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Full Length Research Paper

Antidiabetic activity of *Rosmarinus officinalis* and its relationship with the antioxidant property

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Oxidative stress plays an important role in diabetic pathogenesis. *Rosmarinus officinalis* L. was first used as an antioxidant agent for inhibition of diabetic nephropathy. Oxidative stress induced by Streptozotocin (STZ) has been shown to damage pancreatic beta cell and produce hyperglycemia in rats. In the present study, an attempt was made to examine the action of *R. officinalis* against experimental diabetes as well as the antioxidant potential of the leaf extract. Water extract of *R. officinalis* (200 mg/kg body weight for 21 days) was found to be significantly reducing the blood sugar level. The oxidative stress produced by Streptozotocin was found to be significantly lowered when compared to control rats. This was evident from a significant decrease in blood sugar level and oxidative stress makers including serum TBARS and nitric oxide (NO). Serum enzymatic (glutathione transferase (GST), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidants (vitamin C and reduced glutathione) were found to be increased by the administration of *R. officinalis*. These results indicate that *R. officinalis* extract effectively reduced the oxidative stress induced by Streptozotocin and potential reduction in blood sugar level.

Key words: Diabetes, hypoglycemia, oxidative stress, antioxidant enzymes, Streptozotocin, *Rosmarinus officinalis* L.

INTRODUCTION

Diabetes mellitus is one of the most pressing global health problems. It is estimated that the prevalence of diabetes mellitus will be more than 300 million in 2025 (Ojewole et al., 2006). Type II diabetes is the most common form of the disease and usually involves insulin resistance and β-cell dysfunction (Lupi et al., 2008). The Streptozotocin (STZ) treated rats, a model of type II diabetes, exhibit increased blood glucose and decreased insulin levels on postnatal day one. Therefore, type II diabetes can be studied *in vivo* using this animal model (Portha et al., 2007).

Oxidative stress is produced during normal metabolic process in the body as well as a variety of environmental factors and chemical substances. Oxidative stress has been shown to have a significant effect in the causation of diabetes mellitus as well as diabetes related completions in human beings (Wilson, 1998).

Oxidative stress in diabetes mellitus has been shown to coexist with a reduction in the antioxidant status and glycation of proteins, inactivation of enzymes, and alteration in structural functions of collagen basement membrane (Nirmala et al., 2011).

A previous study has shown that increased thiobarbituric acid reactive substances (TBARS) in rats with STZ-induced diabetes are an indirect measure of the production of free radicals (Semiz and Sen, 2007). Based on ethno botanical information, there are approximately 800 herbal medicinal plants for controlling diabetes mellitus (Chandra et al., 2007). There are some evidence that an increased consumption of fruits and vegetables that contain phytochemicals reduces the risk of chronic diseases, such as diabetes (Myojin et al., 2008), cancer and cardiovascular diseases (Semiz and Sen, 2007; Myojin et al., 2008). Previous studies have reported that plants with hypoglycemic effect might work via different mechanism such as inhibition of glucose absorption, enhanced use of glucose by the liver and increased insulin secretion (Sezik et al., 2005).

One of these plants is Rosemary or *Rosmarinus officinalis* L. (Labiatae) which is an evergreen perennial

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shrub grown in many parts of the world. İt have been used as medicinal plants in folk medicine, but *Rosmarinus* itself was used for asthma, bronchitis, cold, flu, digestive, anemia, palpitation, dizziness, anxiety soothing hearth, pain, hypertension, insomnia, labyrinthitis, sluggishness memory, tachycardia, vitiligo, for high cholesterol and diabetes disease (Çakılcıoğlu and Türkoğlu, 2007, 2007a; Ugulu et al., 2009; Tetik, 2011; Bieski et al., 2012).

Rosemary contains caffeic acid, carnasol, rosmaridiphenol and rosmarinic acid, all of which are potent antioxidants as well as anti-inflammatory agents. Due to its antioxidants, rosemary can help prevent cataracts and the natural acids present in rosemary help in protecting the body's cells and DNA from free radical damage. Rosemary is also a good source of antioxidant vitamin E (alpha tocopherol) and other important antioxidants (Tavaafi et al., 2011). In the present study, we evaluated the effect of *R. officinalis* on blood sugar levels, markers of oxidative stress and antioxidant enzymes in blood in STZ- treated rats.

MATERIALS AND METHODS

Leaves of Rosemary were obtained from the local herbal market of Kingdom Saudi Arabia. Voucher specimens from plant material were deposited at the Herbal Museum, Department of Pharmacology, Faculty of Science, King Abdul-Aziz University of Medical Sciences for identification.

Preparation of the plant sample

The fresh leaves of plant material (5 g) were soaked in 50 ml of boiled water, after 1 h stirring, at room temperature. The supernatant was decanted and the residue was macerated two more days with distilled water. The pooled supernatants were combined and filtered.

Animals and treatment

Adult male albino rats were selected for the study. They were of the same age (2 months) and weight (150 to 200 g). The animals were housed in acrylic cages in standard conditions of temperature prior to the experiments for 1 week in order to adapt to the laboratory condition, fed with commercial diet and water *ad libitum*. The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by King Abdul Aziz University ethical committee was followed regarding experimental treatments.

Experimental design and treatment schedule

The experiment was carried out on 4 groups of five rats in each group to study the effect of plant water extract on STZ -induced diabetes and changes in antioxidants as follows:

Group 1: Healthy control rats, received distilled water.
Group 2: Diabetic control rats. A freshly prepared solution of Streptozotocin or STZ (45 mg/kg body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitonially to overnight fasted rats. STZ injected animals exhibited hyperglycemia within 48 to 36 h (Siddiqui et al., 1987). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.
Group 3: Normal rats administered water extract of rosemary, orally at a dose of 200 mg/kg body weight (dosage determined earlier), and
Group 4: Diabetic treatment rats received 1 ml water extract of Rosemary for 21 days after 36 h STZ injection. The treatment with rosemary 200 mg/kg/day (Tavaafi et al., 2011) was given daily for a period of 3 weeks using gastric cannula (Makino et al., 2002).

No detectable irritation, restlessness or adverse effect (that is, respiratory distress, abnormal locomotion and catalepsy) was observed in any animals after the extract administration.

Starting from the 1st day (3rd day of STZ-injection) of extract administration to diabetic rats, FBG (blood glucose) level was measured in every 7th day using glucometer (Mallick et al., 2007). On the 21th day of extract administration, all the animals were anesthetized (Nesdonal 50 mg/kg, i.p.), blood samples were obtained from hearts of overnight fasted rats by using micro-capillary technique and allowed to clot for 20 min in laboratory temperature and then centrifuged at 10000 rpm for 10 min for serum separation.

Biochemical parameters

The levels of oxidative stress makers including serum malondialdehyde (MDA) as the marker of lipid peroxidation by thiobarbituric acid (TBA) test and nitric oxide (NO) were measured by the method of Thayer (1984) and Ding et al. (1988), respectively. The levels of reactive oxygen species controlled by antioxidant enzymes like GST, CAT and GPx were measured by the method of Habig et al. (1974), Aebi (1983) and Rotruck et al. (1984), respectively. The nonenzymatic scavengers such as reduced glutathione (GSH) and vitamin C were measured by the methods of Moron et al. (1979) and Aye Kyaw (1978), respectively.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). The data were subjected to one-way analyses of variance (ANOVA) and student's t-tests using the Statistical Analysis System (SPSS 15.0) program. For all analyses, p values ≤ 0.05 were considered significant.

RESULTS

Blood glucose concentration

Glucose levels were found to be significantly increased after STZ administration, and there after decreased by administration of rosemary extract. Decrease in serum glucose may be due to the regeneration of beta cells of the pancreas, which were destroyed by STZ. Administration of rosemary extract produced a significant ($p<0.01$) decrease in the blood glucose as compared to diabetic control (Table 1).

Effect of rosemary on serum oxidative stress markers

Diabetes significantly increased serum malondialdehyde

Table 1. Blood glucose level in control, diabetic and treated rats.

Group	FBG (mg/dl)
Healthy control	117 ± 5.2[a]
Diabetic control	371 ± 5.4[c]
Normal + rosemary (200mg/kg)	86.4 ± 3.6[a]
Treated group	137 ±4.6[b]

Values are mean ± SD; values with different superscripts differ significantly ($p < 0.05$).

(MDA) - marker of lipid peroxidation- and NO in comparison with the control group. Treatment of diabetic animals with 200 mg/kg/day rosemary extracts significantly inhibited increase of MDA in comparison with the untreated diabetic animals ($p < 0.05$). These treatments can maintain the level of MDA at the same level compared to that of the control group. The present result revealed that water extracts of rosemary was capable of suppressing NO activity (Table 2).

Effect of rosemary on enzymatic and nonenzymatic antioxidants

The enzymatic antioxidant GST, CAT and GPx levels were found to be lower in diabetic rats compared to that of the control rats. These enzymatic antioxidant levels in diabetic rats treated with rosemary extract significantly ($p < 0.05$) increased to a level closer to the normal values (Table 3).

Figure 1 shows the low levels of nonenzymatic antioxidant vitamin C and reduced glutathione were observed in diabetic rats, when compared to that of control rats. The levels of these antioxidants were significantly increased in diabetic rats by treating with rosemary.

DISCUSSION

Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, muscular degeneration, diabetes mellitus, cancer etc are all contributed by oxidative damage. Hence the enzymatic and nonenzymatic antioxidant levels were measured in diabetic rats (Nirmala et al., 2011).

Increasing blood glucose levels in diabetes leads to overproduction of free radicals, defined as an imbalance between oxidants and antioxidants. Glucose autooxidizes in the presence of transition metal ions generating oxygen-free radicals make the membrane vulnerable to oxidative damage (Bakirel et al., 2008).

Blood MDA levels in STZ-induced diabetic rats increased because of lipid peroxidation (Huang and Zheng, 2006). Previous studies, both *in vitro* and *in vivo*, have shown antioxidation activity to inhibit and reduce the degeneration of cells through its antioxidation activity (Tavaafi et al., 2011).

Nitric oxide synthase (NOS) catalyzes the production of nitric oxide (NO). Inducible nitric oxide synthase (iNOS) is expressed by vascular endothelial cells and smooth muscle cells in response to cytokines, unlike the two other types of NOS, which are constitutive. NO produced by iNOS is implicated in inflammatory diseases (Chaiyasut et al., 2011). Aslan et al. (2007) reported that food and phytochemicals exerts NO –suppressing activity via three different pathways: The blocking of iNOS expression, inactivation of iNOS catalytic function and the scavenging NO; while NO suppressing effect primarily through regulation of cellular iNOS expression. In concomitant with the present results, Kim et al. (2003) found that Labiatae family exhibited strong suppressing activity upon NO production and thus provided further convincing evidence to illustrate, at least partially, the relative benefits of this family as anti -inflammation, anticancer or antioxidant. It seems that the extracts do not only exert NO-suppressing effect through direct scavenging of NO radicals but also through inhibition of NOS catalytic activity and /or suppression of iNOS expression.

Furthermore, the rather striking NO –suppressing activity of rosemary (Labiatae) has been documented according to one of its constituents, namely carnasol, a compound which inhibits iNOS by blocking the activation of tumor necrosis factor â (Grun et al., 2008). Cinnamic aldehyde, a compound found to be present in, specifically, the essential oil cinnamon as well as several flavonoides have been reported to decrease the transcriptional activity of iNOS gene (Bramati et al., 2003).

Moreover, Aslan et al. (2007) demonstrated the suppression of the oxidative stress and inflammatory response related to diabetes through the inhibition of tumor necrosis factor â- (TNF- â) signaling. Natural triterpenes such as ginsenosid Rh1, triterpenes isolated from Panax ginseng, inhibited iNOS expression and the activation of NF- â. In addition, oleanolic acid glycoside isolated from ampelopsis radix, markedly suppressed the activity of NF-â and production of NO.

GST, CAT and GPx constitute a mutually supportive team of defense against reactive oxygen species (ROS). The GST is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals and the hepatic removal of potentially harmful hydrophobic compounds from blood. Decrease in GST activity in diabetic rats might indicate decreased detoxification or free radical scavenging capacity in

Table 2. Effect of *R. officinalis* on oxidative stress markers in serum of control, diabetic and treated rats.

Group	MDA (nmol/ml)	NO (µmol/L)
Healthy control	4.42 ± 0.56[a]	48.3 ±3.8[a]
Diabetic control	9.59 ± 0.64[b]	80.09 ± 3.6[b]
Normal + rosemary (200 mg/kg)	3.75 ±0.27[a]	46.7 ± 2.4[a]
Treated group	4.06 ±0.29[a]	49.12 ±2.7[a]

Values are mean ± SD; values with different superscripts differ significantly ($p < 0.05$).

Table 3. Effect of *R. officinalis* on antioxidant enzymes in serum of control, diabetic and treated rats.

Group	GST(U/ml)	CAT (U/l)	GPx (mU/ml)
Healthy control	46.8 ± 1.05[a]	21.98 ± 1.33[a]	77.09 ± 1.25[a]
Diabetic control	20.39 ± 0.8[b]	8.51 ± 0.09[b]	13 ± 0.4[b]
Normal + rosemary (200 mg/kg)	47.9 ± 0.05[a]	21.33 ± 0.97[a]	76.4 ± 0.96[a]
Treated group	45.56 ± 1.77[a]	23.03 ± 1.31[a]	75.6 ± 1.17[a]

Values are mean ± SD; values with different superscripts differ significantly ($p < 0.05$).

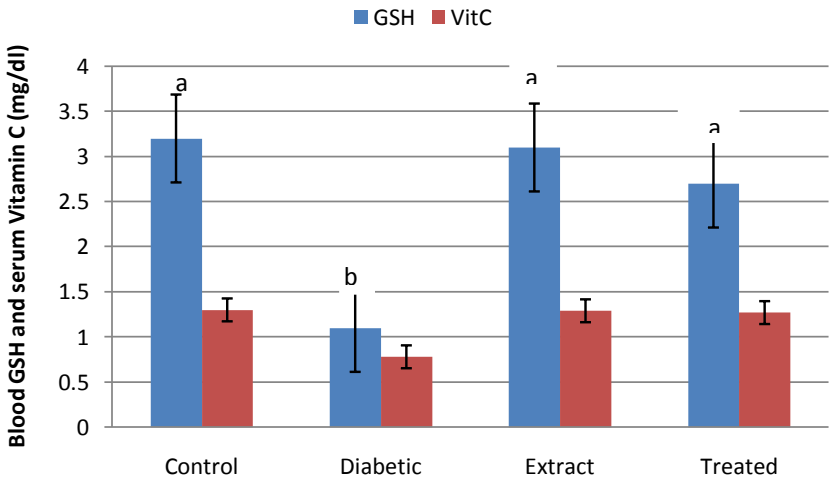

Figure 1. Blood GSH and serum vitamin C levels in experimental animals after treatment (mean ± SD, n = 5). Different lowercase letters indicate significant differences ($p \leq 0.05$).

diabetes. This decrease in GST activity may result from decreased enzyme production or enzyme inactivation. CAT is a hemeprotein, localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen and thus protects the body. GPx catalyses the reaction of hydro peroxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydro peroxide (Sabu and Kuttan, 2004).

In our study, decline in the activities of these enzymes in STZ induced animals and attainment of near normalcy in rosemary treated rats indicates oxidative stress elicited by STZ had been nullified due to the effect of the extract. This observation perfectly agrees with those of Krishnakumar et al. (1999) who have demonstrated hypoglycemic and antioxidant activity of rosemary extract in STZ induced diabetic rats. Similar to the finding in this study, a decrease has been observed in the activities of GST, CAT and GPx in some of the tissues of diabetic rats (Sekar and Govindasamy, 1990).

Glutathione (GSH) is a major non-protein thiol in living organism, which plays a central role in coordinating the body's antioxidant defense processes. Decreased GSH concentration contributes to the pathogenesis of

complications associated with the diabetic state (Meister, 1983). Reduced glutathione, synthesized mainly in the liver, is an important nonenzymatic antioxidant in the antioxidative defense system. Kaplowitz et al. (1985) have remarked that the marked depletion of GSH observed in the tissue of alloxan diabetic mellitus rats, may be due to the utilization of this compound by two antioxidant enzymes, GPx and GST as their substrate.

Vitamin C is an important water soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. It readily oxidizes to dehydroascarbic acid. Human beings have no ability to synthesis vitamin C due to mutation in the gene coded for L-gulonolactone oxidase, an enzyme required for biosynthesis of vitamin via the glucuronic acid pathway. Vitamin C at high doses has been shown to reduce the accumulation of sorbitol in the erythrocytes of diabetes and to inhibit the glycosylation of proteins (Davie et al., 1992). Transport of vitamin C into cell is facilitated by insulin. Many diabetics do not have enough intracellular Vitamin C. Therefore, a relative Vitamin C deficiency exists in many diabetics despite adequate dietary consumption (Cunningham, 1991).

In conclusion, the present study showed that *R. officinalis* extract brings back the blood glucose and also increase the antioxidant level in experimental rats. The aforementioned results show that *R. officinalis* has hypoglycemic activity. Hypoglycemic action of the herbal plant in diabetic rats may be possible through the insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production, or activation of gluconeogenesis in liver and muscle. It may prevent the hepatic injury and pancreas and suppressing the oxidative stress associated with diabetes. Although the exact chemical compounds responsible for the hypoglycemic effects of *R. officinalis* still remain speculative; experimental evidence obtained from this study indicates that *R. officinalis* has an antioxidant activity and also possesses hypoglycemic property.

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REFERENCES

Aebi H (1983). Catalase. In: Bergmeyer HU. Methods in Enzymatic analysis, New York, Academic Press, 3: 276-286.

Aslan M, Deliorman D, Orhan N, Orhan E, Sezik Yesilada E (2007). "*In vivo* antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. plicatum capitulums in Streptozotocin-induced-diabetic rats," J. Ethnopharmacol., 109(1): 54-59.

Aye Kyaw (1978). A simple colorimetric method for ascorbic acid determination in blood plasma. Clin Chem. Acta., 86: 153-157

Bakirel T, Bakirel U, Keleş OU, Ulgen SG, Yardibi H (2008). In vivo assessment of antidiabetic and antioxidant activities of rosemary *(Rosmarinus officinalis)* in alloxan - diabetic rabbits. J Ethnopharmacol., 116: 64-73.

Bieski IGC, Rios SF, de Oliveira RM, Espinosa MM, Macedo M, Albuquerque UP, de Oliveira MDT (2012). Ethnopharmacology of medicinal plants of the Pantanal region (Mato Grosso, Brazil). Evidence-Based Complementary and Alternative Medicine, pp. 1-36.

Bramati LF, Aquilano B, Pietta P (2003). Quantitative characterization of flavonoides by HPLC-UV and determination of the total antioxidant activity," J. Agric. Food Chem., 51(25): 7472-7474.

Chaiyasut C, Kusirisin W, Lailerd N, Lerttrakarnnon P, Suttajit M, Srichairatanakool S (2011). Effects of Phenolic Compounds of Fermented Thai Indigenous Plants on Oxidative Stress in Streptozotocin-Induced Diabetic Rats. Evidence-Based Complementary and Alternative Medicine, 2011: 1-10.

Chandra A, Mahdi AA, Ahmad S, Singh RK (2007). Indian herbs result in hypoglycemic responses in Streptozotocin-induced diabetic rats. Nutr. Res., 27(3): 161-168.

Cunningham J (1991). Reduced mononuclear leukocyte ascorbic acid content in adults with insulin–dependent diabetes mellitus consuming adequate dietary Vitamin C. Metabolism, 40: 140-149.

Çakılcıoğlu U, Türkoğlu İ (2007). Plants used to lower blood sugar in Elazığ Central District, In: I. Int. medicinal and aromatic plants conf. on culinary herbs. Acta Horticulturae. 826: 97-104.

Çakılcıoğlu U, Türkoğlu İ (2007a). Plants and fruits used for cholesterol treatment by the folk in Elazig. Phytologia Balcanica, 13: 239-245

Davie SJ, Gould BJ, Yudkim JS (1992). Effect of Vitamin C on glycosylation of protein. Diabètes, 41: 167-73.

Grun FA, van Dorsten DM, Jacobs et al. (2008). "GC-MS methods for metabolic profiling of microbial fermentation products of dietary polyphenols in human and in vitro intervention studies". J. Chromatogr. B, 871(2): 212–219.

Ding AH, Nathan CF, Stuchr DJ (1988). Release of reactive nitrogen intermediates and reactive oxygen intermediate from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. J. Immunol., 141, 2407–2412.

Habig WR, Pbst MJ, Jakpoly WB (1974). Glutathione transferase. A first enzymatic step in mercaturic acid formation. J. Biol. Chem.,.249: 7130-7139.

Huang SS, Zheng Rl (2006). Rosmarinic acid inhibits angiogenesis and its mechanism of action *in vitro*. Cancer Lett., 239:271-280.

Kaplowitz N, Ookhtens M, Aw TV (1985). The regulation of hepatic glutathione. Ann. Rev. Pharmacol. Toxicol., 25: 715-744.

Kim DO, Jeong SW, Lee CY (2003). Antioxidant capacity of Phenolic phytochemicals from various cultivars of plums. Food Chem., 81: 321-326.

Krishnakumar K, Augusti KT, Vijayammal PL (1999). Hypoglycemic and antioxidant activity of Saacia oblonga wall extract in Streptozotocin-induced diabetic rats. Ind. J. Physiol. Pharmacol., 43(3): 510.

Lupi R, Del PS, Prato S (2008). [beta]-cell apoptosis in type 2 diabetes: Quantitative and functional consequences. Diabetes Metab., 34: S56-S64.

Makino T, Ono T, Liu N, Nakamura T, Muso E, Honda G (2002). Suppressive effects of rosmarinic acid on mesangio proliferative glomerulonephritis in rats. Nephron, 92:898-904.

Mallick C, Chatterjee K, GuhaBiswas M, Ghosh D (2007). Antihyperglycemic effects of separate and composite extract of root of Musa paradisiaca and leaf of *Coccinia Indica* in Streptozotocin induced diabetic male albino rat, Afr. J. Trad. CAM, 4: 362-371.

Meister A (1983). Selective modification of glutathione metabolism. Science, 20: 472-477.

Moron MS, De Pierre JN, Mannervik V (1979). Levels of glutathione, glutathione reductase, and glutathione -S- transferase in rat lung and liver. Biochem. Biophys. Acta., 582: 67-68.

Myojin C, Enami N, Nagata A, Yamaguchi T, Takamura H, Matoba T (2008). Changes in the Radical-Scavenging Activity of Bitter Gourd (*Momordica charantia* L.) during Freezing and Frozen Storage with or without Blanching. J. Food Sci., 73(7): C546-C550.

Nirmala A, Saroja S, Devi GG (2011). Antidiabetic Activity of Basella rubra and its Relationship with the Antioxidant Property. British Biotechnol. J., 1(1): 1-9.

Ojewole JAO, Adewole SO, Olayiwola G (2006). Hypoglycemic and hypertensive effects of *Momordica charantia* Linn. (Cucurbitaceae)

whole-plant aqueous extract in rats. Cardiovasc. J. S. Afr., 17(5): 227-232.
Portha B, Movassat J, Cuzin TC, Bailbe D, Giroix MH, Serradas P, Dolz M, Kergoat M (2007). Neonatally Streptozotocin-induced (n-STZ) diabetic rats: A family of type 2 diabetes models. In E. Shafrir (ed.) Animal Models of Diabetes: Frontiers in Research. CRC Press, New York, pp. 223-250.
Rotruck JT, Pope AL, Ganther HE, Swanson AB (1984). Selenium: Biochemical roles as a component of glutathione peroxidase. Science, 179: 588-590.
Sabu CM, Kuttan R (2004). Antidiabetic activity of Aegle marmelos and its relationship with its antioxidant properties. Ind. J. Physiol. Pharmacol., 48(1): 81-88.
Sekar N, Govindasamy S (1990). Insulin mimetic role vanadate on plasma membrane insulin receptor. J. Biosci., 15: 115.
Siddique O, Sun Y, Lin JC, Chien YW (1987). Facilitated transdermal transport of insulin. J. Pharm. Sci., 76: 341-45.
Semiz A, Sen A (2007). Antioxidant and chemoprotective properties of Momordica charantia L. (bitter melon) fruit extract. Afr. J. Biotechnol., 6(3): 273-277.
Sezik E, Aslan M, Yesilada E, Ito S (2005). Hypoglycemic activity of Gentiana olivieri and isolation of the active constituent through bioassay-directed fractionation techniques. Life Sci., 76(11): 1223-1238.
Tavaafi M, Ahmadvand H, Khalatbari A, Tamjidipoor A (2011). Rosmarinic acid ameliorates diabetic nephropathy in uninephrrectomized diabetic rats. Iranian J. Basic Sci., 14(3): 275-283.
Tetik F (2011). A research on the ethnobotanical valued plants in Malatya province. Yüksek lisans Tezi. Fırat Üniversitesi, Fen Bilimleri Enstitüsü, Elazığ
Thayer WS (1984). Serum lipid peroxides in rats treated chronically with Adriamycin. Biochem. Pharmacol., 33: 2259-2263.
Ugulu I, Baslar S, Yorek N, Dogan Y (2009). The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province, Turkey. J. Med. Plants. Res., 3: 345-367.
Wilson RL (1998). Free radicals and tissue damage, mechanistic evidence from radiation studies. In: Biochemical mechanisms of Liver Injury. New York, Academic Press, pp. 123-125.