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Variation in body weight and some hematological parameters in streptozotocin-induced diabetic rats, treated with *Teucrium orientale*

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Abstract

Teucrium orientale (TO) have previously been shown to have hypoglycemic and antioxidant effects on experimental diabetic animals. This study was designed to examine the effects of methanol extract from *T. orientale* on hematological parameters and body weight in experimental diabetic rats. In this research 32 male albino Wistar rats, with body weights of 200 – 240 g were randomly allocated into four groups with 8 rats per each. Diabetes was induced in rats by intraperitoneal injection of streptozotocin (STZ) (60 mg/kg). Methanol extracts were administered at a dose of 200 mg kg^{-1} by gavage over a 21-day period. The results indicate that the administration of *T. orientale* to diabetic rats resulted in a significant increase in body weight ($p<0.01$) and in the levels of packed cell volume (PCV), erythrocytes, leukocytes and lymphocytes, while neutrophil levels were decreased compared with diabetic group ($p<0.05$). It may be concluded that TO extract improve hematological properties in experimental diabetic rats following repeated treatment for 3 weeks.

KEY WORDS: TEUCRIUM ORIENTALE, HEMATOLOGICAL PARAMETERS, STREPTOZOTOCIN, BODY WEIGHT

Introduction

Diabetes mellitus (DM) is related to a group of metabolic alterations that continues to be major health problem worldwide (1). It is characterized by absolute or relative deficiencies in insulin secretion, action or both associated with chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism (2). Diabetes is recognized for severe complications such as diabetic nephropathy, neuropathy and retinopathy (3-5). The complications are major causes of morbidity and mortality in DM.

Although insulin treatment and other chemical therapies can control the disease to various extents, the complications are very common, whose pathologic base is microangiopathy (6). Abnormalities in hemorheology have been related to the development of diabetic micro- and macroangiopathy. On the other hand, alterations in hemorheology might also be a consequence of underlying vessel wall injury in diabetic patients with manifest microangiopathy (7).

Plants with an antioxidant property still remain a major source for drug discovery in spite of the great development of synthetic molecules are used for the management and/or control of complication of diabetes mellitus such hemolytic anemia and affecting arteries. Unfortunately, only a few of such medicinal plants have been scientifically validated (8).

Teucrium orientale (TO) L. var. orientale is a wild aromatic plant belonging to the labiatae family (9). Our recent study has reported the anti-diabetic property of this plant (10). According to a previous survey, TO possess potent antioxidant and DPPH radical scavenging activities that these activities related to the presence of flavonoids and other phenolic compounds (9).

Therefore this study is designed to investigate the possible effects of the methanol extract of TO, under study on body weight and hematological properties in streptozotocin-induced diabetic rats.

Methods

Chemicals and reference marker compound Streptozotocin (STZ) was purchased from Pharmacia and Upjohn (USA), Ethylenediaminetetraacetic acid (EDTA) from Merck (Darmstadt, Germany) and other salts were obtained from Merck (Darmstadt, Germany).

Plant material

Aerial parts (stems, leaves, flowers) of *T. orientale*, growing wild in Iran, were collected in May 2010 from Mishow-Dagh, Marand (North-Weast of Iran).The aerial parts of the plant were gently washed in tap water and completely dried under room temperature (25±2°C) for 2 weeks protected from direct heat or sunlight. Preparation of *T. oriental* methanol extract (TO) The powdered plant material (160 g) was extracted with methanol (MtOH) (90%), at room temperature overnight. The extraction was repeated three-times and the solvent was evaporated in vacuum, and dried extracts were stored at 4°C until use (11).

Animals

The study was conducted on thirty matured Wistar male albino rats, were obtained from the

experimental animal care centre of faculty of pharmacy, Tehran university of medical science, 15 weeks old, weighing 200 -250 g which were housed in colony cages (four rats per cage) at an ambient temperature of 25 ± 2°C with 12 h-light and 12 h-dark cycle. The rats were fed normal diets purchased commercially from vendors and also had free access to water *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for one week and then randomly divided into various groups.

Induction of experimental diabetes

STZ-induced diabetes mellitus was produced in a batch of normoglycemic male Wistar rats (fasting blood glucose level of 75 ± 5 mg/dl). STZ freshly dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, was immediately injected intraperitoneally (60 mg/kg) (12, 13). This single dose of streptozotocin

produced type-I diabetes mellitus after 24 h of injection and this diabetic state is maintained throughout the experimental schedule.

Treatment of animals

Rats were divided into four groups of eight rats each: Group I (C): normal control rats. Group II (STZ): diabetic rats, received STZ in single dose (60 mg/kg , intraperitoneal way). Group III (TO): TO treated rats, received only TO (200 mg/kg bw, oral gavage) for 21 days. Group IV (STZ + TO): TO-treated diabetic rats received by oral gavage, 3 days after STZ treatment, 200 mg/kg bw of TO for 21 days. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The treatment was started on the third after STZ injection and continued for 21 days.

Blood Sample Collection

At the end of experiment, animals were anesthetized by infusion of ketamine (60 mg/kg) and xylazine (10 mg/kg). Five ml of blood was collected from each animal. Part of the blood sample was put in an EDTA bottles for hematological determinations (7).

Hematological analysis

The CBC was performed on an automated hematology analyzer using well mixed whole blood to which EDTA was added to prevent clotting. CBC count was measured by Sysmex XS800i, hematology analyzer with flourescance technology (Diamond Diagnostics-USA)

Weight assessment

The weight of each rat was monitored daily as an index of the physical status of the animals over the period of study using compression spring balance (BAW-660-M).

Results

Figure 1 shows the body weight changes in the normal and experimental animals in each group. The mean body weight of the diabetic rats decreased compared to extract-treated rats. There was a significant reduction in body weight of the diabetic rats compared with normal and TO treated diabetic rats. After administration of methanol extract of TO for 21 days there was significant increase in the body weight of diabetic rats ($p<0.01$).

The hematology parameters of each group are presented in table 1. In normal, non-diabetic rats treated with TO, considerable increases in red blood cell count (RBC) and packed cell volume (PCV) and hemoglobin (Hb) were observed when compared with the normal untreated rat ($p < 0.05$).

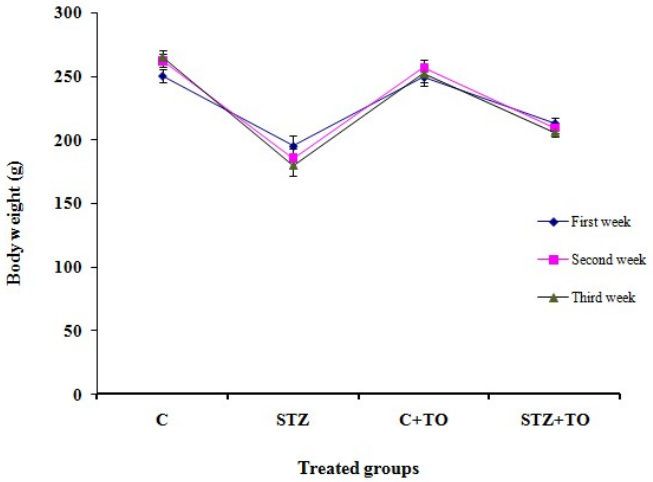

Figure 1. Effect of TO orally for 21 days on body weight in normal and STZ-induced diabetic rats. Values are mean±S.E. of eight animals. Differences of $p<0.01$ were considered significant. Significant differences: a (STZ), (STZ + TO) and (C+TO) vs. C; b (STZ + TO) and (C+TO) vs. (STZ)

The RBC in diabetic group was significantly lower than control group ($p < 0.05$). The RBC count of diabetic untreated rat was reduced significantly ($p < 0.05$), while there were significant ($p < 0.05$) increases in RBC count of diabetic rats treated with Oral administration of TO. The white blood cell (WBC) and lymphocytes (LYM) of the normal (nondiabetic) rats treated with TO significantly increased ($p < 0.05$) while neutrophils (NEU) was significantly reduced ($p < 0.05$).

Discussion

Diabetes mellitus describes a metabolic disorder

of multiple etiology, which is characterized by chronic hyperglycemia (14). Recurrent or persistent hyperglycemia during diabetes causes non-enzymatic glycosylationof body proteins (15). Some biomolecules, such as hemoglobin (Hb) and RBC membrane proteins, are modified by glycation (16). This structural alteration may lead to impaired protein function, and perhaps contribute to the long-term complications of diabetes. Lipid peroxidation is a marker of oxidative stress and also one of the prime factors involved in RBC damage caused by free radicals during diabetes (17). Glycosylation of membrane proteins and lipid peroxidation may lead to RBC membrane hemolysis and anemia (18). In the present study, TO administration was shown to decrease lipid peroxide and serum glucose level that leading to a decrease susceptibility of RBC to hemolysis.

The present data also show that an increase of 9.21×10^9/L in WBC was recorded for of TO treated rats as against WBC of the control rats (7.91×10^9/L). Also the value of LYM for the treatment group with TO extract animals which were given the plant extract increased to 67% from 77% recorded for the control rats. This may go a long way to suggesting that extract of TO must have influenced the defense mechanism of the test rats. So the continuous exposure of the body systems of animals to these medicinal products (herbs) may cause lymphocytosis, which may then account for the use of this plant for medicinal purposes (19-21).

During the 21-day experimental period the body weight is reduced in diabetic rats, whereas there was a significant gain of body weight in TO treated rats. The failure of STZ-induced diabetic rat to gain weight has already been reported (22, 23). The administration of TO restored these levels significantly (P <0.01) towards normal. The ability of the methanol extract to restore body weight seems to be a result of its ability to reduce hyperglycemia (10). Diabetic rats treated with the methanol extract showed an increase in body weight compared to diabetic control. This may also be due to the protective effect of the extract in controlling muscle wasting i.e. reversal of gluconeogenesis (24). Oxidative stress has been shown to play a key role in the pathogenesis of diabetes as such, antioxidants may have a role in the alleviation of diabetes (25). The progressive increase in weight suggests that methanol extract TO can attenuate the toxicity of STZ, particularly at high dose. It might be possible that treatment with TO can lead to a better utilization of nutrients in the diet and thus a gain in weight. In conclusion, the increase in RBC, PCV, WBC and LYM counts following administration of the methanol extract of *T. orientale* may signify the positive effects on the haemopoietic system of experimental rats and might be capable of improving the hematological abnormalities associated with pathophysiology of diabetes mellitus.

References

1. Langhi C, Cariou B. Cholesterol metabolism and beta-cell function. Med Sci (Paris) 2010; 26(4):385–90.
2. Rahmati R, Karimi M, Parivar K, Kadivar M. Construction of an optimized lentiviral vector containing pdx-1 gene for transduction of stem cells towards gene therapy diabetes type 1. Armaghane-danesh 2013; 6:493-484
3. Teimouri F, Amirkabirian N, Esmaily H, Mohammadirad A, Aliahmadi A, Abdollahi M. Alteration of hepatic cells glucose metabolism as a non-cholinergic detoxication mechanism in counteracting diazinon-induced oxidative stress. Hum Exp Toxicol 2006; 25(12):697-703.
4. Babu PVA, Sabitha KE, Shyamaladevi CS. Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. Chem Biol Interact 2006; 162:114-20.5.
5. Wolffe SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med 1991; 10: 339–52.
6. Muhammad NO, Akolade JO, Usman LA, Oloyede OB.Haematological parameters of alloan-induced diabetic rats treated with leaf essential oil of Hoslundia Opposite (VAHL). Excli Journal 2012; 11:670-676.
7. Vague P, Raccah D, Juhan-Vague I. Hemobiology, vascular disease, and diabetes with special reference to impaired fibrinolysis. Metabolism 1992; Suppl.1: 2-6.
8. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001; 109: 69–75.
9. Cakir A, Mavia A, Kazaz C, Yildirim A, Kufrevioglu OI. Antioxidant activities of the extracts and components of Teucrium orientale L. var. orientale. Turk J Chem 2006; 30:483 – 494.
10. Dehghan G, Tahmasebpour N, Hosseinpourfeizii MA, Sheikhzadeh F, Banan Khojasteh SM. Hypoglycemic, antioxidant and hepato- and nephroprotective effects of Teucrium orientale in streptozotocin diabetic rats. Pharmacologyonline 2013; 1:189-182.
11. Dehghan G, Shafiee A, Ghahremani M, Ardestani S, Abdollahi M. Antioxidant potential of various extracts fromFerula szovitsiana in relation to their phenolic contents. Pharm Biol

2007; Vol. 45: No. 9, pp. 1–
12. Kaleem M, Asif M, Ahmed, QU, Bano B. Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. Singapore Med J 2006;47: 670-5.
13. George BO, Osioma E, Falodun A .Effect of At iko (Aframomum Sceptrum) and African Nutmeg (Monodora Myristicca) on reduced glutathione, Uric acid levels and liver marker enzymes in Streptozotocin-induce diabetic rats. Egyptian J Biochem 2010; 28(2): 67-78.
14. Negre-Salvayre A, Coatrieux C, Ingueneau C, Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. Br J Pharmacol 2008; 153:6-20.
Kennedy, L. and Baynes, JW. Non-enzymatic glycosilation and the chronic complications of diabetes: An Overview. Diabetologia 1984; 24: 93-98.
16. Koga T, Keiko M, Terao J. Protective effet of vitamin E analog, phosphatidylchromanol, against oxidative hemolysis of human erythrocytes. Lipids 1980; 33:589-595.
17. Sen S, Kar M, Roy A, Chakraborti AS. Effect of nonenzymatic glycation on function and structural properties of hemoglobin. Biophys Chem 2005; 113:289-298.
18. Kolanjiappan K, Manoharan S, Kayalvizhi M. Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin Chim Acta 2002; 326: 143–9.
19. Shivani M and Veena B. K. Effect of ethanolic extract of Parthenium hysterophorus on haematological parameters in rat. The Bioscan(esme jornale hamine ba the shoro mishe) 2010; 5(3):437-440.
20. Subba PV, Mangla A and Prakash KM. Clinical and immunological studies on person exposed to Parthenium hysterophorus L. Experianta 1976; 33: 1387-1388.
21. George MI and Adegoke AO. Effect of vitamin E on haematological parameters in albino rats treated with Gasoline. J Sci Res 2012; 4 (2): 437-444.
22. Marrif HI, Ali BH and Hassan KM. Some pharmacological studies on Artemisia herba –alba (Asso) in rabbits and mice. J Ethnopharmacol 1995; 49: 51-55.
23. Mansi K and Lahham J. Effects of Artemisia sieberi Besser (A. herba-alba) on heart rate and some hematological values in normaland alloxan-induced diabetic J. basic appl. sci 2008; 4(2): 57-62.
24. Burke JP, Williams K, Narayan KM, Leibson C, Haffner SM and Stern MP. A population perspective on diabetes prevention: whom should we target for preventing weight gain? Diabetes Care 2003 ;(7): 1999-2004.
25. Eslamizad , Tajvar A, Mohammadirad A S, Abdollahi M. Study on the oxidative stress status among cement plant workers. Hum Exp Toxicol 2008; 27(6):463-9.

Parameters	Contorol	Diabetes	Contorol+TO	Diabetes+TO
RBC(x 10^6/mm^3)	8.6 ± 0.76	6.5 ± 0.46[a]	9.21 ± 0.96[ab]	7.21 ± 0.66[ab]
WBC(x 10^9/L)	7.91 ± 0.45	6.9 ± 0.54	9.21 ± 0.5[ab]	8.21 ± 0.55[b]
Hb (g/dl)	15.21 ± 2.26	11.7 ± 1.8[a]	16.11 ± 2.36[ab]	13.21 ± 1.96[ab]
Neutrrophils%	24± 2.26	34 ± 3.66[a]	14± 2.76[ab]	28± 3.26[b]
Basophils%	2 ± 0.26	3 ± 0.16	2 ± 0.16	3 ± 0.26
Eosinophils%	4 ± 0.58	3 ± 0.36	3± 0.45	4± 0.56
Lymphocytes%	67 ± 3.26	56 ± 4.48[a]	77 ± 5.26[ab]	61± 6.26[ab]
Monocots%	3 ± 0.44	4 ± 0.76	4 ± 0.58	4 ± 0.57
PCV%	46 ± 4.26	37 ± 3.08[a]	51 ± 4.45[ab]	40 ± 3.76[a]

Table 1. Hemorheological parameters of control, STZ-diabetic and diabetic treated rats after three weeks treatment with TO.

Values are mean±S.E. of eight animals; RBC= red blood cell; WBC=white blood cell; Hb = hemoglobin.PCV= packed cell volume. Differences of $p<0.05$ were considered significant. Significant differences: a (STZ), (STZ + TO) and (C+TO) vs. C; b (STZ + TO) and (C+TO) vs. (STZ)