

## CHARD (*BETA VULGARIS* L. VAR *CICLA*) EXTRACT INHIBITS POLYOL PATHWAY AND HYPERGLYCAEMIA – INDUCED OXIDATIVE STRESS IN RAT LENS

ISMET BURCU TURKYILMAZ<sup>1</sup>, BERTAN BORAN BAYRAK<sup>1</sup>, OZLEM SACAN<sup>1</sup>, LEVENT KABASAKAL<sup>2</sup>, GOKSEL SENER<sup>2</sup>, REFIYE YANARDAG<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Faculty of Engineering, Istanbul University-Cerrahpasa, 34320, Avcilar, Istanbul, Turkey

<sup>2</sup>Department of Pharmacology, Faculty of Pharmacy, Marmara University, 81010, Haydarpasa, Istanbul, Turkey

\*corresponding author: [refiyeyanardag@yahoo.com](mailto:refiyeyanardag@yahoo.com)

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### Abstract

In this study, the protective effect of chard extract on the lens of rats with hyperglycaemia was investigated. Rats were randomly divided into five groups. Control, Streptozotocin (STZ) - hyperglycaemic animals, STZ - induced hyperglycaemic animals given chard extract (2 g/kg bw/day), STZ - induced hyperglycaemic animals given insulin (6 U/kg bw/day), STZ - induced hyperglycaemic animals given chard extract and insulin. Hyperglycaemia was induced by i.p. injection of STZ in a single dose of 60 mg/kg body weight. Fourteen days after hyperglycaemia induction, chard extract, insulin, and chard + insulin were administered for 45 days, to the respective groups. In the lens tissues of the hyperglycaemic group, lipid peroxidation levels, the activities of aldose reductase, sorbitol dehydrogenase, and antioxidant enzymes increased, while glutathione levels decreased. Chard, insulin and chard + insulin combination reversed the eye abnormalities of the hyperglycaemic animals. As a result, it can be suggested that chard extract has a protective effect against lens damage due to diabetes.

### Rezumat

În studiul de față a fost investigat efectul protector al extractului de sfeclă roșie asupra cristalinului șobolanilor cu hiperglicemie. Șobolanii au fost împărțiți în cinci grupuri: martor, animale cu hiperglicemie indusă cu streptozotocină (STZ), animale cu hiperglicemie indusă cu STZ cărora li s-a administrat extract de sfeclă roșie (2 g/kg corp/zi), animale hiperglicemice STZ cărora li s-a administrat insulină (6 U/kg corp/zi), animale hiperglicemice STZ cărora li s-a administrat extract de sfeclă și insulină. Hiperglicemia a fost indusă prin injectarea i.p. de STZ într-o singură doză de 60 mg/kg corp. La paisprezece zile de la inducerea hiperglicemiei, s-au început administrările de extract de sfeclă roșie, insulină și extract de sfeclă roșie + insulină, timp de 45 de zile. În cristalinele grupului hiperglicemic, nivelurile de peroxidare lipidică, activitățile aldozo-reductazei, sorbitol dehidrogenazei și enzimelor antioxidante au crescut, în timp ce nivelurile de glutatation au scăzut. Sfecla roșie, insulina și combinația de sfeclă roșie + insulină au antagonizat anomaliile oculare ale animalelor hiperglicemice. Ca și concluzie, se poate sugera că extractul de sfeclă roșie are un efect protector asupra modificărilor determinate de diabet, survenite la nivelul cristalinului.

**Keywords:** lens, chard, diabetes mellitus, oxidative damage

### Introduction

Diabetes mellitus is a serious metabolic disease characterized by increased hyperglycaemia, associated with deficiency or insufficient release of insulin and destruction of pancreatic  $\beta$ -cells [1]. Hyperglycaemia is reported to be associated with severe damage to different organs like lens, liver, lung, etc. [2-4]. Stadler K report insinuated that although few amounts of free radicals are necessary, they are at the centre of cell signalling mechanisms [5]. However, the breakdown of balance will lead to oxidative stress, which aids the progress of diabetes and other diseases. Increased polyol and hexosamine pathways, non-enzymatic glycation of proteins, lipid peroxidation products among others are indicators for determining the extent of diabetes related tissue damage. In addition, the increased

glucose metabolism *via* tricarboxylic acid (TCA) cycle, leads to alteration of NADH and FADH<sub>2</sub> levels – which are related to electron transport chain (ETC) in mitochondria. Excess electron flow in ETC elevates the consumption of oxygen, thereby resulting in an increased generation of superoxide radicals [6]. This process ultimately results in the formation of ROS products.

Numerous maladies like cardiovascular diseases related to atherosclerosis, hypertension, diabetes and associated complications are some of the inevitable results of nutritional factors [7]. Plants are still considered as potential therapeutic instruments in either modern or traditional medicine. Chard, a leafy plant that grows all over the world, is a vegetable belonging to the *Chenopodiaceae* family. It grows easier when compared to the cultivating processes of celery and spinach.

Salad can be prepared from chard leaves and/or cooked like spinach. The stems of this plant are also used for cooking [8]. It has some unique nutritional properties, being rich in vitamins and minerals composition [9]. In addition, chard is alternatively used in order to diminish excess blood glucose levels in diabetic individuals. The blood glucose lowering effect of chard has been reported by several researchers [10, 11]. Chard and other *Beta vulgaris* species have been found to contain some important chemical substances like saponins and flavonoids, which help to regulate the blood glucose levels [12-14].

Lens represent one of the most affected organs in diabetic conditions. Its closed system is prone to protect itself against deleterious effects of oxidative stress. The consequence of diabetes on lens is the onset of cataract due to excess glucose, beyond the handling capacity of the tissue. Thus, the functionality of lens tissue can be affected if no alternative protective mechanism is utilized [2]. Some foods, vitamins and minerals are recommended for diminishing the deleterious effects of diabetes. Chard can be a good candidate for preventing lens damage owing this to its unique composition. In this work, we aimed to research the possible protective effects of chard aqueous extract on hyperglycaemic rat lens due to STZ.

## Materials and Methods

### *Plant material*

Chard was purchased from Istanbul, Turkey. The chard leaves were dried under room temperature, after they were carefully washed using distilled water. After the drying process, they (100 g) were extracted with distilled water (1 L) by boiling for 8 hours. The water of the filtrate was evaporated with a rotary evaporator. The extracts were dissolved in distilled water based on required dosage before been administered to the rats.

### *Experimental animals*

Forty male Sprague-Dawley rats of 6 - 7 months, weighing 380 - 420 g were used. The experimental process and protocol was approved by Marmara University Animal Care and Use Committee (No: 68.2008.mar). They were allowed the access to tap water *ad libitum*. The animals were randomly assigned into five groups, 8 animals each, as follows: Group I (n = 8) were control animals given only citrate buffer; Group II (n = 8) were streptozotocin (STZ) - induced hyperglycaemic animals; Group III (n = 8) were STZ - induced hyperglycaemic animals given chard extract (2 g/kg bw/day; by gavage); Group IV (n = 8) were STZ - induced hyperglycaemic animals administered with insulin (Humulin M, Lilly, Indianapolis, USA) (6 U/kg bw/day) intraperitoneally (i.p.); Group V (n = 8) were STZ - induced hyperglycaemic animals given chard extract and insulin. STZ was injected as a single dose (60 mg/kg body weight) for inducing hyperglycaemia. STZ was prepared daily by dissolving in

freshly prepared 0.01 M citrate buffer of pH 4.5. Fourteen days after rats were rendered hyperglycaemic, the chard extract was given to Group III, insulin was injected to Group IV, while chard + insulin was administered to Group V for 45 days. The experiment has ended at 60<sup>th</sup> day, all the rats were decapitated, and lens tissues were sampled from animals.

### *Biochemical assays*

In the 1<sup>st</sup> and 60<sup>th</sup> day of the experiment, fasting blood glucose was determined by a glucose analyser (MediSense Optium Xceed Glucometer, Abbott, Ireland). The blood samples were taken from the tail vein [15]. Biochemical parameters were determined from all the lens tissues of each group. Both of the right and left lens were homogenized in cold NaCl (0.9%) to obtain a 10% (w/v) homogenate. After the centrifugation process, the supernatants were collected and used for determination of glutathione (GSH), lipid peroxidation (LPO), protein levels and several enzymes activities. GSH contents were determined by the Beutler method [16]. LPO contents were assessed by the Ledwozyw *et al.* method [17]. Aldose reductase (AR) activity was determined according to Hayman and Kinoshita [18]. Sorbitol dehydrogenase (SDH) activity in lens tissues was estimated by Barretto and Beutler method [19]. Catalase (CAT) activity was measured in lens tissue according to Aebi method [20]. Superoxide dismutase (SOD) activity was determined by the method of Mylroie *et al.* [21]. Glutathione peroxidase (GP<sub>x</sub>) activity was evaluated in lens tissue according to Wendel [22]. Glutathione reductase (GR) activity was measured according to Beutler [23]. The protein contents of all samples were determined by the Lowry *et al.* method [24].

### *Statistical analysis*

Biochemical results were assessed *via* unpaired *t*-test and ANOVA variance analysis. The statistical evaluations were determined by the NCSS statistical computer package. The obtained values of the experiments were presented as mean  $\pm$  SD. The Mann-Whitney test was chosen for the analysis of control and experimental groups. The *p* values < 0.05 were accepted as significant difference.

## Results and Discussion

The data of fasting blood glucose levels, and the protective effects of chard extract on its levels have been published in a previous study by Gezgin-Oktayoglu *et al.* [15]. The GSH contents of lens tissues were significantly decreased in the hyperglycaemic animals as compared to the control group ( $p < 0.0001$ ). Chard, insulin and chard + insulin treated hyperglycaemic rats, GSH contents increased when compared to hyperglycaemic rats ( $p < 0.0001$ ). In the hyperglycaemic group, LPO contents were higher than that of control group in a significant manner ( $p < 0.0001$ ). Administration of chard extract,

insulin, chard extract + insulin decreased the lens LPO levels of hyperglycaemic rats significantly ( $p < 0.0001$ ) (Table I).

AR and SDH activities of hyperglycaemic rat lens significantly increased ( $p < 0.0001$ ). Treatment of chard extract, insulin and chard + insulin to the hyperglycaemic rats significantly decreased AR and SDH activities ( $p < 0.0001$ ) (Table II).

CAT, SOD, GP<sub>x</sub> and GR activities of all groups are shown in Table III. These enzymes activities increased in the lens tissues of hyperglycaemic rats ( $p < 0.0001$ ). Chard extract and insulin, chard extract + insulin treatment decreased all the enzymes activities ( $p < 0.0001$ ) (Table III).

**Table I**

Glutathione (GSH) and lipid peroxidation (LPO) levels in lens tissue of rats

Groups	GSH (nmol GSH/mg protein)*	LPO (nmol MDA/mg protein)*
Control	18.57 ± 2.09	0.34 ± 0.04
Hyperglycaemic	4.29 ± 0.09 <sup>a</sup>	0.57 ± 0.09 <sup>a</sup>
Hyperglycaemic + Chard	17.95 ± 1.52 <sup>b</sup>	0.38 ± 0.05 <sup>b</sup>
Hyperglycaemic + Insulin	8.05 ± 1.21 <sup>b</sup>	0.26 ± 0.03 <sup>b</sup>
Hyperglycaemic + Chard + Insulin	24.78 ± 1.09 <sup>b</sup>	0.15 ± 0.02 <sup>b</sup>
P <sub>ANOVA</sub>	0.0001	0.0001

\*Mean ± SD; <sup>a</sup> $p < 0.0001$  vs. control group; <sup>b</sup> $p < 0.0001$  vs. hyperglycaemic group

**Table II**

Aldehyde reductase (AR) and sorbitol dehydrogenase (SDH) activities in lens tissue of rats

Groups	AR (U/g protein)*	SDH (U/g protein)*
Control	3.08 ± 0.38	7.10 ± 0.49
Hyperglycaemic	8.61 ± 1.46 <sup>a</sup>	20.19 ± 1.56 <sup>a</sup>
Hyperglycaemic + Chard	2.94 ± 0.69 <sup>b</sup>	4.26 ± 0.72 <sup>b</sup>
Hyperglycaemic + Insulin	2.83 ± 0.44 <sup>b</sup>	5.71 ± 0.54 <sup>b</sup>
Hyperglycaemic + Chard + Insulin	4.10 ± 0.29 <sup>b</sup>	9.64 ± 0.65 <sup>b</sup>
P <sub>ANOVA</sub>	0.0001	0.0001

\*Mean ± SD; <sup>a</sup> $p < 0.0001$  vs. control group; <sup>b</sup> $p < 0.0001$  vs. hyperglycaemic group

**Table III**

Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GP<sub>x</sub>) and glutathione reductase (GR) activities in lens tissue of rats

Groups	CAT (U/mg protein)*	SOD (U/g protein)*	GP <sub>x</sub> (U/g protein)*	GR (U/g protein)*
Control	2.09 ± 0.07	10.60 ± 0.77	37.89 ± 3.49	34.04 ± 2.13
Hyperglycaemic	8.56 ± 0.44 <sup>a</sup>	34.15 ± 4.61 <sup>a</sup>	74.60 ± 5.54 <sup>a</sup>	87.77 ± 3.05 <sup>a</sup>
Hyperglycaemic + Chard	2.51 ± 0.21 <sup>b</sup>	2.42 ± 0.03 <sup>b</sup>	32.71 ± 3.15 <sup>b</sup>	15.77 ± 0.83 <sup>b</sup>
Hyperglycaemic + Insulin	2.43 ± 0.11 <sup>b</sup>	4.89 ± 0.90 <sup>b</sup>	30.30 ± 1.79 <sup>b</sup>	18.90 ± 2.26 <sup>b</sup>
Hyperglycaemic + Chard + Insulin	1.80 ± 0.10 <sup>b</sup>	0.62 ± 0.10 <sup>b</sup>	21.17 ± 0.51 <sup>b</sup>	30.42 ± 2.78 <sup>b</sup>
P <sub>ANOVA</sub>	0.0001	0.0001	0.0001	0.0001

\*Mean ± SD; <sup>a</sup> $p < 0.0001$  vs. control group; <sup>b</sup> $p < 0.0001$  vs. hyperglycaemic group

Diabetes mellitus is a complicated metabolic disease characterized by hyperglycaemia. In such circumstances, insulin becomes vital because of its regulating effect on glucose uptake by cells in peripheral tissues [25]. However, insufficient insulin levels result to elevated free glucose concentrations, which subsequently react with other biologic components to form ROS. As a consequence, different approaches are assessed for possible effects on the elevation of  $\beta$ -cells number, or finding pharmaceutical agents and herbal remedies with antidiabetic effects [25-27]. The insulin treatment decreased fasting blood glucose levels in the diabetic group as expected. Either singular or used in combination with insulin, chard extract induced lower glucose levels to a level similar to that caused by insulin administration. Chard has probably decreased glucose levels by

increasing the activities of glucose transporters as previously proposed by Gezgin-Oktayoglu *et al.* [15]. In diabetic conditions, the level of ROS in mitochondria are elevated [28]. Therefore, antioxidant systems (either enzymatic or non-enzymatic) must be utilized by tissues such as lens in order to protect their self from the increasing levels of ROS in the system. GSH is a unique non-enzymatic antioxidant, whose depletion is associated with increased ROS levels and elevated concentrations of GP<sub>x</sub> substrates [29]. Besides that, lipids can be oxidized, thus allowing compounds such as malondialdehyde to cross membranes/barriers and attack other components [30]. A report by Oztay *et al.* [31] indicates that the depletion of GSH and the elevation of LPO have positive correlations for diabetics. In the present study, chard extract, insulin and chard extract + insulin combination restored the

GSH and LPO contents in hyperglycaemic animals' lens tissues. These singular or dual effects of chard extract and insulin can be attributed to its decreasing effect on blood glucose level *via* promoting the utilization of glucose by lens and other tissues, thereby diminishing ROS levels.

AR and SDH are important enzymes in the polyol pathway. By passive diffusion, glucose can easily enter due lens without needing insulin. However, polyol pathway products can't easily pass out through the lens membrane, and thereafter begin to accumulate [32]. The accumulation of sorbitol and fructose initiates problems like increased osmotic pressure [33], as well as the elevation of ROS and other free radical species in the tissue. These have led researchers to continuously search for compounds or herbal formulations capable of inhibiting enzymes of the polyol pathway. The polyphenol contents of herbs can be suitable inhibitors for these enzymes [34-36]. In this study, an increase in AR and SDH activities was observed in the hyperglycaemic group. Administration of chard, insulin and their combination decreased the activities of these enzymes. The diminished activity of AR and SDH in the chard administered group may be due to flavonoids and polyphenol contents of the plant as previously declared by Sacan and Yanardag [8]. Insulin and chard + insulin combination may have conducted to a decrease of the excess glucose levels and protect lens tissue against further damage.

Hyperglycaemia alters the antioxidant status and this in addition to the presence of polyol products makes lens tissue more vulnerable to ROS [37]. Superoxide radicals are scavenged by SOD to form H<sub>2</sub>O<sub>2</sub>, a substrate for CAT. In addition, GP<sub>x</sub> converts H<sub>2</sub>O<sub>2</sub> to water *via* GSH. The GSH is regenerated *via* the action of GR – an enzyme that converts oxidized glutathione to its reduced form for continual GP<sub>x</sub> activity. The outcome of the current study indicates the elevated activities of antioxidant enzymes in the lens of the hyperglycaemic group. This suggests increased concentration of radicals in the tissue. The results in our study are consistent with Wojnar *et al.* [38]. Treatment of chard extract, insulin and chard extract + insulin decreased the activities of the aforementioned enzymes. Chard, insulin and their combination are considered to be capable of decreasing hyperglycaemia and ROS levels. Chard might have positively influenced insulin action *via* its unique antioxidant capacity as earlier reported by Sener *et al.* [39].

## Conclusions

In the current work, the possible protective effects of chard extract either alone or in combination with insulin on STZ - induced hyperglycaemic lens damage was investigated. It can be concluded that chard exhibited remarkable antioxidant and blood glucose

lowering effects against STZ - induced hyperglycaemia in the experimental animals.

## Conflict of interest

The authors declare no conflict of interest.

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