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Araştırma makalesi

Effect of Oleuropein on Element Distributions in Liver of Diabetic Rats

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Abstract

There is accumulating evidence demonstrating that the metabolism of many trace elements is modified in diabetes mellitus. In addition, essential elements and minerals are key to nutrition and sound health. Oleuropein, a major phenolic compound in olives, is known to reduce the blood glucose levels in alloxan induced diabetic rats and rabbits. The purpose of this study was to compare the levels of essential trace elements, selenium (Se), manganese (Mn), copper (Cu), chromium (Cr) and zinc (Zn) in Streptozotocin (STZ)-induced diabetic rats and to evaluate the effects of oleuropein on trace elements levels. Animals were apportioned into 4 groups of 8 rats each. The control group was fed with standard rat provender and got no added treatment. In the oleuropein group, 20 mg/kg dosage of oleuropein was given to normal animals intraperitoneally (i.p) for 28 days. In the diabetic group, STZ was injected to rats at a single dose of 50 mg/kg i.p. The last group, 20 mg/kg dosage of oleuropein was given to diabetic animals i.p for 28 days. In this study, trace elements levels were evaluated by using ICP-MS and MDA, SOD and CAT levels were evaluated by using spectrophotometers methods. Trace elements levels were significantly ($p<0,05$) decreased in diabetic rats liver but oleuropein was significantly ($p<0,05$) increased trace element levels in this group. In the diabetic group, serum blood glucose levels were significantly ($p<0,05$) increased and STZ increased total oxidant status (TOS), malondialdehyde (MDA) in the liver, whereas it decreased superoxide dismutase (SOD), catalase (CAT) and total antioxidant capacity (TAS) in diabetic rats liver. As a consequence, oleuropein treatment shows an antioxidant and in diabetes by reducing oxidative stress and it was increased trace element levels.

Keywords: *Oleuropein, diabetes, trace elements, antioxidant, liver.*

Özet

Diabetes mellitusta birçok eser elementin metabolizmasının değiştirildiğini gösteren kanıtlar vardır. Ek olarak, temel elementler ve mineraller beslenme ve sağlığın anahtarıdır. Zeytinlerde önemli bir fenolik bileşik olan Oleuropein, alloksanın neden olduğu diyabetik sıçanlarda ve tavşanlarda kan glikoz seviyelerini düşürdüğü bilinmektedir. Bu çalışmanın amacı, Streptozotosin (STZ) ile indüklenmiş diyabetik sıçanlarda temel eser elementlerin, selenyum (Se), manganez (Mn), bakır (Cu), krom (Cr) ve çinko (Zn) düzeylerinin karşılaştırılması ve Oleropeinin eser element düzeylerine etkisini değerlendirmektir. Hayvanlar, her biri 8 sıçandan oluşan 4 gruba ayrıldı. Kontrol grubu standart fare yemi ile beslendi ve ilave tedavi almadı. Oleropein grubunda 28 gün boyunca normal hayvanlara intraperitoneal olarak (i.p) 20 mg / kg oleuropein verildi. Diyabetik grupta, STZ sıçanlara tek bir dozda 50 mg / kg (i.p). Son grupta, 28 mg diyabetik hayvanlara 20 mg / kg oleuropein verildi. Bu çalışmada, eser element düzeyleri ICP-MS kullanılarak

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değerlendirilmiş ve MDA, SOD ve CAT düzeyleri spektrofotometre yöntemleri kullanılarak değerlendirilmiştir. Diyabetik sıçan karaciğerlerinde eser element düzeyleri anlamlı düzeyde ($p < 0,05$) azalırken, bu grupta oleuropein iz element düzeylerinde anlamlı artış ($p < 0,05$) saptandı. Diyabetik grupta serum kan glukoz düzeyleri anlamlı derecede ($p < 0,05$) artmış ve STZ karaciğerde total oksidan statü (TOS), malondialdehid (MDA), süperoksit dismutaz (SOD), katalaz (CAT) , total antioksidan kapasite (TAS) azalmıştır.Sonuç olarak, oleuropein tedavisi oksidatif stresi ve diyabeti azaltarak bir antioksidan etki gösterdi ve eser element düzeylerini arttırdı.

Anahtar Kelimeler: *Oleuropein, diyabet, eser elementler, antioksidan, karaciğer.*

1. Introduction

Diabetes mellitus is a bunch of metabolic disorders characterized by hyperglycemia. There are several types of diabetes caused by complicated genetic reactions and environmental factors. Hyperglycemia is caused by defects in insulin secretion, reduced glucose utilization and increased glucose production depending on the type of diabetes. End organ damage is the result of metabolic disorder in patients with diabetes, which is accompanied by other important problems for patients and health care system.

Oleuropein is also known as prevent the progression of hepatic steatosis [1]. Poudyal et al. reported that olive leaf extracts contain polyphenols such as oleuropein and its major metabolite, hydroxytyrosol, which ameliorate the cardiovascular, hepatic, and metabolic symptoms in rat models with obesity and diabetes induced by high-carbohydrate and high-fat diets (HFD) [2]. Olive leaf extracts were reported to lower blood cholesterol and lipid concentrations in cholesterol-fed rats [3,4].

Antioxidants deactivate free radicals and this situation leads to lipid peroxidation inhibition [5]. The molecules that are capable of inhibiting the oxidation of other molecules by repressing ROS are known as antioxidants [6]. Synthetic antioxidants may have contrary effects on the human body; thereby, much attention has been put toward several natural antioxidants [3]. Antioxidants from dietetic sources which are also known as non-enzymatic antioxidants include lipid-soluble vitamins such as vitamin E (alphatocopherol), vitamin A (carotenoids) and right along with watersoluble vitamin C (ascorbic acid) [7-9].

Zn is an important micronutrient, which has a major role in synthesis, storage, secretion and function of insulin and its metabolism is altered in diabetes [10,11]. Zn deficiency is correlated with insulin resistance [12]. In diabetic patients, chronic hyperglycemia due to glycosylation and peroxidation leads to increased oxidative stress and thereby proteins and lipids structure are changed [13].

Selenium depletion may lead to the development of vascular complications and microalbuminuria in diabetic patients by the way of increasing oxidative stress [14]. Selenium is a significant component of the enzymes that defend cells from the contrary effects of reactive oxygen species and free radicals [15].

Cu binding glycosylated proteins can cause oxidative reactions, increased oxidative stress and free radicals production in diabetes [16,17]. Cu binding glycosylated proteins can cause oxidative reactions, increased oxidative stress and free Cu ions by accelerating oxidative stress in diabetes. Therefore, plasma level of Cu is increased which can induce oxidative activity. In contrast, antioxidant defense system reduces damages by decreasing oxidative stress [18,19].

Chromium (Cr) is another element, which affects insulin action. The requirement of insulin would be reduced with sufficient dietary intake of Cr, which is very critical to prevent adverse effects of diabetes. Cr deficiency in diet causes impaired glucose tolerance. Chromium supplementation has an anti-diabetic effect and improves fasting blood sugar (FBS), insulin function, insulin-receptor binding, increased pancreas beta cells sensitivity and glucagon action [19].

In the light of this literary information, we aimed to investigate whether the effect of oleuropein , which known as antioxidant, on the level of varying elements in diabetes rat liver.

2. Materials and Methods

2.1. Chemicals

Oleuropein and STZ were obtained from Sigma-Aldrich (Sigma-Aldrich Chemical Co. St. Louis, MO, USA). All other chemicals and reagents were of analytical reagent grade and bought from trading sources.

2.2. Animals and Experimental Design

Healthy male Wistar rats, 60 days old and weighing 180–200 g, were obtained from the Breeding Laboratories of the Experimental Animal Research and Application Center (Afyon, Turkey Animal). The animals were held at room temperature (25°C) and relative humidity (50–55%) in a 12 h light/dark cycle with *ad libitum* accession to a standard gnawer diet and water. The rats were let to acclimatise to the animal facility for at least seven days before the experiment started. Before starting the experiment, rats were fed with standard gnawer diet for one week for the purpose of adaption to the laboratory conditions.

The animals fasted overnight and diabetes was generated by using a single intraperitoneal injection (i.p) of a freshly produced solution of STZ (50 mg/kg body weight) in dissolved in 0.1 M citrate buffer (pH 4.5) [20]. The animals were let to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Rats of control group were injected with 0.9% saline only. 48 h after the STZ injection, STZ rats' fasting blood glucose concentration were measured by using glucometer, ACCU-CHEK (Bayer, Germany) and diabetes was confirmed. The rats with blood glucose level more than 200 mg/dl were regarded as diabetic and were used in the experiment. After the injection of STZ, the treatment was started on the second day and this was regarded as the first day of treatment.

The animals used in the experiment were equally distributed into four groups:

- Group 1 (n:8) General control: The group which was fed on a normal diet and not subjected to any procedure.
- Group 2 (n :8) diabetic group: The group which was given streptozotocin (STZ) "40 mg/kg for 28 days.
- Group 3 (n:8) oleuropein group: The group which was given 20 mg/kg/day oleuropein for 28 days [21].
- Group 4 (n:8) diabetic +oleuropein group: The group which was given 40 mg/kg streptozotocin +20 mg/kg/day oleuropein.

The rats were allocated into 4 groups and each group had 8 animals. Normal diet and tap water were supplied to both the control and treatment groups for a period of 28 days. The experimental protocols were confirmed by the Animal Care and Use Committee at Afyon Kocatepe University (2015/372-14) and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.3. Tissue Collection

Following the experimental procedures, the rats were sacrificed after an overnight fasting period (12 hours) under anesthesia with an injection of 65 mg/kg i.p ketamine and 7 mg/kg i.p xylazine. Liver samples from the rats were kept in tubes and all samples were stored at -80°C until use.

2.4. Measurement of elements, MDA, SOD, CAT, TAS, and TOS in liver

Malondialdehyde (MDA) level is LPO marker and it was verified by using the method of Ohkawa et al. in tissue homogenates by the reaction of thiobarbituric acid with MDA and spectrophotometric measurement at 532 nm [22]. The concentration of MDA was presented as nmol/g for tissue. The activities of superoxide dismutase (SOD) were measured according to Sun et al. by using the spectrophotometry at 560 nm; activities were presented in the unit of U/mg protein in tissue homogenate [23]. Catalase (CAT) activities were obtained according to Aebi [24]. The reaction mixture was consisted of 2.90 mL 50 mmol/L phosphate buffer (pH 7.0), 5 mL 10 mmol/L H_2O_2, and 50 mL sample. The reduction of H_2O_2 was followed at 240 nm for 45 s at room temperature. CAT activities were presented as nmol/mg protein in tissue homogenates. Total antioxidant status (TAS) levels were calculated according to Erel's method, which represents the quantity of plasma antioxidants, and were obtained by using a kit from Rell Assay Diagnostics in Turkey [25]. The results were presented as millimol Trolox equivalents per liter (mmol Trolox equiv./L). Also, the total oxidant status (TOS) levels were obtained by using a novel automated measurement method developed by Erel [26]. The results were calibrated with hydrogen peroxide and presented in the unit of micromolar hydrogen peroxide equivalent per liter (μmolH_2O_2 equiv./L). Zn, Cu, Cr, Se , Mn liver levels were determined by using ICP-MS (Termo scientific qtegra).

3. Statistical analysis

Data obtained from experimental animals were presented as means and standard deviation of means (±SD), and analyzed by using one-way analysis of variance (ANOVA), followed by Duncan post-hoc tests on the SPSS (20) computer software program. A difference in the mean values of $P<0.05$ was regarded as significant.

4. Results

4.1. Effect on blood glucose levels

Animals induced by STZ regularly exhibited hyperglycemia ($P<0.05$) compared to initial glucose levels. Oleuropein treatment led to reduction in the elevated serum glucose levels in STZ diabetic rats, as measured at the end of the study ($p<0.05$).

4.2. Liver MDA, SOD, CAT, TAS, and TOS levels

There were significant increases of MDA and TOS levels whereas decreases TAS and CAT and SOD levels in the diabetic group compared to control group (Table 1). Additionally, oleuropein treatment decreased to their levels in diabetic rats compared to diabetic groups ($p<0.05$). These results supported that the administration of oleuropein alleviated the STZ-induced alteration of oxidative stress and antioxidant status compared to the diabetic group ($P<0.05$).

Table 1
MDA, SOD, CAT, TAS TOS levels

Group	MDA (nmol/g tissue)	SOD (IU/mg protein)	CAT (IU/mg protein)	TAS (mmol Trolox Equiv./L)	TOS (μmol H_2O_2 Equiv./L)
Group 1	4.04±0.78^{c}	50 2.04±0.42^{a}	23.04±4.42^{a}	3.54±0.17^{a}	4.52±0.6^{c}
Group 2	18.4±2.11^{ba}	23.53±7.23^{c}	15.63±2.08^{c}	1.54±0.15^{c}	7.52±0.4^{a}
Group 3	3.65±0.73^{ac}	50 1.90±0.22^{a}	23.53±7.23^{a}	3.21±0.49^{a}	4.32±0.4^{c}
Group 4	9,04±0,42^{cb}	34.02±2.96^{b}	19.46±1.05^{b}	2.95±0.18^{b}	5.41±0.1^{b}

*a,b,c In the same column values with different letters show statistically significant differences in liver MDA,SOD, CAT levels and liver TAS, TOSlevels ($p<0.05$).Mean±SD: Standard Deviation

4.3. Liver Element Levels

Liver Zn, Cr, Mn Cu, and Se levels significatlly ($p<0,05$) decreased in diabetics rats as compeared controls and these significatlly ($p<0,05$) increased in oleuropein groups compeared diabetics groups (Table 2).

Table 2
Element levels

Group	Zn	Se	Mn	Cu	Cr
Group 1	4.58 ± 0.21^{a}	9.19 ± 1.36^{a}	1.68 ± 0.26^{a}	32.20 ± 9.14^{a}	8.19 ± 1.26
Group 2	2.46 ± 0.97^{c}	8.86 ± 2.42^{b}	0.06 ± 0.20^{c}	19.79 ± 3.87	6.86 ± 2.12^{c}
Group 3	4.32 ± 0.72^{a}	9.25 ± 1.84^{a}	1.63 ± 3.10^{a}	40.56 ± 7.57	8.25 ± 1.84^{a}
Group 4	3.65 ± 0.52^{b}	7.06 ± 1.15^{b}	1.21 ± 0.65^{b}	39.91 ± 8.96	7.06 ± 1.15^{b}

*a,b,c In the same column values with different letters show statistically significant differences in liver element levels ($p<0.05$). Mean±SD: Standard Deviation

5. Discussion

The content of metal concentration in different organs has influence on metabolism (enzyme activity) of living organisms.

In this study, investigated association between diabetes of mineral elements and oleuropein effect on these elements. Diabetes is a chronic metabolic disease affecting the whole world. Especially peoples living in the developing part of the world are becoming more victim of diabetes because of globalization; urbanization and sedentary life. Generally, research findings in the past revealed the strong associations between disturbed blood parameters and end glycated products in the blood sample of diabetes patients. For example, findings were reported an abnormal relationship between plasma trace metal element amount and hyperglycemia in the blood sample of diabetic patients.

However, osmotic dieresis due to hyperglycemia has been mentioned as a potential factor for the presences of disturbed trace metal elements in the blood sample of diabetic patients [27].

In the current study, Zn^{+2} was the only trace metal element strongly shows significant plays a life-sustaining role in many biological processes and plays a significant role in the function of insulin and carbohydrate metabolism [28].

In this study, Zn levels significantlly decreased in diabetic rat group compared to control group. These findings compatible with previous studies. Previous studies have linked zinc to glycemic control in individuals with diabetes [28-30]. From our results, one can clearly observe the high Zn nonuptake in diabetic rats. The trivalent metal element Cr^{+3} enhances the action of insulin in the uptake of glucose at the cellular level (36). Cr^{+3} serves as an important antioxidant element that improves glucose intolerance and dyslipidemia [31,32]. ln this study, Cr^{+3} levels decreased in diabetic groups.

Mg^{+2} plays an extremely significant role in the activation and modulation of many enzymes that are involved in carbohydrate and insulin metabolism.

Mg^{+2} and Cr^{+3}in the blood would play a greater role in controlling metabolic crisis among diabetes mellitus patients [32,33]. In previous studies, shown that trace element levels changed in diabetic rat models [34,35].

Zinc may affect other elemental levels (Cr, Mn Cu, Se) that were found in the diabetic group powerfully support the results of researchers who declare that zinc has insulin-like effects [36,37].

In this study, oleuropein increased that decreased element levels. This can be explained the antioxidant properties of oleuropein. At the same time, oleuropein has been reported to have beneficial anti-diabetic functions such as reduction of glycemia and enhanced glucose tolerance in diabetic animal models [38,39].

The observed changes can influence especially the enzyme activity where metals are cofactors and finally the organism homeostasis. Probably during treatment of some diseases, it would be desirable supplementation with chosen elements, which can help to regulate activity of enzymes with metal cofactors. In the similar investigation it would be important to measure not only elements content, but also enzymes activity.

In summary, here we report, for the first time to our knowledge, that oleuropein is a novel natural compound inducing anti-diabetic function and antioxidant effect. Our data suggested that its element levels changed in diabetics and oleuropein is effective on element levels due to its antioxidant effects.

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