PLASMA PROTEIN OXIDATION IN DIABETIC RATS AFTER SUPPLEMENTATION WITH *Teucrium polium* EXTRACT

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Summary

The protein oxidation provoked by free radicals has been demonstrated to play a significant role in the pathogenesis of diabetes. The aqueous extract of *Teucrium polium* (Lamiaceae) has long been used in Iran as an anti-diabetic agent. The high insulinotropic and antihyperglycemic activity of *Teucrium polium* crude extract using both animal and/or isolated rat pancreatic islets has recently been published. In the present study, we investigated whether *Teucrium polium* extracts (TPE) could have antioxidant effect on oxidative protein damage parameters such as protein carbonyl (PCO), advanced oxidation protein products (AOPP), and protein thiol (P-SH), as well as oxidative stress parameters such as total thiol (T-SH), nonprotein thiol (Np-SH), and lipid peroxidation in the plasma of diabetic rats. For diabetes induction, STZ was injected intraperitoneally at a single dose of 40 mg kg^{-1} to a group of rats. The TPE (equivalent to 0.5 g plant powder per kg body weight) was administered orally (i.g) to a group of STZ diabetic rats for 30 consecutive days. PCO, and AOPP levels were increased, P-SH, Np-SH, and T-SH levels were all decreased in the plasma of diabetic rats. Moreover, plasma lipid peroxidation levels were significantly increased in diabetic rats compared to those of the control group. TPE supplementation of diabetic rats significantly decreased the level of protein oxidation markers such as PCO, and AOPP along with lipid peroxidation in the plasma. In addition, treatment of diabetic rats with TPE also significantly increased the P-SH level as well as Np-SH, and T-SH in the plasma of plant-treated rats. Our data clearly indicated that TPE, by decreasing oxidative stress, may be effective in preventing oxidative protein damages which are thought to be involved in the pathogenesis of diabetes and its complications.

Introduction

There are convincing experimental and clinical evidences that the generation of reactive oxygen species (ROS) is increased in both type of diabetes and that the onset of diabetes is closely associated with oxidative stress. Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to damage

of cellular organelles and enzymes, increased lipid and protein oxidation and development of complication of diabetes mellitus (1,2). The role of oxidative protein damage in the pathogenesis of the diabetic state is being investigated extensively. The protein oxidation provoked by free radicals has been demonstrated to play a significant role in the pathogenesis of diabetes (3,4). Radical-mediated damage to proteins may be initiated by electron leakage, metal-ion dependent reactions and autoxidation of lipids and sugars. Accumulation of modified proteins disrupts cellular function either by loss of catalytic and structural integrity or by interruption of regulatory pathways (5). Major molecular mechanisms leading to structural changes in proteins are metal-catalyzed protein oxidation characterized by protein carbonyl (PCO) formation, loss of protein thiol (P-SH) groups and advanced oxidation protein products (AOPP) formation (6). In the case of excessive production of ROS, endogenous protective mechanisms may not be sufficient to limit ROS and the damages they cause. To maintain the balance between oxidative and antioxidative process and to avert oxidative stress, an external source of the antioxidant protection system is consequently crucial (7).

Various natural products have long been used in traditional medical systems for treating diabetes. Most of them contain different antioxidants with potent scavenging activities for ROS. Therefore, it might be assumed that these products or isolated natural compounds could play a very important role in the treatment of diabetes (8). The aqueous extract of *Teucrium polium* (Lamiaceae) has been used in some parts of Iran as an antidiabetic agent. The high insulinotropic and anti-hyperglycemic activity of *Teucrium polium* crude extract using both animal and/or isolated rat pancreatic islets has recently been published (9). Due to its rich content of compounds with antioxidant activities, recently world wide attention has been paid to its clinical applications (10). Therefore, it seems interesting to elucidate whether *Teucrium polium* extract is capable of preventing the oxidative protein damages which are though to be involved in the pathogenesis of diabetes.

Materials and methods

Plant material

Aerial parts of *Teucrium polium L.* were collected from Fars province during spring. A voucher herbarium specimen (No.570) was deposited in the herbarium of the school of pharmacy, Shaeed Beheshti University of medical sciences, Tehran, Iran. The plant aerial parts were air-dried, protected from direct sunlight, and then powdered. The powder was kept in a closed container at 10 ^{o}C.

Extraction

The powdered plant material (250 g) was extracted three times with ethanol-water (7:3, v/v), at room temperature. The combined extracts were concentrated under reduced pressure and the volume was adjusted to 500 ml (containing 0.5 g plant powder per ml).

Experimental animals and procedure

Male wistar albino rats (n=30), 5-7 months old with a weight of 200-250 g (purchased from Pasteur Institute, Tehran, Iran) were housed under conventional conditions and were allowed free access to food and water ad libitum. All experiments were carried out according to the guidelines for the care and use of experimental animals and

approved by state veterinary administration of the University of Tehran. Streptozotocin (STZ; Sigma, USA; 40 mg/kg body weight) was dissolved in 0.1 M sodium citrate buffer at pH 4.5 just before use and injected intraperitoneally (i.p) to 22 rats. Control rats (group1, n=8) received with the same route of administration an equivalent volume of citrate buffer. One week after STZ administration, the diabetic rats with blood glucose levels higher than 280 mg/dl were selected and distributed in two groups (2 and 3). The plant extract was administered by gavages (i.g.) to 7 rats of group 3 in a dose of 1 ml/rat (equivalent to 0.5 g plant powder/kg body weight) for 30 consecutive days. The blood glucose levels of rats in each group were determined using glucose oxidase kit according to manufacture's instructions (Pars azmoon, Tehran, Iran). The rats were then sacrificed to collect the blood. Serum was separated immediately by centrifugation (3000 rpm, 10 minutes). The protein concentration was determined by the method of Lowry (11) using bovine serum albumin as the standard.

Analyses of oxidative stress markers of the plasma

Plasma PCO levels were measured spectrophotometrically by using the method of Reznick et al. (12). PCO groups react with 2,4-dinitrophenylhydrazine (DNPH) to generate chromophoric dinitrophenylhydrazones. The absorbance was measured at 360 nm, using the molar extinction coefficient of DNPH ($\varepsilon = 2.2\times10^4$ $cm^{-1}M^{-1}$). AOPP levels were determined according to Cakatay et al. method (6). The concentration of AOPP for each sample was calculated by using the extinction coefficient of 26 l mM^{-1} cm^{-1} and the results were expressed as μM. Total thiols (T-SH) concentration was determined by using 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) as described by Sedlak and Lindsay (13). After protein precipitation with 10% TCA (trichloroacetic acid), plasma Np-SH level was determined in the resulting supernatant in the same manner. P-SH levels were determined from the difference between T-SH and Np-SH. Sample absorbances were measured at 412 nm. Plasma malondialdehyde (MDA) levels, an index of lipid peroxidation, were measured by the double heating method of Draper and Hadley (14). The method is based on the spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA. The concentration of MDA was calculated based on the absorbance coefficient of the TBA-MDA complex ($\varepsilon = 1.56\times10^5$ cm^{-1} $M^{-1)}$ and it was expressed as nmol/mg protein.

Statistical analyses

Statistical analyses were performed using student's t-test. All data are presented as means ± S.D. The statistical significances were achieved when $P< 0.05$.

Results and discussion

A significant increase in the level of blood glucose and a decrease in body weight were observed in diabetic rats when compared to control rats. Administration of TPE to diabetic rats significantly decreased the level of blood glucose and increased body weight gain to near control level (Table 1).

Several clinical studies show increase in levels of oxidative stress markers in diabetic patients, for instance malondialdehyde and 8-epi-prostaglandin F2 (indices of lipid peroxidation), 8-hydroxy deoxyguanosine (index of DNA damage) (7). Nevertheless

there is controversy about which markers of oxidative stress are most reliable and suitable for clinical practices.

Table 1. Serum glucose levels and body weight of control, diabetic and TPE-treated rats. Each measurement has been done at least in triplicate and values are the means ± S.D. for six rats in each group.

Parameters	Control	Diabetic	TPE-treated
Initial body weight (g)	236 ± 11	222 ± 6	210 ± 7
Final body weight (g)	305 ± 24	154 ± 7	247 ± 7
Initial serum glucose (mg/dl)	87 ± 19	284 ± 9*	336 ± 21
Final serum glucose (mg/dl)	88 ± 14	294 ± 10*	98 ± 8**

*significantly different from normal rats (P< 0.05).
**significantly different from diabetic rats (P<0.05).

The use of oxidative modification products of proteins as a marker have some advantages over other markers, because of their relatively early formation, greater stability and reliability and also their longer lifespan (3,6). Specific markers used to determine protein oxidation and oxidative stress in the control, diabetic and plant-treated rats are given in Table 2. Plasma PCO, and AOPP levels as well as MDA concentration were increased in the diabetic rats compared with those of the control rats (Table 2). Under TPE supplementation, plasma PCO, AOPP and MDA levels were significantly decreased in the plant-traded rats compared with those of the diabetic rats. In addition, there was significant difference between the plasma P-SH, T-SH, and Np-SH levels in the three groups of rats. Plasma P-SH, T-SH, and Np-SH levels were decreased in the diabetic rats compared with those of control group. After administration of TPE, plasma P-SH, T-SH, and Np-SH levels were restored to normal levels compared with those of diabetic group.

Table 2. Changes in the concentration of protein carbonyl (PCO), advanced oxidation protein products (AOPP), protein thiol (P-SH), nonprotein thiol (Np-SH), total thiol (T-SH) and malondialdehyde (MDA) levels in plasma of control and experimental animals. Each measurement has been done at least in triplicate and values are the means ± S.D for six rats in each group.

Parameters	Control	Diabetic	Diabetic+TPE
PCO (nmol/mg protein)	0.74±0.12	1.31±0.08*	0.92±0.11**
AOPP (μM)	14.3±2.2	31.8±4.4*	20.6±3.7**
P-SH (nmol/mg protein)	123±11	81±8*	113±10**
T-SH (nmol/mg protein)	182±14	114±21*	174±15**
Np-SH (nmol/mg protein)	59±8	33±4*	61±7**
MDA (μM)	1.8±0.15	3.5±0.33*	2.12±0.40**

*Significantly different from normal (P< 0.05)
**Significantly different from diabetic group (P< 0.05).

Major molecular mechanisms leading to structural changes in proteins under diabetic condition are metal-catalyzed protein oxidation characterized by PCO formation, loss of P-SH groups and AOPP formation. Carbonyl (CO) groups (aldehydes and ketones) are produced on protein side chains (especially of Pro, Arg, Lys, and Thr) when they are oxidized. In deed, measurement of PCO has been used as a sensitive assay for

oxidative damages of proteins (3). It has already been shown that free radicals cause oxidation of protein SH groups in plasma. Albumin is the most abundant plasma protein and is a powerful extracellular antioxidant.
The measurement of plasma thiol (T-SH) is a good reflection of excess free radical generation, since the conformation of albumin is altered, allowing SH groups to be oxidized (6,13). Recently, the occurrence of protein oxidative damage in diabetic patients was confirmed by a novel marker, AOPP which is a good reflection of excess free radical generation in vivo. AOPP, defined as dityrosine containing cross linked protein products, are significantly elevated in diabetic patients compared to healthy individuals (6,15). In our intervention, we found an increased production of plasma PCO and AOPP and loss of P-SH in diabetic rats. Treatment with TPE, however, significantly reduced PCO and AOPP contents and increased P-SH levels in the plant-treated rats compared to diabetic subjects. This clearly indicate that TPE, by decreasing oxidative stress, may be effective in preventing oxidative protein damages which are thought to be involved in cellular damages under the diabetic condition. Lipid peroxidation is one of the characteristic features of chronic diabetes and may bring about protein damage and inactivation of membrane-bound enzymes either through direct attack by free radicals or through chemical modification by its end products such as MDA and 4-hydroxynonenal (16). In the present study, induction of diabetes in rats uniformly resulted in an increase in MDA levels. However, treatment of the diabetic rats with TPE significantly decreased MDA content.

The present study demonstrates that TPE has potential antioxidant properties with the final affection on inhibiting protein oxidation and reducing oxidative stress under diabetic condition. These results clearly reveal that *Teucrium polium* extract might act as a potential antioxidant candidate for biological systems susceptible to free radical-mediated reactions and therefore, it might reduce the risk of chronic diseases such as diabetes. Based on these data, additional studies are needed to characterize the bioactive compounds of the plant which are the cause of the observed results.

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