Protective effect of chard extract on glycoprotein compounds and enzyme activities in streptozotocin-induced hyperglycemic rat lungs

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In the present study, the protective effect of chard on glycoproteins, hydroxyproline, advanced oxidation protein products and enzyme activities in the lung tissue of streptozotocin (STZ) – induced hyperglycemic rats were examined. Male, Sprague Dawley rats were grouped as control, hyperglycemic, chard, insulin, chard+insulin given hyperglycemic rats. Hyperglycemia was induced by as a single dose of STZ (60 mg/kg) administered intraperitoneally. Fourteen days after the rats were made hyperglycemic, chard extract was administered to rats 2 g/kg/day by gavage and/or insulin a dose of 6 U/kg/day for 45 days. Glycoprotein components, prolidase activity and levels of hydroxyproline and advanced oxidation protein products were significantly increased in the lung tissues of hyperglycemic rats; on the other hand, paraoxonase and arylesterase activities were decreased. Treatment with chard and/or insulin reversed these effects. These results suggested that chard possesses a significant beneficial effect on abnormal glycoprotein metabolism and enzyme activities in STZ- induced hyperglycemic rats.

Keywords: chard, glycoprotein, hyperglycemic, lung, rat.

INTRODUCTION

Diabetes mellitus is a disease affecting many organs such as liver, kidney, pancreas, lungs and eyes. As the lung has abundant connective tissue and diffuse microvascular circulation, it is thought to be a target organ for diabetic disease. Oxidative stress is considered to be the main factor in the development of diabetic complications and tissue injury. Many traditional herbs, through their hypoglycemic and antioxidant properties, may also protect the organs involved in diabetes mellitus.

Carbohydrates seem to play a central role in the development of chronic diabetic complications. Glycoproteins, which are carbohydrate linked protein macromolecules found on the cell surface, are some of the principal components of animal cells. Hexose, hexosamine, fucose and sialic acid are the basic sugar components found in glycoproteins and glycosaminoglycans. Glycoprotein metabolism plays a major role in the pathogenesis of diabetes mellitus. Glycoproteins have multiple and complex functions and are found as hormones, enzymes, blood group substances and as constituents of extracellular membranes [1]. They play an important role in functions such as cell differentiation and recognition, membrane transport and absorption of macromolecules [2]. In hyperglycemic state, high blood glucose levels accelerate the synthesis of basement membrane components, such as glycoproteins [3].

Chard (Beta vulgaris L. var. cicla) is a biennial leaf vegetable cultivated worldwide, in Northern India, South America, the Mediterranean countries and USA. It is an important crop due to its year round availability, high yield and low cost. Several studies have demonstrated that chard has antioxidant and antiacetylcholinesterase [4], antidiabetic [5, 6], anticancer [7], antimicrobial [8], hepatoprotective [9] and other biological activities. Phytochemical screening of B. vulgaris varieties have revealed the presence of some saponins, flavonoid glycosides [10], flavonoids, vitamin C, vitamin E, carotenoids and minerals [11]. In the present study, the protective effect of chard on glycoprotein levels and enzyme activities in hyperglycemic lung tissue were examined.

EXPERIMENTAL

Preparation of chard extract

Chard leaves were collected from Istanbul, Turkey. Plant material was washed with distilled water and dried at room temperature. The chard was identified by Prof. Dr. Neriman Ozhatay (Faculty of Pharmacy, Istanbul University). Dried chard leaves (100 g) were extracted with 1000 mL of distilled water and boiled for 30 min. The extract was filtered and the filtrate was evaporated under reduced pressure using a rotary evaporator. The chard extract yield was 39.48 % (w/v). Chard extract was dissolved in distilled water in order to obtain 43.33 g/55 ml extract solution.

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Male Sprague–Dawley rats weighing 380-420 g and aged 6-7 months were used in the study. All experimental procedures were approved by Marmara University Animal Care and Use Committee. (No: 68.2008.mar). The rats were divided into five groups. Group I (n= 8): control rats given citrate buffer; Group II (n= 8): STZ-induced hyperglycemic rats; Group III (n= 8): STZ-induced hyperglycemic rats given chard extract; Group IV (n= 8): STZ-induced hyperglycemic rats given insulin; Group V (n= 8): STZ-induced hyperglycemic rats given chard extract+insulin. Fourteen days after the rats were rendered hyperglycemic, chard extract (2g/kg/day, by gavage), insulin (6 U/kg/ day, by subcutaneous injection) and chard extracts plus insulin at the mentioned doses were administrated to rats for 45 days. On day 60, lungs of rats were removed and used for the analysis of glycoprotein components and enzyme analysis. Hyperglycemia was induced by a single intraperitoneal injection of (60 mg/kg) STZ freshly dissolved in citrate buffer (pH=4.5; 0.01 M).

Biochemical estimations

Fasting blood glucose levels after 18 h of fasting were determined using an automated glucose analyzer [6]. Rats with more than 200 mg/dl fasting plasma glucose were considered as having hyperglycemia. The lungs were homogenized in 0.9% NaCl to make up a 10% (w/v) homogenate. Hexose and hexosamine contents were estimated by the method of Winzler [12] and fucose was determined by the method of Dische and Shettles [13] in lung tissue homogenates. Sialic acid was estimated by the method of Lorentz et al. [14], Furlong et al. [15], Gan et al. [16] and Chinard [17] methods were used to determine the paraoxonase (PON), arylesterase (ARE) and prolidase activities, respectively. Lung hydroxyproline and advanced oxidation protein products (AOPP) contents were assayed by the method of Reedy and Enwemeka [18] and Witko-Sarsat et al. [19], respectively. The protein content was estimated by the method of Lowry [20].

Statistical analysis

Biochemical results were evaluated using an unpaired \( t \)-test and ANOVA variance analysis using the NCSS statistical computer package. The values were expressed as mean \( \pm \) SD. Comparison between control and experimental groups was performed using the Mann-Whitney test. \( p < 0.05 \) was considered as significant.

RESULTS

Lung tissue glycoprotein levels are presented in Table 1. The levels of glycoproteins containing hexose (\( p < 0.001 \)), hexosamine, fucose and sialic acid were significantly increased in the hyperglycemic groups (\( p < 0.0001 \), respectively). Administration of chard, insulin and chard+insulin reversed these changes in the glycoprotein components in the lungs of hyperglycemic rats except hexose values (\( p < 0.005 \) and \( p < 0.0001 \), respectively).

The PON, ARE, prolidase, hydroxyproline and AOPP activities in the lung tissue are presented in Table 2. In the hyperglycemic group, the activities of PON, ARE were decreased while prolidase, hydroxyproline and AOPP activities were increased (\( p < 0.005 \), respectively). Administration of chard, insulin and chard+insulin significantly increased the lung paraoxonase PON and ARE activities in the hyperglycemic groups (\( p < 0.005 \), respectively) while lung prolidase activities, hydroxyproline and AOPP levels were significantly decreased in the hyperglycemic rats. The effect of chard was better than that of insulin and insulin-chard.

DISCUSSION

Diabetes mellitus is a chronic disease which affects various organs such as liver, brain, kidney, skin and lung. Experimental diabetes models have an important place in analyzing diabetes complications and determining treatment approaches. Lung is a target organ in diabetes mellitus. Diabetes mellitus causes a decrease in lung elasticity and neuropathy, which in turn partially affects basic lung functions [21]. Generally, abnormalities in glycoprotein metabolism are observed in both natural and experimental diabetes [22]. Altered metabolism of glycoproteins plays a major role in the pathogenesis of diabetes mellitus. Insulin deficiency and high levels of blood glucose in diabetic condition may result in an increased synthesis of glycoprotein components, which results in thickening of the basal membrane [23]. The increase in tissue glycoprotein components has been associated with the severity and duration of diabetes. In diabetes mellitus, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars such as glucose, to yield a Schiff-base intermediate. Intermediate products in these reactions undergo Amadori rearrangement to form stable ketoamine derivatives [24].
According to this results, it can be suggested that other parameters except hexose (Table 1). In this study, we have observed increased levels of hexose, hexosamine, fucose and sialic acid in the lung tissue of STZ-induced hyperglycemic rats. Previous studies have shown that decrease in hyperglycemia could lead to a decrease in glycoprotein components [25, 26].

In this study, we have observed increased levels of hexose, hexosamine, fucose and sialic acid in the lung tissue of STZ-induced hyperglycemic rats. Previous studies have shown that decrease in hyperglycemia could lead to a decrease in glycoprotein components [25, 26]. In this study, chard, insulin and chard-insulin treatment to diabetic rats significantly decreased lungs glycoprotein components to near-normal levels by virtue of its antihyperglycemic effects. The most effective treatment was insulin therapy on glycoprotein levels. This could be due to the decreased hyperglycemic state with increased levels of insulin in diabetic rats [27]. In this study, it has been determined that chard decrease hexose, hexosamine, fucose and sialic acid levels in hyperglycemic rats because it increases insulin secretion from B cells of the pancreas [28]. In diabetic rats with the use of chard and insulin + chard glycoprotein levels were decreased in all other parameters except hexose (Table 1). According to this results, it can be suggested that chard decreases glycoprotein levels in lungs because its antihyperglycemic effects [28]. This results show the efficiency of chard in modulating the altered glycoprotein metabolism in diabetic rats. Diabetes is characterized by high glucose concentration which leads to an increase in the production of reactive oxygen species (ROS) via several mechanisms (glucose autooxidation, stimulation of polyol pathway and formation of advanced glycation end products). The resulting oxidative stress can play a key role in diabetes pathogenesis. ROS are known to be responsible for the oxidative damage of DNA, nucleotides, proteins, lipids, carbohydrates and cell membrane structures [29].

PON is known to have an antioxidant function [30]. In humans, PON gene family has three members (PON 1, PON 2, PON 3) [31]. PON 1 has three known enzymatic molecules including paraoxonase, arylesterase and dyazoxonase. PON 1 activity is reduced in diseases associated with high oxidative stress such as coronary heart disease, dyslipidemia, cancers, inflammatory processes, hyperlipidemia, diabetes mellitus and certain neuropathies, chronic hepatitis, HDL deficiencies and Gulf War Syndrome [32-34]. Most studies have found that PON 1 activity is reduced in diabetic patients such as Type I and Type II with some

### Table 1. Lung tissue hexose, hexosamine, fucose and sialic acid levels of all groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexose (mg glucose/mg protein)*</th>
<th>Hexosamine (µg hexosamine /mg protein)*</th>
<th>Fucose (µg fucose /mg protein)*</th>
<th>Sialic acid (µmole sialic acid /g protein)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.63 ± 1.17</td>
<td>5.33 ± 0.39</td>
<td>10.23 ± 0.06</td>
<td>139.78 ± 9.76</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>30.65 ± 5.95*</td>
<td>16.55 ± 1.99*</td>
<td>22.41 ± 0.58*</td>
<td>442.26 ± 12.33*</td>
</tr>
<tr>
<td>Hyperglycemic + Chard</td>
<td>17.60 ± 1.44b</td>
<td>4.68 ± 0.41c</td>
<td>7.51 ± 1.28c</td>
<td>133.25 ± 18.22c</td>
</tr>
<tr>
<td>Hyperglycemic + Insulin</td>
<td>15.61 ± 1.37c</td>
<td>4.51 ± 0.94c</td>
<td>7.62 ± 0.65c</td>
<td>113.97 ± 7.66c</td>
</tr>
<tr>
<td>Hyperglycemic + Chard + Insulin</td>
<td>24.14 ± 2.08</td>
<td>11.98 ± 1.18b</td>
<td>10.17 ± 1.33c</td>
<td>169.73 ± 6.06c</td>
</tr>
</tbody>
</table>

*Mean ± SD; *P<0.001 vs control group; bP<0.005 vs hyperglycemic group; *P<0.001 vs hyperglycemic group; bP<0.001 vs control group

### Table 2. Lung tissue PON, ARE and prolidase activities, hydroxyproline and AOPP levels of all groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PON (U/g tissue)*</th>
<th>ARE (U/g protein)*</th>
<th>Prolidase (U/g protein)*</th>
<th>Hydroxyproline (µmole sialic acid /g protein)*</th>
<th>AOPP (nmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.05 ± 2.76</td>
<td>7.09 ± 0.60</td>
<td>53.50 ± 1.27</td>
<td>10.00 ± 0.43</td>
<td>9.29 ± 0.40</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>22.83 ± 2.11a</td>
<td>4.87 ± 0.38b</td>
<td>110.10 ± 5.15a</td>
<td>22.48 ± 1.55a</td>
<td>11.08 ± 0.82a</td>
</tr>
<tr>
<td>Hyperglycemic + Chard</td>
<td>49.15 ± 3.38b</td>
<td>11.88 ± 1.00b</td>
<td>38.58 ± 4.79b</td>
<td>2.03 ± 0.35b</td>
<td>8.49 ± 0.57b</td>
</tr>
<tr>
<td>Hyperglycemic + Insulin</td>
<td>29.72 ± 1.92b</td>
<td>8.90 ± 0.88b</td>
<td>56.26 ± 6.34b</td>
<td>20.87 ± 0.97b</td>
<td>8.50 ± 1.17b</td>
</tr>
<tr>
<td>Hyperglycemic + Chard + Insulin</td>
<td>31.63 ± 2.35b</td>
<td>5.40 ± 0.56</td>
<td>49.18 ± 2.92b</td>
<td>5.65 ± 0.30b</td>
<td>8.98 ± 0.83b</td>
</tr>
</tbody>
</table>

*Mean ± SD; *P<0.005 vs control group; bP<0.005 vs hyperglycemic group
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Chronic inflammation is closely associated with angiogenesis and PON1 activity decreases during inflammation [37]. SH groups play a role in PON 1 activities. In the present study, lung PON and ARE activities were reduced in the diabetic rats which is consistent with previous diabetic rat study [38]. Decreased PON and ARE activities in diabetes mellitus might be associated with hyperglycemia and oxidative stress. In this study, paraoxonase and ARE activities were increased after treatment with chard, chard+insulin and insulin. This fact may be due to the antioxidant effect of polyphenols, tannins and anthocyanins as well as to their direct effect on enzyme activities [11].

Prolidase activity has been reported as a marker for oxidative stress for many diseases like diabetes, diabetic neuropathy, nonulcer dyspepsia, chronic liver diseases, erectile dysfunction, osteoporosis, and so forth [39, 40]. Increased prolidase activity is an indicator of fibrosis. Fibrosis may occur in some lung diseases. This enzyme plays an important role in the recycling of proline for synthesis and cell growth [41]. In our study, we found a significant increase in lung prolidase activity in hyperglycemic rats. We observed that chard, insulin and chard+insulin inhibited prolidase activity. These results indicate that chard extract ameliorates oxidative stress and hyperglycemia by inducing antioxidant defense in the lung of hyperglycemic rats. Based on these results, it could be argued that prevention of inflammation and oxidative stress was achieved in the lung tissue of hyperglycemic rats. Collagen is one of the proteins which contain the amino acid hydroxyproline. Some authors consider hydroxyproline as a marker of collagen content. Excessive production of collagen has been documented in disorders associated with proline derivatives such as lung fibrosis [42]. Hydroxyproline levels were increased in the lung tissues of hyperglycemic rats. This increase indicates the presence of pulmonary fibrosis in lung tissues. Surfactant protein (SP-D) is a useful and early diagnostic marker for pulmonary fibrosis. It may serve as a specific marker for lung injury. In our previous study [43], SP-D levels were determined in the lung tissue. In the present study, our data showed that STZ-induced pulmonary fibrosis led to an increase in hydroxyproline content. Treatment with chard, insulin and chard + insulin prevented these changes induced by STZ in rat and exerted a protective effect on STZ-induced pulmonary fibrosis. Administration of chard, insulin and chard+insulin restored the hydroxyproline levels to normal in the lung tissue. The reduction of hydroxyproline in rat lung via chard treatment alone and in combination with insulin was first shown in the present study. In our manuscript, we determined that chard which was used on hyperglycemic rats was more effective than insulin on enzyme activity and hydroxyproline level (Table 2). This effect can be because of antioxidant and hypoglycemic properties of chard [4, 28].

AOPP, the dityrosine containing and cross linking protein products, are amongst the markers which indicate oxidative stress-based protein damage [44]. Accumulation of AOPP in the tissue plays an important role in the long-term complications of diabetes. Gradinaru et al. has mentioned the elevation of AOPP levels in diabetic patients [45]. We have also found an increased production of AOPP in the lungs of hyperglycemic rats. Treatment with chard, chard+insulin and insulin significantly reduced AOPP levels in hyperglycemic rats. This result indicated that chard, insulin and chard+insulin may be effective in preventing oxidative protein damages by reducing oxidative stress.

These results demonstrated that chard, insulin and chard+insulin consumption for 45 days may exert beneficial effects on the levels of glycoprotein, hydroxyproline, AOPP and PON, ARE and prolidase activities. Chard may have beneficial role in diabetic rats which may be due to the enhancement of insulin. Chard can be used as an effective indicator to demonstrate its effects in controlling the complications of diabetes.

REFERENCES

В настоящей статье изучен защитный эффект на цвекло върху глюкопротеините, хидроксипролината, продуктите от окисление на протеина и ензимната активност в белодробната тъкан на стрептозоцин (СТЦ)-индуцирани хипергликемични плъхове. Мякки плъхове от вида Sprague Dawley са групирани както следва: контролна група, хипергликемична група, третирани с цвекло, третирани с инсулин и третирани с инсулин и цвекло. Хипергликемията е индуцирана чрез единочно интратерапевтично вкарване на СТЦ (60 mg/kg). След 14 дни на плъховете се дава със стомашна сонда екстракт от цвекло 2 g/kg/ден и/или инсулин 6U/kg/ден в продължение на 45 дни. Глюкопротеиновите компоненти, пролидазната активност, нивата на хидроксипролин и продуктите от окисление на протеина значително нарастват в белодробната тъкан, докато параксоксназата и арилестеразната активност намаляват. Третирането с цвекло и/или инсулин обръща тези ефекти. Резултатите показват, че цвеклото притежава значително благоприятно действие върху абоанормалния глюкопротеинов метаболизъм и ензимната активност в СНЦ-индуктирани хипергликемични плъхове.