Limited Effects of Parsley (*Petroselinum crispum*) on Protein 
Glycation and Glutathione in Lenses of Streptozotocin-Induced 
Diabetic Rats

Füsun Özcêlik¹, Ayşen Yarat¹, Refiye Yanardağ², Tuğba Tunali¹, Özlem Özsoy², Nesrin Emekli¹ and Ali Üstüner²

¹Department of Biochemistry, Faculty of Dentistry, Marmara University, Nişantaşı; ²Department of Chemistry, Faculty of 
Engineering, Istanbul University, Avcilar; ³Department of Ophthalmology, Cerrahpaşa Medical Faculty, Istanbul University, 
Istanbul, Turkey

Abstract

Parsley (*Petroselinum crispum*), which has hypoglycaemic 
activity, has been used as a folk remedy for diabetes. This study investigated the effect of parsley administration 
on diabetes-induced impairments in rat lenses. Administration of parsley extract did not prevent a decrease 
in glutathione nor an increase in protein glycation, although it did significantly prevent an increase in blood 
glucose. SDS-polyacrylamide gel electrophoresis revealed no significant differences in any protein bands between any 
of the groups.

Keywords: Lens, diabetes, parsley, glutathione, protein, 
glycation, electrophoresis.

Introduction

Glycation or nonenzymatic glycosylation (NEG) of lens 
proteins and damage to antioxidant systems increases 
during hyperglycaemia (Bresnic, 1982; Cotlier, 1987; 
Davson, 1990). An increase in lens protein glycation results 
in protein functional and conformational changes (Luthra & 
Balasubramanian, 1993; Swamy et al., 1993; Yarat et al., 
1995; King & Branskota, 1994; Chait & Bierman, 1994) and 
a decrease in reduced glutathione (GSH) causes oxidation of 
protein sulphhydryl groups which adversely affects protein 
linkages, solubility and transparency, and leads to free 
radical-induced damage of the lens. These effects may lead 
to opacification of lens (Bresnic, 1982; Cotlier, 1987; 

There is an increasing use of herbal remedies for reducing 
blood glucose levels. The following plants found in 
Turkey, which are known to lower blood glucose levels in 

A recent study from our laboratory cites many investiga-
tions of the hypoglycemic activity of parsley (*Petro-
selinum crispum*) hereafter referred to as "parsley", from 
the Umbellifera. It has also been reported to have antimis-
crobial, antianemic, anticoagulant, antihyperlipidemic, anti-
hepatotoxic, diuretic and rheumatoid effects, and to have 
been used as a folk remedy for treating high blood pressure, eczema, kneeache, impotence and nose 
bleeding. Phytochemical screening of parsley has revealed the presence of monoterpene (β-pinene), sesquiterpenes, 
carotenoids, phthalides, furanocoumarins, coumarins, 
myristicin, apio, ascorbic acid and flavonoids (Tunali et al., 
1999).

Using streptozotocin (STZ)-induced diabetic rats, the 
present study has investigated the extent to which adminis-
tration of parsley extract effected diabetically induced 
changes in NEG, GSH and proteins in lens homogenates.
Materials and Methods

Preparation of aqueous parsley extract

Parsley leaves were collected from Büyükçekmece, Istanbul, during June and July. They were carefully washed with tap water and left to dry in the shade at room temperature. They were then stored until required in well sealed cellophane bags. These leaves (100 g quantities) were then boiled in 1000 ml of distilled water for 30 min and filtered. Extracts were removed from the filtrate by evaporation under reduced pressure until dry. These extracts were again dissolved in distilled water before being administered to normal and STZ-induced diabetic rats.

Animals and treatment

Sixty-two male, 6-month-old, Swiss Albino rats were used in this study. They were divided into four groups designated as follows: C = control, Cp = control + parsley, D = diabetic, Dp = diabetic + parsley. The rats in the diabetic groups were fasted for 18 h and rendered diabetic on day zero by one intraperitoneal injection of 65 mg/kg STZ (Sigma) in a freshly prepared citrate buffer (1 mM sodium citrate and HCl, pH 4.5). On day 14, parsley extracts were administered at 2 g/kg by gavage, daily for 14 days, to the parsley groups (Cp, Dp). On day 42 after STZ injection, after an 18 h period of fasting, tail blood samples were taken from all rats under ether anesthesia. The rats were then sacrificed by giving excessive ether. Intracapsular extracts were then made from their lenses. Blood glucose was determined in the blood samples by the o-toluidine method (Relander & Raiha, 1963).

Both lenses of each rat were homogenized together with a glass homogeniser in 2 ml of saline solution and kept in a deep-freezer. The total protein, protein glycation and glutathione levels of the lens homogenates were determined and protein electrophoresis was carried out.

Lens total protein levels were measured by the method of Lowry et al. (1951) and lens protein glycation was assessed by the 2-thiobarbituric acid method (Parker et al., 1981). The latter involved hydrolysing each 0.5 ml homogenate with 0.5 ml of 0.5 M oxalic acid in an autoclave for 1 h at 124 ± 1°C. To this, 0.5 mL 40% trichloroacetic acid (w/v) was added, mixed, centrifuged at 1500 x g for 10 min, and filtered using filter paper. Absorbance at 443 nm was recorded. Then, 0.75 ml of supernatant was incubated in 0.25 mL of 0.05 M 2-thiobarbituric acid at 37°C for 30 min. After standing for 15 min at room temperature, absorbance was again measured at 443 nm and the differences between the first and second absorbances were calculated. The protein glycation values were expressed as nmol of fructose per mg protein.

Commercial fructose (Sigma) was used as a standard.

Reduced glutathione levels of the lenses were determined by using Ellman's reagent (Beutler, 1975). Here, a precipitation solution, containing metaphosphoric acid, EDTA and NaCl was added to each 0.2 ml of homogenate. This was mixed and centrifuged at 1500 x g for 10 min. Then, 0.3 M Na₃HPO₄ was added to an aliquot of the supernatant followed by Ellman's reagent, 0.04% 5,5'-dithio-bis-2-(nitrobenzoic acid), in 1% sodium citrate. After standing for 5 min at room temperature, the absorbance at 412 nm was recorded. Reduced glutathione (Sigma) was used as a standard. The concentration of lens glutathione was expressed as nmol of glutathione per mg protein.

Electrophoretic examination of lens proteins was carried out by SDS-polyacrylamide gel electrophoresis as described by Laemmli (1970). Schleicher and Schuell Profile System mini-electrophoresis was performed with Sigma low molecular weight protein standards (SDS-7, Dalton Mark VII-L). After electrophoresis, scans of Coomasie blue stained protein bands were obtained using a densitometer (Helena Laboratories TCL plus). Peak areas were measured with a planimeter (Placom-Sokkisha, Rp-90N, digital) and the protein percentage calculated in each band.

Statistical analysis

The results were evaluated using unpaired t-test, Anova analysis and regression analysis, using the NCSS statistical computer package.

Results and Discussion

The mean levels of blood glucose and body weights for the 4 groups are given in Table 1. Prior to inducing diabetes, the groups were checked for differences in weight and blood glucose, but none was found (p = 0.973 and 0.999, respectively) (Table 1). On day 14, immediately before parsley administration, the blood glucose (BG) levels were determined. BG was significantly higher in the diabetic group compared with the control group (p = 0.0001, Table 1). At 42 days, body weight (BW) was significantly lower (p = 0.0001) and BG significantly higher (p = 0.001) than at day 0 for the non-parsley diabetic group (D). The same applied for the Dp group (p = 0.0001 and 0.002, respectively) (Table 1) and the reduction in BG between day 14 and 42 was significant (p = 0.002) showing the parsley to moderate the diabetic-induced increase in blood glucose. However, the 42 day BG for the Dp group was not significantly lower than that of the D group (p = 0.114).

As the chemical composition of parsley extract is at present not well known, but it can be noted that the BG reducing effect would have been due to substances other than non-phenolic flavonoids, ascorbic acid and phthalides, since these substances would have been oxidized during the harsh 30 min boiling treatment.

Table 2 shows values and differences between groups for lens NEG, GSH and total protein at day 42. Although macroscopic evaluation revealed no opacification of rat lenses in any groups, NEG was significantly higher than controls (C; Cp) in both diabetic groups (D, Dp) (p = 0.0001). NEG in the Dp group was lower than in the D group, but the difference was not significant (p = 0.358, Table 2).
NEG of lens proteins causes alterations in their structure and function. Consistent with results of other studies (Bresnic, 1982; Cotlier, 1987; Davson, 1990; Luthra & Balasubramanian, 1993; Swamy et al., 1993; Yarat et al., 1995; King & Branskota, 1994; Chait & Bierman, 1994), NEG levels in the diabetic groups were found to be higher than in controls. Various means of preventing these NEG level increases have been investigated with in vivo and in vitro studies (Yarat et al., 1998a). However, there have been no prior diabetic studies related to parsley and the lens. A trend was observed where parsley administration caused a non-significant decrease of about 11% in lens NEG in diabetic group (Dp; Table 2).

GSH was significantly lower than controls (C, Cp) in both diabetic groups (D, Dp) ($p_{\text{int}} = 0.001, 0.029$, Table 2). It was about 30% higher in the Dp group than in the D group, although the difference was not significant ($p_{\text{int}} = 0.155$, Table 2). Diabetes impairs the function of the lens antioxidant system and glutathione is one of the antioxidant factors. Decreased lens glutathione has been reported in experimental diabetes and in diabetic patients (Cotlier, 1987; Davson, 1990; Yarat et al., 1995; Kamei, 1993). Our findings were consistent with these, the decrease being about 53% in the diabetic rat group. Investigations have shown the decrease of lens glutathione to be inhibited by aldose reductase inhibitors (Yarat et al., 1998a). GSH at 42 days was about 12% lower (non-significant) in the Cp group compared with the C group (Table 2). This effect may have been due to xenobiotics found in parsley extracts, free radical enhancer xenobiotics having been reported to cause a 30–35% decrease in reduced glutathione of the lenses with in vitro treatments (Bluyn & Bluyn, 1991).

Yarat et al. found vitamin B6 to cause decrease of lens glutathione in both control and diabetic groups and attributed this to B6 causing increased GSH consumption (Yarat et al., 1998b). The anticancer agent, Adriamycin, has also been found to accelerate GSH decrease in rats on a riboflavin-free diet (Dutta et al., 1990).

In another of our lens studies, in which the experimental conditions were the same, it was found that chard (Beta vulgaris L. var. cicla) significantly inhibited both NEG increase and GSH reduction (Yarat et al., 1998a).

Lens total protein was only found to differ significantly between the control (C) and diabetic (D) groups ($p_{\text{int}} = 0.008$, Table 2). The protein bands obtained by Laemmli SDS-PAGE were in the same position for every sample and found at the same molecular weights as in previous studies (Yarat et al., 1995; 1998a,b). There were no significant differences in the concentrations in any of the bands between any of the groups (not shown).

No significant correlations were found between any of the lens parameters. In summary, uncontrolled induced diabetes caused a significant decrease in lens GSH and an increase in blood glucose and NEG of the lens proteins. Administering
parsley extracts was not found to significantly moderate the GSH and NEG effects although it did moderate the BG increase.

References