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 complications in human beings (Wilson, 1998).

Oxidative stress in diabetes mellitus has been shown to coexist with a reduction in the antioxidant status andglycation 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 plant.
shrub grown in many parts of the world. It have been used as medicinal plants in folk medicine, but *Rosmarinus* itself was used for asthma, bronchitis, cold, flu, digestive, anemia, palpititation, 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, carnosol, rosmarinic acid 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 markers 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...
(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 carnosol, a compound which inhibits iNOS by blocking the activation of tumor necrosis factor α (Grun et al., 2008). Cinnamic aldehyde, a compound found to be present in, specifically, the essential oil cinnamon as well as several flavonoids 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 α- (TNF-α) signaling. Natural triterpenes such as ginsenosid Rh1, triterpenes isolated from Panax ginseng, inhibited iNOS expression and the activation of NF-α. In addition, oleanolic acid glycoside isolated from ampelopsis radix, markedly suppressed the activity of NF-α 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>
<thead>
<tr>
<th>Group</th>
<th>FBG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>117 ± 5.2a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>371 ± 5.4c</td>
</tr>
<tr>
<td>Normal + rosemary (200mg/kg)</td>
<td>86.4 ± 3.6a</td>
</tr>
<tr>
<td>Treated group</td>
<td>137 ±4.6b</td>
</tr>
</tbody>
</table>

Values are mean ± SD; values with different superscripts differ significantly (p < 0.05).
Table 2. Effect of *R. officinalis* on oxidative stress markers in serum of control, diabetic and treated rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/ml)</th>
<th>NO (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>4.42 ± 0.56a</td>
<td>48.3 ±3.8a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>9.59 ± 0.64b</td>
<td>80.09 ± 3.6b</td>
</tr>
<tr>
<td>Normal + rosemary (200 mg/kg)</td>
<td>3.75 ±0.27a</td>
<td>46.7 ± 2.4a</td>
</tr>
<tr>
<td>Treated group</td>
<td>4.06 ±0.29a</td>
<td>49.12 ±12.7a</td>
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Table 3. Effect of *R. officinalis* on antioxidant enzymes in serum of control, diabetic and treated rats.

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

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 ≤ 0.05).

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

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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$_2$O$_2$) 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 dehydroascorbic 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 insulimimetic 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.

ACKNOWLEDGEMENT

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REFERENCES


