıçanları 3 gruba ayrıldı. Kontrol sıçanları; Streptozotosin (STZ) ile diyabetik yapılan sıçanlar; pazı ekstresi verilen STZ diyabetik sıçanlar; Sıçanların diyabet olmasından on dört gün sonra, sıçanlara pazı ekstresi (2 g/kg/gün, gavaj ile) belirtilen dozda 45 gün uygulandı. 60. günde sıçan karaciğerleri çıkarıldı ve glikoproteinler ve ileri oksidasyon protein ürünlerinin analizi için %10 (w/v) karaciğer homojenizatları hazırlandı.

Bulgular: Yapılan deneyler sonucunda diyabetik sıçanların glikoprotein parametreleri olan heksoz, heksozamin, fukoz, siyalik asit ve ileri oksidasyon protein ürün seviyelerinin arttığı bulunmuştur. Pazı ekstresinin uygulaması, incelenen biyokimyasal parametre değerlerinin tersine çevirdiği belirlenmiştir.

Sonuç: Elde edilen sonuçlardan pazı ektresinin diyabetik sıçanlar üzerinde koruyucu bir etkiye sahip olduğu ileri sürülebilir.

Anahtar Kelimeler: Şeker hastalığı, pazı, karaciğer, glikoprotein

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Submitted/Başvuru: 12.02.2021 **Revision Requested/Revizyon Talebi:** 23.02.2021
Last Revision Received/Son Revizyon: 08.03.2021 **Accepted/Kabul:** 08.03.2021

INTRODUCTION

Diabetes mellitus is a serious disease of worldwide importance. It is caused by genetic factors, carbohydrate-heavy diets and lifestyles, and/or obesity. In the USA, it is projected that diabetes mellitus will be increased by 54% between 2015 and 2030, and its resultant death rate will increase by 38% annually (1). These statistics show that diabetes mellitus will increase year by year. For that reason, diabetes and the need for new treatment methods maintain their popularity in terms of research.

In diabetes mellitus, patients are not able to synthesis or use insulin sufficiently; therefore, blood glucose levels remain high (2). Glycoproteins are carbohydrate-linked proteins found on the cell surface. These compounds have important biological roles and are ubiquitous components of hormones, enzymes, membranes, and antibodies. The increasing interest in the biological importance of glycoproteins is indirectly or directly related to some diseases, including diabetes mellitus (3,4). There are fucose, sialic acid, hexose, and hexosamine in glycoproteins and glycosaminoglycans. They serve as biomarkers for some biological events such as secretion and absorption of macromolecules and cell differentiation, as well as recognition and membrane transport (5). Evidence has shown that people with diabetes have altered concentrations of glycoproteins when compared to non-diabetics. Some studies suggest that tissues like those in the liver and kidneys have elevated levels of glycoproteins due to insulin deficiency and high levels of blood glucose (6).

Diabetes mellitus can give rise to oxidative stress and carbonyl stress, thus leading to protein oxidations. Protein oxidation occurs under the influence of chlorinated oxidants, such as hypochlorous acid and chloramines. Among the important markers of protein oxidation, advanced oxidation protein products (AOPP) occur. Accumulation of AOPP in tissues has an important role in long-term diabetes; it is an important marker for determining diabetic damage (7).

Medicinal plants are an important aspect of alternative medicine; their continued popularity and use are linked to their valuable therapeutic functions and compositions/agents. They are valuable alternatives in the treatment of diabetes (8). Chard (*Beta vulgaris* L. var cicla, chenopodiaceae family) is one of the vegetables grown widely in Turkey, North India, South America, Mediterranean countries, and the USA. Some studies have shown that chard extracts exhibit diverse biological effects, including anticancer and anti-inflammatory (9), antimicrobial (10), hepatoprotective (11), antioxidant and anti-acetylcholinesterase (12), and anti-diabetic (13,14) activities. These biological activities are closely associated with the phytochemical composition/content of chard. *B. vulgaris* is reported to contain some saponins, flavonoid glycosides (15), flavonoids, vitamin C, vitamin E, carotenoids and minerals (16), folic acid, phospholipid, glycolipid, and some fatty acids (11).

This study investigated the effects of chard on glycoproteins and the advanced oxidation protein product levels in diabetic rats' livers.

MATERIALS AND METHODS

Preparation of Chard Extract

Chard leaves were purchased from a local market in Istanbul, Turkey. The chard plant was inspected by Prof. Dr. Neriman Ozhatay (Faculty of Pharmacy, Istanbul University). For the extraction of chard leaves, 100 g of dry chard leaves were weighed and boiled in 1 liter of distilled water for 8 hours. The water in the extract was then removed using an evaporator under reduced pressure.

Experimental Animal Design

The male Sprague-Dawley rats of 6-7 months old, weighing 380-420 g, were chosen to be used in this experiment. Ethical approval was obtained from Marmara University Animal Care and Use Committee (No: 68.2008.mar). The animals were grouped as follows:

Control rats (C) given citrate buffer (n= 8).

STZ-induced diabetic rats (D), (n= 8).

STZ-induced diabetic rats given chard extract (D+Chard), (n= 8).

The rats were made diabetic by intraperitoneal administration of 60 mg/kg STZ dissolved in 0.01 M citrate buffer (pH=4.5). A dose of chard extract (2 g/kg/day, gavage) was administered to the rats for 45 days, 14 days after diabetes. Liver samples were taken on the 60th day.

Biochemical Assays

The blood glucose levels of the rats were determined by an automatic glucose analyser 18 hours after STZ administration. The data/results have been previously published (14). Rats with fasting blood glucose greater than 200 mg/dl were considered to be diabetic. The liver tissues taken were homogenized (10% (w/v)) in ice-cold saline. From the liver tissue homogenates, hexose, hexosamine, and fucose levels were quantified by the methods of Winzler (17) and Dische and Shettles (18). For the sialic acid levels, the method of Lorentz et al. was used (19). Liver AOPP contents were assayed by the method of Witko-Sarsat et al. (20). The total protein level was determined according to the Lowry method (21).

Statistical Analysis

The GraphPad Prism 6.0 program (GraphPad Software, San Diego, California, USA) was used for statistical analysis. All data were expressed as means±standard deviation (SD). The results were calculated using t-test and analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. *P*-values below 0.05 were considered statistically significant.

RESULTS

In experimentally induced diabetes, it was statistically evaluated whether the damage caused by diabetes was lessened or not by the application of chard. The significance between groups was compared using the one-way ANOVA test, and it was decided that it was significant when the p-value was equal

to or below 0.05. The lower the values specified, the higher the significance of the changes in the tissue would be.

Liver tissue glycoprotein levels are presented in figures 1-4. The levels of hexose ($p \leq 0.0001$), hexosamine ($p \leq 0.01$), fucose ($p \leq 0.05$), and sialic acid ($p \leq 0.001$) were increased significantly in the diabetic groups. The administration of chard extract to the diabetic group reversed these changes in the liver ($p \leq 0.0001$, $p \leq 0.001$, $p \leq 0.001$ and $p \leq 0.01$, respectively).

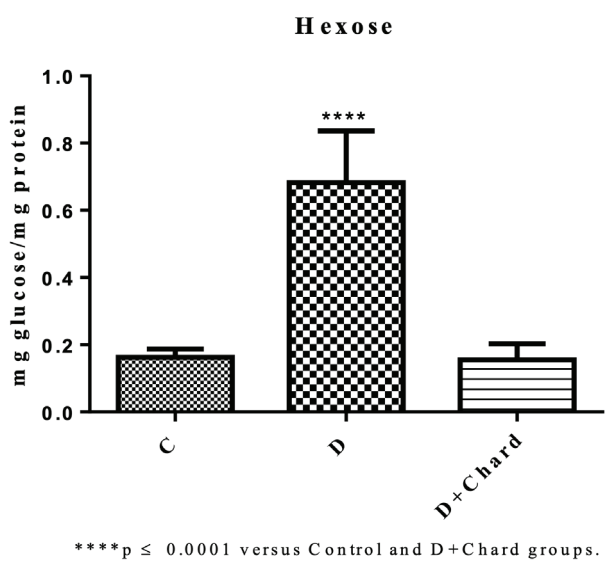

Figure 1. Liver tissue hexose levels of all groups. C (Control rats); D (STZ-induced diabetic rats); D+Chard (STZ-induced diabetic rats given chard extract).

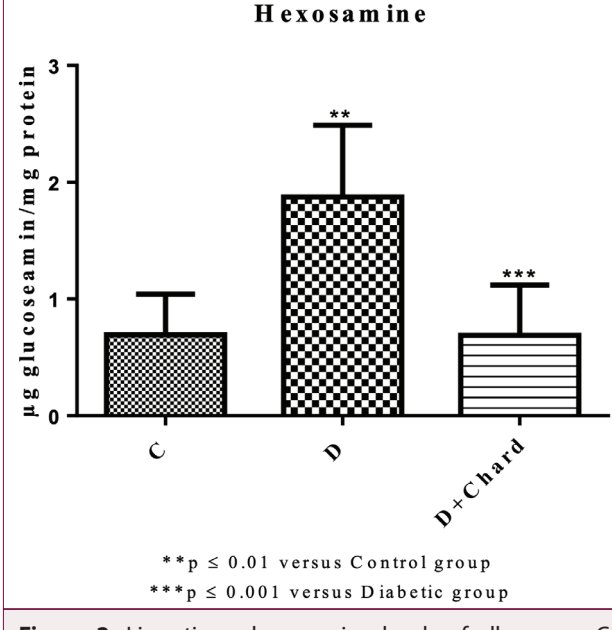

Figure 2. Liver tissue hexosamine levels of all groups. C (Control rats); D (STZ-induced diabetic rats); D+Chard (STZ-induced diabetic rats given chard extract).

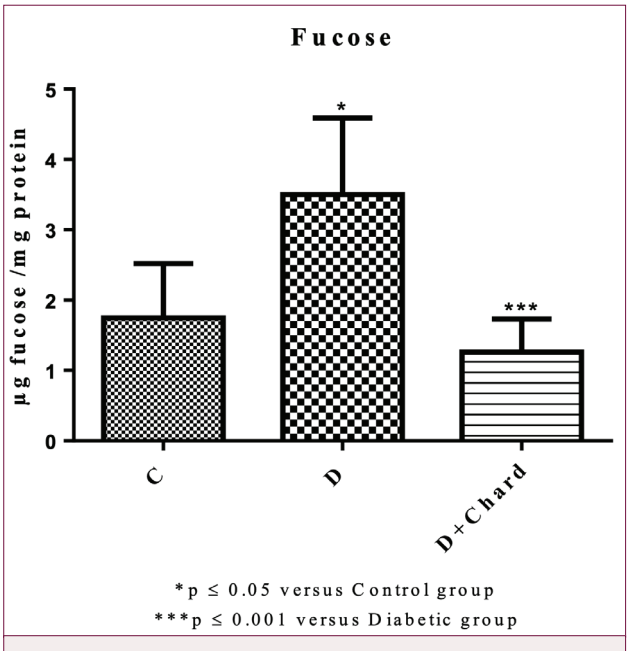

Figure 3. Liver tissue fucose levels of all groups. C (Control rats); D (STZ-induced diabetic rats); D+Chard (STZ-induced diabetic rats given chard extract).

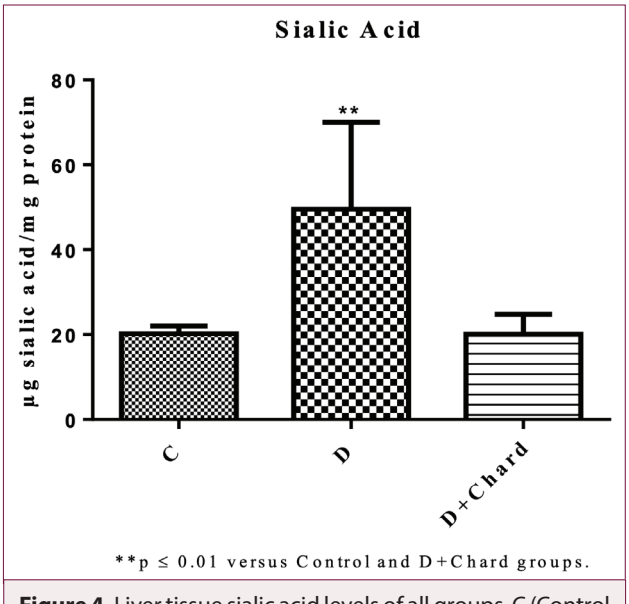

Figure 4. Liver tissue sialic acid levels of all groups. C (Control rats); D (STZ-induced diabetic rats); D+Chard (STZ-induced diabetic rats given chard extract).

The AOPP levels in the liver tissue are presented in Figure 5. In the diabetic group, the level of AOPP was increased ($p \leq 0.001$). The administration of chard reversed AOPP level in the diabetic group ($p \leq 0.001$).

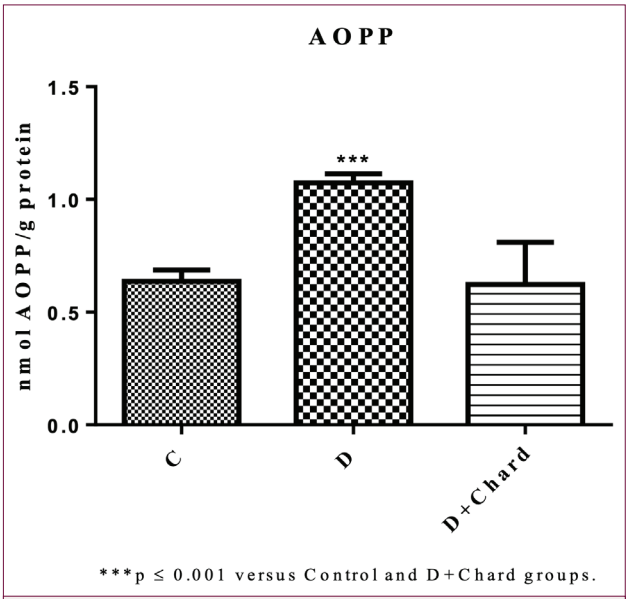

Figure 5. Liver tissue AOPP levels of all groups. C (Control rats); D (STZ-induced diabetic rats); D+Chard (STZ-induced diabetic rats given chard extract).

DISCUSSION

Diabetes mellitus is a chronic disease that can affect many tissues, such as those in the liver, kidney, brain, skin, and lung. Therefore, experimental diabetes models are very important for the understanding and treatment of diabetes. The STZ-induced diabetes model is very similar to human diabetes and is, therefore, often the preferred diabetes model (22).

Throughout history, people have used herbs for medicinal purposes. These medicinal plants have been used to control diabetes and other diseases due to their perceived pharmacological effect. These herbs have low toxicity and have less side effects compared to several conventional drugs. Changes in insulin levels affect glucose and glycogen metabolism in the liver. Therefore, the liver can be an important target tissue in diabetes, and protecting it from the harmful effects of diabetes is paramount (23). The glycoprotein components of the liver are assayed because of their biological importance in diabetes (24). Thus, hexose, hexosamine, fucose, and sialic acid levels were determined in the present study.

Hexosamines are amino sugars in which an amino group replaces a hydroxyl group, the first step of the synthesis of blood and tissues glycoprotein. The level of hexosamines is very important for understanding the harmful effects of diabetes. Several studies have shown that hexose and hexosamine levels are increased in the plasma and tissues of diabetic rats. In our previous study, we reported that the hexose and hexosamines levels in the lungs of diabetic rats were elevated. Treatment with chard extract was seen to reduce hexose and hexosamine levels when compared to the diabetic group (25). In our present study, the chard treatment of diabetic rats significantly decreased liver hexose and hexosamines levels to near-normal levels. This is likely due to the antihyperglycemic effects of chard leaves, which possibly increased insulin secretion.

Fucose is an essential sugar responsible for cell-to-cell communication. Its metabolism is a marker for several diseases, including diabetes mellitus. Haptoglobin, alpha-1-acid glycoprotein, and alpha-1-antitrypsin, which are proteins, are synthesized by the liver and have a potential role in increasing fucose level. The metabolism and synthesis of these proteins may be disrupted in diabetic conditions, thereby causing an increase in the level of fucose. Owing to an increased glucose level and subsequently higher glycation in diabetic states, fucose level might be increased (24). A significant increase in liver fucose levels of diabetic rats was observed in the present study. Chard extract reversed this abnormality (the level of fucose).

Sialic acid is derived from neuraminic acid and can be found in glycoproteins structures. It plays a role in intracellular signalling, protease resistance, cell-cell recognition, conformational stabilization, and protein targeting (26). The sialic acid levels of serum and tissues are seen as a marker in diabetes (28), in which, the amount of sialic acid increases. This increase in insulin-independent tissues may be due to an increase in sialic acid synthesis and enzyme activity of sialyltransferase. In the present study, chard extract treatment decreased the sialic acid levels of diabetic rats.

AOPP are cross-linked proteins containing dityrosine and are safe markers for assessing the oxidative modification of proteins (29). They usually are produced during oxidative stress or by myeloperoxidase in activated neutrophils through the interaction of hypochlorous acid and chloramines. The accumulation of AOPP in tissues plays an important role in the long-term complications of diabetes. Gradinaru et al. stated that AOPP levels are elevated in diabetic patients (30). An increased production of AOPP in the livers of diabetic rats was observed in the present study. Treatment with chard extract significantly reduced the AOPP levels in diabetic rats. This result indicates that chard may be effective in preventing oxidative protein damage by reducing oxidative stress through its flavonoids contents.

The outcome of the present study showed that chard has a beneficial role in diabetic rats' livers by decreasing the levels of glycoprotein metabolites and AOPP as well. The results also demonstrated that consumption of chard for 45 days may stabilise the levels of glycoproteins and AOPP. Therefore, chard can be a potentially effective vegetable for controlling and deescalating the complications of diabetes.

Ethics Committee Approval: Ethical approval was obtained from Marmara University Animal Care and Use Committee (No: 68.2008.mar).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - L.K., G.S., R.Y.; Data Collection - O.E., L.K., G.S., O.S., R.Y.; Analysis and/or Interpretation - O.E., O.S., R.Y.; Writing - O.E., O.S., R.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This work was supported by Istanbul University-Cerrahpaşa Scientific Research Projects (Project number: BYP-46945).

Etik Komite Onayı: Marmara Üniversitesi Hayvan Bakım ve Kullanım Kurulu'ndan (No: 68.2008.mar) etik onay alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Çalışma Konsepti - L.K., G.S., R.Y.; Veri Toplama - O.E., L.K., G.S., O.S., R.Y.; Veri Analizi/Yorumlama - O.E., O.S., R.Y.; Yazma - O.E., O.S., R.Y.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma İstanbul Üniversitesi-Cerrahpaşa Bilimsel Araştırma Projeleri Birimi tarafından desteklenmiştir (Proje no: BYP-46945).

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