ELSEVIER

Pathophysiology 18 (2011) 111–115

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Antihyperglycemic and antihyperlipidemic effect of *Rumex patientia* seed preparation in streptozotocin-diabetic rats

Reza Sedaghat [a], Mehrdad Roghani [b,*], Maedeh Ahmadi [c], Faezeh Ahmadi [c]

[a] *Department of Anatomical Sciences and Pathology, School of Medicine, Shahed University, Tehran, Iran*
[b] *Department of Physiology, School of Medicine and Medicinal Plant Research Center, Shahed University, Tehran, Iran*
[c] *School of Medicine, Shahed University, Tehran, Iran*

Received 16 February 2010; accepted 18 March 2010

Abstract

Background and objective: *Rumex patientia* (RP) could exert beneficial health effects to ameliorate metabolic diseases. The effect of subchronic feeding of RP seeds was evaluated on serum glucose and lipid profile in streptozotocin (STZ)-diabetic rats. **Methods:** Wistar rats were divided into control, RP-treated control, diabetic, glibenclamide-treated diabetic, and RP-treated diabetic groups. For induction of diabetes, streptozotcin was administered at a dose of 60 mg/kg. Meanwhile, RP-treated groups received RP seed powder mixed with standard pelleted food at a weight ratio of 6% for 4 weeks. Serum glucose and lipid levels were determined before the study and at 2nd and 4th weeks after the study in addition to the oxidative stress markers in hepatic tissue. **Results:** Serum glucose was significantly lower in RP-treated diabetic rats at 2nd and 4th weeks as compared to untreated diabetics ($p < 0.05$ and $p < 0.01$, respectively). Serum total cholesterol and triglyceride levels did not show significant reductions in RP-treated diabetic rats as compared to untreated diabetics. Serum HDL-cholesterol, however, significantly increased ($p < 0.05$) and LDL-cholesterol showed a significant reduction ($p < 0.05$) in RP-treated diabetic rats as compared to untreated diabetics ($p < 0.05$). RP also attenuated the increased malondialdehyde (MDA) content and reduced activity of superoxide dismutase (SOD) in hepatic tissue. **Conclusion:** Subchronic treatment of diabetic rats with RP could lessen the abnormal changes in blood glucose level and to improve lipid profile regarding HDL- and LDL-cholesterol in part due to its attenuation of lipid peroxidation in hepatic tissue.

Keywords: *Rumex patientia*; Diabetes mellitus; Blood glucose; Blood lipids

1. Introduction

Chronic hyperglycemia in diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, and cardiovascular system [1]. In addition to hyperglycemia, several other factors such as dyslipidemia or hyperlipidemia are also involved in the development of cardiovascular complications in diabetes which are the major causes of morbidity and mortality [2]. Diabetic patients also exhibit abnormal antioxidant status, auto-oxidation of glucose, and excess glycosylated proteins [3–4]. Oxidative stress in diabetes leads to tissue damage, with lipid peroxidation, inactivation of proteins, and protein glycation as intermediate mechanisms for its complications [5] including retinopathy, nephropathy, and coronary heart disease [6–7].

Several approaches are presently available to reduce the hyperglycemia in diabetes mellitus including insulin therapy which suppresses glucose production and augments glucose utilization, treatment by sulfonylureas, which stimulates pancreatic islet cells to secrete insulin; metaformin, which acts to reduce hepatic glucose production; α-glucosidase inhibitors, which interfere with glucose absorption. Unfortunately, all of these therapies have limited efficacy and various side effects and thus searching for new classes of compounds is essential to overcome these problems [8]. Recent interests are focusing on the use of medicinal plants with antidiabetic and antioxidant potential in reducing the ensuing complications in diabetic patients [9]. Plant-based pharmaceuticals have been employed in the management of various mankind diseases [10]. They are as essential part of human diet and

* Corresponding author. Fax: +98 2188966310.
E-mail address: mehjour@yahoo.com (M. Roghani).

0928-4680/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pathophys.2010.03.001

are present in plant extracts that have been used for centuries in oriental medicine. Antioxidant properties, ROS scavenging and cell function modulation of medicinal plants and their effective substances could account for the large part of their pharmacological activity [11–12]. Antioxidant and free radical scavenging activity [13], gastroprotective and anti-ulcerogenic activity in gastric tissue [14], protecting pancreatic B cells against STZ-induced damage [15], and anti-inflammatory effect [16] of *Rumex patientia* L. (RP) have already been reported. In addition, *R. patientia* seed (grain) aqueous extract has been used for the treatment of type I diabetes induced by STZ [17]. The aim of this study was to assess hypoglycemic and hypolipidemic effect of subchronic feeding of *R. patientia* seeds in streptozotocin-diabetic rats.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) (190–240 g) were housed in an air-conditioned colony room at 21 ± 2 °C and supplied with standard pellet diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with NIH guidelines for the care and use of laboratory animals.

2.2. Experimental protocol

Male Wistar rats ($n = 40$) were divided into equal-sized control, RP-treated control, diabetic, glibenclamide-treated (positive control), and RP-treated diabetic groups. The rats were rendered diabetic by a single intraperitoneal injection of 60 mg kg^{-1} STZ freshly dissolved in cold normal saline. Age-matched normal animals that received an injection of an equivalent volume of normal saline comprised a non-diabetic control group. Diabetes was confirmed by the presence of hyperglycemia, polyphagia, polydipsia, polyuria and weight loss after 1 week. One week after STZ injection, blood samples were collected and serum glucose concentrations were measured using glucose oxidation method (Zistshimi, Tehran). Only those animals with serum glucose higher than 250 mg dL^{-1} were selected for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. *R. patientia* seeds were prepared from Isfahan Natural Resources Center, systemically identified with a voucher number of 2006-69-1 (Shahid Beheshti Biology Department, Tehran), dried under shade and finally ground. Plant-mixed food pellet at a weight ratio of 6 was prepared using pellet-making device (Nooran Co., Tehran). This ratio was chosen according to our pilot study. Glibenclamide was orally administered as 600 μg/kg/day in 10% Cremophor. Changes in body weight, food consumption and water intake were regularly recorded during the experimental period. In addition, serum triglyceride, total cholesterol, and HDL-cholesterol levels were spectrophotometrically measured using appropriate enzymatic kits (Zistshimi, Tehran). LDL and very low density lipoprotein (VLDL) cholesterol levels were calculated by the following formula:

$$\text{VLDL} = \frac{\text{Triglyceride}}{5}$$

$$\text{LDL} = \text{Total cholesterol} - \text{HDL cholesterol} - \text{VLDL}$$

2.3. Assay of MDA concentration and SOD activity in hepatic tissue

After removing liver and its cleansing of extra tissues, it was blotted dry and weighed, then made into about 5% tissue homogenate in ice-cold 0.9% NaCl solution. A supernatant was obtained from tissue homogenate by centrifugalization (1000 × *g*, 4 °C, 10 min). The MDA concentration (thiobarbituric acid reactive substances, TBARS) in the supernatant was measured. Briefly, 1.0 mL of 20% trichloroacetic acid and 1.0 mL of 1% TBARS reagent were added to 100 μL of supernatant, then mixed and incubated at 100 °C for 80 min. After cooling on ice, samples were centrifuged at 1000 × *g* for 20 min and the absorbance of the supernatant was read at 532 nm. TBARS results were expressed as MDA equivalents using tetraethoxypropane as standard [18].

For SOD activity, a competitive inhibition assay was performed by using xanthine/xanthine oxidase reaction-generated superoxide radicals to reduce nitro blue tetrazolium (NBT) quantitatively to blue formazan. Conversion of superoxide radicals to hydrogen peroxide by superoxide dismutase inhibited dye formation and served as a measure of superoxide dismutase activity. Briefly, 0.5 mL of supernatant was incubated with xanthine (50 μmol/L) and xanthine oxidase (2.5 μmol/L) in 50 mmol/L of potassium phosphate buffer (pH 7.8, 37 °C) for 40 min and NBT was added. Blue formazan was then monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50% maximum was defined as 1 nitrite unit (NU) of SOD activity [18]. The protein content of the supernatant was measured with Bradford method using bovine serum albumin as the standard.

2.4. Drugs

Streptozotocin was obtained from Pharmacia and Upjohn (USA). All other chemicals were purchased from Merck (Germany) and Temad (Iran).

2.5. Data and statistical analysis

All values were given as means ± S.E.M. Statistical analysis was carried out using repeated measure and one-way ANOVA followed by Tukey post hoc test. A statistical p value less than 0.05 considered significant.

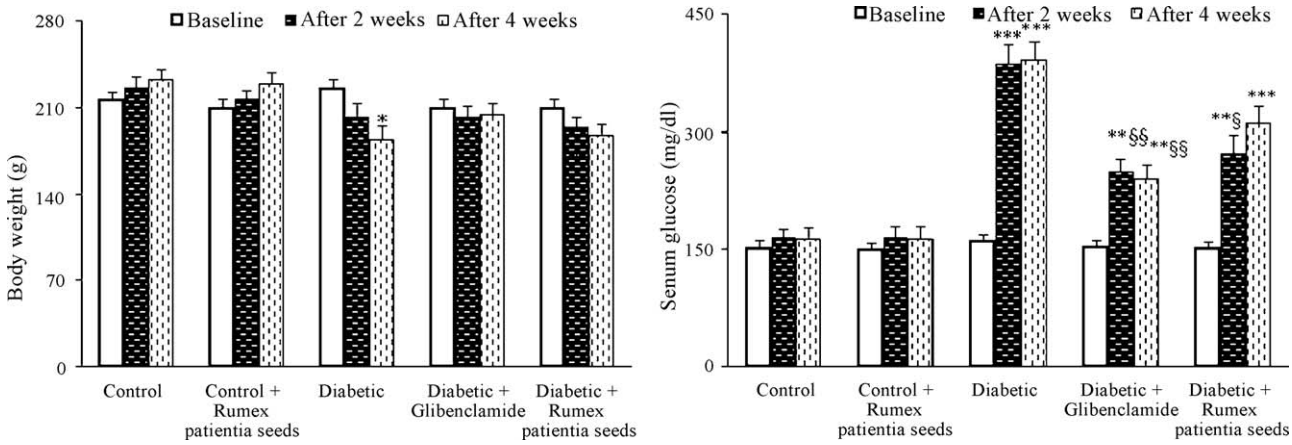

Fig. 1. Body weight and serum glucose levels in control and STZ diabetic rats receiving *Rhumex patientia* seed preparation after 2 and 4 weeks treatment (means ± S.E.M). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (as compared to baseline in the same group); $^{\S}p<0.05$, $^{\S\S}p<0.01$ (as compared to diabetic in the same week).

3. Results

Body weight and serum glucose measurements (Fig. 1) indicated that before diabetes induction, there were no significant differences among experimental groups. After 4 weeks, the weight of the vehicle-treated diabetic rats was significantly decreased as compared to control rats ($p<0.05$) and RP-treated diabetics showed a less non-significant decrease as compared to vehicle-treated diabetics. Although glibenclamide administration to diabetic group prevented body

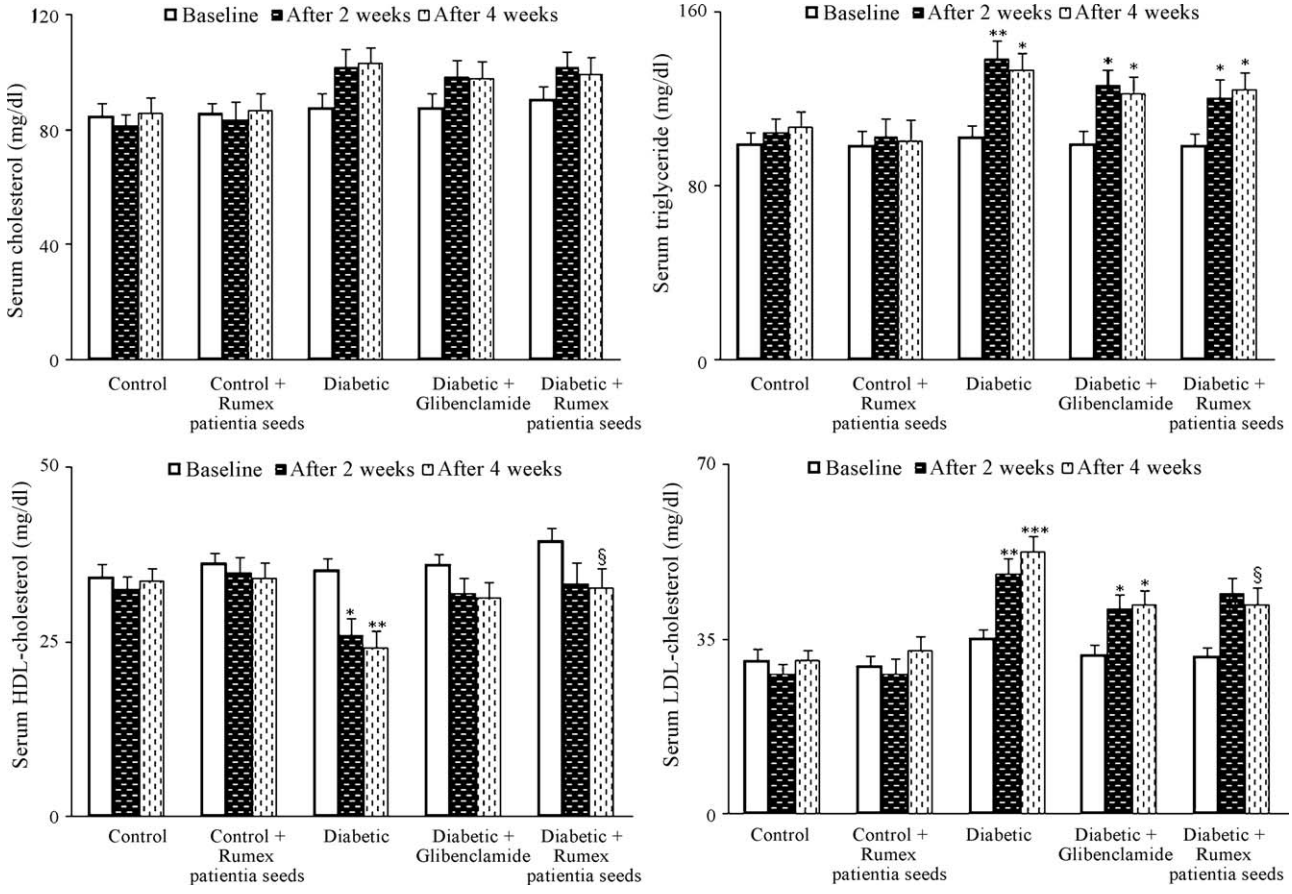

Fig. 2. Serum total cholesterol, triglyceride, HDL- and LDL-cholesterol concentrations in STZ diabetic and control rats receiving *Rhumex patientia* seed preparation after 2 and 4 weeks treatment (means ± S.E.M). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.005$ (as compared to baseline in the same group); $^{\S}p<0.05$ (as compared to diabetic in the same week).

Table 1
MDA concentration and SOD activity in hepatic tissue in control and STZ diabetic rats receiving *Rhumex patientia* seed preparation.

Groups	Hepatic tissue	
	MDA (μmol g^{-1} protein)	SOD activity (kNU g^{-1} protein)
Control ($n=6$)	4.3 ± 0.3	158.6 ± 7
Control + *Rumex patientia* ($n=5$)	4.6 ± 0.4	163.2 ± 6
Diabetic ($n=6$)	$7.8 \pm 0.5^{**}$	$82.3 \pm 8^{***}$
Diabetic + *Rumex patientia* ($n=6$)	$5.7 \pm 0.5^{*,\#}$	$123.4 \pm 7^{*,\#}$

* $p<0.05$ (as compared to control).
** $p<0.01$ (as compared to control).
*** $p<0.005$ (as compared to control).
$^{\#}$ $p<0.05$ (as compared to diabetic).

weight reduction, this group showed a lower weight gain compared to controls. Untreated diabetic rats had also an elevated serum glucose level over those of control rats ($p<0.001$) and treatment of diabetic rats with RP caused a significant decrease in the serum glucose ($p<0.05$) only at 2nd week relative to vehicle-treated diabetics. In addition, RP treatment of control rats did not produce any significant change regarding serum glucose level and glibenclamide-treated diabetic group showed a marked lower serum glucose relative to untreated diabetic group ($p<0.01$) and serum glucose level in this group was higher than control one ($p<0.01$) (Fig. 1).

After 4 weeks from the diabetes induction a non-significant increase in total cholesterol and a significant increase in triglyceride ($p<0.05$), LDL-cholesterol ($p<0.005$) and a significant reduction in HDL-cholesterol ($p<0.01$) were observed compared with the baseline data (data before the study) and RP treatment significantly improved only HDL- and LDL-cholesterol. Regarding RP-treated control group, there was not any significant improvement regarding lipid profile (Fig. 2).

With respect to hepatic biochemical markers (Table 1), STZ-induced diabetes resulted in an elevation of MDA content and decreased SOD activity ($p<0.01$–0.005) in this tissue and treatment of diabetic group with RP for 4 weeks significantly lowered MDA content and significantly attenuated the reduced activity of SOD ($p<0.05$). Meanwhile, there was no significant change in RP-treated control group relative to control animals regarding these parameters.

4. Discussion

R. patientia seed feeding for 4 weeks exhibited a hypoglycemic effect and it improved serum lipid profile regarding HDL- and LDL-cholesterol. Although glucose-lowering effect of *R. patientia* seed was not significantly in the control group. The subchronic *R. patientia* treatment showed a marked hypoglycemic effect in diabetic rats, indicating hypoglycemic mechanism of *R. patientia* to be different in diabetic and non-diabetics. A study by Degirmenci et al. showed that RP could protect and in part restore secretory function of beta cells in pancreatic tissue, in this way exerting its antihyperglycemic and antidiabetic effect [15]. One of the main constituents of *R. patientia* plant is catechins [19]. Several studies carried out on catechins have indicated their anti-diabetic potential [20–22]. Such compounds have been suggested to inhibit hepatic gluconeogenesis through a ROS-dependent pathway. Catechins also mimic the cellular effects of insulin such as reducing gene expression of rate-limiting gluconeogenic enzymes [23–24]. Furthermore, catechins in a similar manner like the hormone insulin may increase tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 and it reduces phosphoenolpyruvate carboxykinase gene expression in a phosphoinositide 3-kinase-dependent manner. Catechins also mimic insulin by increasing phosphoinositide 3-kinase, mitogen-activated protein kinase, and p70(s6k) activity [25].

In addition to its hypoglycemic effect, *R. patientia* seed was also able to improve some lipid metabolites including HDL- and LDL-cholesterol levels in diabetic rats and non-significantly to reduce level of serum triglyceride and total cholesterol. Diabetes has been reported to be associated with profound alterations in lipid and lipoprotein profile [26]. Lowering of plasma or tissue lipid levels leads to a decrease in the risk of micro- or macrovascular disease and related complications [27]. *R. patientia* may have improved lipid profile directly or indirectly through reducing blood glucose in diabetic animals. ROS and NO have also possible roles in pathogenesis of diabetes complications [28]. Possible sources of oxidative stress in diabetes condition include an increased production of ROS, especially from enhanced glycation and lipoxidation processes [29]. Substantial evidence suggests that *R. patientia* elicit anti-oxidant properties by attenuating the lipid peroxidation caused by various forms of free radicals [13] and in this way may have affected lipid profile. This fact was verified by a lower MDA content and an increased activity of SOD in hepatic tissue of treated diabetics in this study.In conclusion, subchronic treatment of diabetic rats with *R. patientia* seeds at a weight ratio of 6% could prevent abnormal changes in blood glucose and to improve lipid profile regarding HDL- and LDL-cholesterol in diabetic rats. More studies are warranted to evaluate whether such therapy can be administered as an auxiliary beneficial therapeutic regimen in diabetic population.

Acknowledgment

This study was financially supported by a research grant from School of Medicine affiliated to Shahed University (Tehran, Iran) (1386).

References

[1] A.I. Vinik, E. Vinik, Prevention of the complications of diabetes, Am. J. Manag. Care 9 (2003) S63–S80.
[2] C.A. Reasner, Reducing cardiovascular complications of type 2 diabetes by targeting multiple risk factors, J. Cardiovasc. Pharmacol. 52 (2008) 136–144.
[3] R.B. Nawale, V.K. Mourya, S.B. Bhise, Non-enzymatic glycation of proteins: a cause for complications in diabetes, Indian J. Biochem. Biophys. 43 (2006) 337–344.
[4] T. Nishikawa, E. Araki, Impact of mitochondrial ROS production in the pathogenesis of diabetes mellitus and its complications, Antioxid. Redox Signal. 9 (2007) 343–353.
[5] S.P. Wolffe, Z.Y. Jiang, J.V. Hunt, Protein glycation and oxidative stress in diabetes mellitus and ageing, Free Radic. Biol. Med. 10 (1991) 339–352.
[6] T.J. Lyons, Oxidized low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes? Diabet. Med. 8 (1991) 411–419.
[7] E. Valezquez, P.H. Wincour, P. Kestsven, K.G. Alberti, M.F. Laker, Relation of lipid peroxides to macrovascular disease in type 2 diabetes, Diabet. Med. 8 (1991) 752–758.
[8] D.E. Moller, New drug targets for type 2 diabetes and the metabolic syndrome, Nature 414 (2001) 821–827.
[9] G. Suji, S. Sivakami, Approaches to the treatment of diabetes mellitus: an overview, Cell Mol. Biol. 49 (2003) 635–639.
[10] K. Shapiro, W.C. Gong, Natural products used for diabetes, J. Am. Pharm. Assoc. 42 (2002) 217–226.
[11] D.W. Laight, M.J. Carrier, E.E. Anggard, Antioxidants, diabetes and endothelial dysfunction, Cardiovasc. Res. 47 (2000) 457–464.
[12] M. Ajay, M.R. Mustafa, Chronic treatment with flavonoids prevents endothelial dysfunction in spontaneously hypertensive rat aorta, J. Cardiovasc. Pharmacol. 46 (2005) 36–40.
[13] I.A. Lone, G. Kaur, M. Athar, M.S. Alam, Protective effect of *Rumex patientia* (English Spinach) roots on ferric nitrilotriacetate (Fe-NTA) induced hepatic oxidative stress and tumor promotion response, Food Chem. Toxicol. 45 (2007) 1821–1829.
[14] I. Gürbüz, A.M. Ozkan, E. Yesilada, O. Kutsal, Anti-ulcerogenic activity of some plants used in folk medicine of Pinarbasi (Kayseri, Turkey), J. Ethnopharmacol. 101 (2005) 313–318.
[15] I. Degirmenci, M.C. Ustuner, Y. Kalender, S. Kalender, H.V. Gunes, The effects of acarbose and *Rumex patientia* L. on ultrastructural and biochemical changes of pancreatic B cells in streptozotocin-induced diabetic rats, J. Ethnopharmacol. 97 (2005) 555–559.
[16] H. Süleyman, L.O. Demirezer, A. Kuruüzüm, Z.N. Banoğlu, F. Göçer, G. Ozbakir, A. Gepdiremen, Antiinflammatory effect of the aqueous extract from *Rumex patientia* L. roots, J. Ethnopharmacol. 65 (1999) 141–148.
[17] H.V. Gunes, I. Degirmenci, M. Aydin, B. Bozan, E. Aral, Z. Tunalier, The effects of *Rumex patientia* L. and *Urtica dioica* L. on some blood and urine parameters, and liver and kidney histology in diabetic rats, Turk. J. Med. Sci. 29 (1999) 227–232.
[18] M. Roghani, T. Baluchnejadmojarad, Chronic epigallocatechin-gallate improves aortic reactivity of diabetic rats: underlying mechanisms, Vascul. Pharmacol. 51 (2009) 84–89.
[19] Y. Yuan, W.S. Chen, S.Q. Zheng, G.J. Yang, W.D. Zhang, H.M. Zhang, Studies on chemical constituents in root of *Rumex patientia* L., Zhongguo Zhong Yao Za Zhi 26 (2001) 256–258.
[20] E.K. Song, H. Hur, M.K. Han, Epigallocatechin gallate prevents autoimmune diabetes induced by multiple low doses of streptozotocin in mice, Arch. Pharm. Res. 26 (2003) 559–563.
[21] M. Roghani, T. Baluchnejadmojarad, Hypoglycemic and hypolipidemic effect and antioxidant activity of chronic epigallocatechin-gallate in streptozotocin-diabetic rats, Pathophysiology 17 (2010) 55–59.
[22] S. Anton, L. Melville, G. Rena, Epigallocatechin gallate (EGCG) mimics insulin action on the transcription factor FOXO1a and elicits cellular responses in the presence and absence of insulin, Cell Signal. 19 (2007) 378–383.
[23] M.E. Waltner-Law, X.L. Wang, B.K. Law, R.K. Hall, M. Nawano, D.K. Granner, Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production, J. Biol. Chem. 277 (2002) 34933–34940.
[24] Y. Koyama, K. Abe, Y. Sano, Y. Ishizaki, M. Njelekela, Y. Shoji, Y. Hara, M. Isemura, Effects of green tea on gene expression of hepatic gluconeogenic enzymes in vivo, Planta Med. 70 (2004) 1100–1102.
[25] Q.F. Collins, H.Y. Liu, J. Liu, Z. Liu, M.J. Quon, W. Cao, Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5′-AMP-activated protein kinase, J. Biol. Chem. 282 (2007) 30143–30149.
[26] A.J. Krentz, Lipoprotein abnormalities and their consequences for patients with type 2 diabetes, Diabetes Obes. Metab. 5 (2003) S19–27.
[27] B.G. Brown, X.Q. Zhao, D.E. Sacco, J.J. Albers, Lipid lowering and plaque regression: new insights into prevention of plaque disruption and clinical events in coronary disease, Circulation 87 (1993) 1781–1791.
[28] H. Chen, E.C. Carison, L. Pellet, J.T. Moritz, P.N. Epstein, Overexpression of metallothionein in pancreatic beta-cells reduces streptozotocin-induced DNA damage and diabetes, Diabetes 50 (2001) 2040–2046.
[29] D. Bonnefont-Rousselot, J.P. Bastard, M.C. Jaudon, J. Delattre, Consequences of the diabetic status on the oxidant/antioxidant balance, Diabetes Metab. 26 (2000) 163–176.