SUMMARY

This study was designed to evaluate and compare the efficacy of infusion of Ruta graveolens (R. graveolens) and its pharmacologically active constituent, rutin, on impaired glucose tolerance, lipid profile and oxidative stress in nicotinamide-streptozotocin-induced (type 2) diabetic albino rats. The study also suggested the probable physiological and molecular mechanisms of action of these treatments in this animal diabetic model. R. graveolens infusion and rutin were orally administered to diabetic rats at a dose of 125 and 50 mg/kg body weight/day, respectively, for 30 days. The results obtained revealed that both R. graveolens infusion and rutin led to significant amelioration of hyperglycemia, hyperlipidemia, serum insulin and C-peptide concentrations, liver glycogen content and the activities of hexokinase, glucose-6-phosphatase and glycogen phosphorylase as well as oxidative stress in diabetic rats. On the other hand, the in vitro and in situ studies indicated that R. graveolens infusion and rutin significantly enhanced insulin release from isolated islets of Langerhans, insulin binding to its receptors in rat diaphragm and peripheral glucose uptake by the rat diaphragm, whereas intestinal glucose and cholesterol absorption was significantly decreased. In addition, both treatment agents decreased resistin expression in adipose tissue, while rutin only was found to be effective in increasing adipose tissue peroxisome proliferator-activated receptor (PPAR) γ expression. Based on these results, the study suggested both R. graveolens and rutin to exhibit antihyperglycemic and antihyperlipidemic properties via their insulinogenic effects, decreasing intestinal glucose and cholesterol absorption, improving peripheral insulin action, affecting mediators of insulin resistance, enhancing peripheral glucose uptake and decreasing hepatic glucose output in addition to the ameliorating effect on the antioxidant status in this condition.

INTRODUCTION

Diabetes mellitus (DM) has been classified into 3 categories, insulin dependent DM (IDDM), non-insulin dependent DM (NIDDM), and other specific types of diabetes (1). NIDDM is a much more prevalent form, responsible for 90% of the disease
prevalence (1-3). It is a complex and heterogeneous disorder presently affecting more than 100 million people worldwide and causing serious socioeconomic problems (4).

Peroxisome proliferator-activated receptors (PPARs) have been identified as molecular focuses for drugs that target lipoprotein and glucose abnormalities associated with insulin resistance (5). There are three isoforms of PPAR: PPARα, PPARδ and PPARγ (6). The expression of PPARα is greatest in tissues with active metabolism, such as the liver, striated muscle and kidney, whereas PPARδ has a very broad expression pattern that has made identifying its role more difficult. PPARγ is highly expressed in fat, colon, placenta and macrophage (7,8). Two isoforms of PPARγ, PPARγ1 and PPARγ2, can respond to the same signals and activate the same target genes: lipoprotein lipase, fatty acid binding protein, acyl-CoA-synthetase and CD36 (9,10).

Resistin, an adipocyte-derived protein, was originally found in a screen for genes that were induced during adipocyte differentiation, and it is found to be down-regulated in mature adipocytes exposed to thiazolidinediones (11). It has been reported that resistin is expressed exclusively in adipocytes and is linked with the traits that are related to obesity and insulin resistance (12). The insulin-resistant effects of resistin are thought to account for the activation of glucose 6-phosphatase, which subsequently prevents glycogen synthesis and increases the rate of glucose production (13).

In recent years, there has been renewed interest in the treatment of different diseases using herbal drugs as the World Health Organization (WHO) has also recommended evaluation of the effectiveness of plants in conditions where we lack safe modern drugs (14). The treatment of diabetes with synthetic drugs is generally not preferred because of its high cost and the range of side effects thus caused; hence, the development of traditional or alternative medicine is needed (15). Several plants of Rutaceae family are used in traditional medicine worldwide (16). The most common medicinal plant of this family is Ruta (R.) graveolens, known as rue and native to Europe. The plant is now available all over the world, although preferably grown in Mediterranean climate (16). This plant has been in medicinal use for various clinical conditions from ancient times, but the rationality of its use is still controversial. Rue contains various active compounds like flavonoids, coumarin derivatives, furoquinolines, volatile oils, undecanone and others (17). According to the available literature (18), R. graveolens plant contains approximately 2% of rutin.

Flavonoids, and particularly quercetin derivatives, have received special attention as dietary constituents in the last few years. Epidemiological studies have pointed out their possible role in preventing cardiovascular disease and cancer (19-23). This health-promoting activity seems to be related to the antioxidant (free-radical scavenging) activity to flavonoids (24). Quercetin (3,3’,4’,5,7-pentahydroxy flavone) is one of the most common native flavonoids occurring mainly in glycosidic forms such as rutin (5,7,3’,4’-OH, 3-rutinose) (23,25). Rutin is abundantly present in onions, apples, tea and red wine (20). Rutin exhibits multiple pharmacological activities including antibacterial, antitumor, antiinflammatory, anti-diarrheal, antiulcer, antimutagenic, myocardial protecting, vasodilator, immunomodulator and hepatoprotective activities (26). However, studies of the antidiabetic properties of R. graveolens and its pharmacologically active constituent, rutin, are scarce. Thus, this study aimed to assess the antidiabetic efficiency of the plant infusion and rutin in nicotinamide-streptozotocin (NA-STZ) diabetic rats and to suggest the probable mechanisms of action in this animal diabetic model.

MATERIALS AND METHODS

Experimental animals

White male albino rats (Rattus norvegicus) weighing about 150-200 g were used. They were obtained from the animal house of the National Research Center, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12-hour light/dark cycle. Moreover, they had free access
to water and were supplied daily with standard diet of known composition ad libitum. All animal procedures were in accordance with the recommendations of the Canadian Committee for Care and Use of Animals (Canadian Council on Animal Care) (27).

**Preparation of plant extract**

*R. graveolens* (sadab) was obtained from Experimental Station of Medical Plants (ESMP), Faculty of Pharmacy, Cairo University, Egypt. Its leaves were air dried and then powdered with an electric grinder. The infusion (water extract) was prepared according to the method described by Swanston-Flatt *et al.* (28). Powdered plant material was added to boiling water and infused for 15 minutes. The infusion was filtered and the filtrate was freshly used. The herb infusion was orally administered by gastric tube at a dose level of 125 mg/kg body weight (b.w.) per day for 30 days.

**Preliminary phytochemical screening of R. graveolens**

The air dried powder of *R. graveolens* was subjected to tests for detection of the presence of carbohydrates and/or glycosides, tannins, alkaloids and/or nitrogenous bases, flavonoids, saponins, unsaturated sterols and/or triterpenes and resins (29).

**Rutin**

Rutin in the form of rutin hydrate was purchased from Sigma Company, USA. It was suspended in distilled water and freshly prepared just before the administration. It was orally administered by gastric tube at a dose level of 50 mg/kg b. w./day (23) for 30 days.

**Induction of diabetes mellitus**

Type 2 DM was experimentally induced in animals fasted for 16 hours by intraperitoneal injection of 120 mg/kg b.w. nicotinamide dissolved in NaCl solution (0.9%) 30 minutes before intraperitoneal injection of 50 mg/kg b. w. STZ (Sigma Company) dissolved in citrate buffer (pH 4.5) (30). Ten days after STZ injection, rats were screened for serum glucose levels.

**Experimental design**

The rats were divided into 4 groups of 6 rats, as follows: group I consisting of normal control rats were orally administered an equivalent volume of vehicle (distilled water); group II was considered as diabetic control and orally given an equivalent volume of vehicle (distilled water); group III were orally treated with infusion of *R. graveolens* at a dose level of 125 mg/kg b.w.; and group IV were orally treated with rutin (50 mg/kg b.w.) dissolved in distilled water. All treatments were given daily for 30 days by gastric intubation. By the end of the experiment, animals were sacrificed and blood samples, visceral adipose tissue, pancreas and liver were obtained.

**Histologic study**

After sacrifice and dissection, pancreas was immediately excised from each animal, fixed in 10% neutral buffered formalin and transferred to Histopathology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, for preparation for blocking in paraffin wax and sectioning. Pancreas was stained with modified aldehyde fuchsin satin method (31).

**Biochemical study**

On the day before sacrifice, oral glucose tolerance test (OGTT) was performed in normal, diabetic control and diabetic rats treated with *R. graveolens* infusion and rutin. Blood samples were obtained from lateral tail vein of rats deprived of food overnight (10-12 hours). Successive blood samples were then taken at 0, 30, 60, 90 and 120 minutes following the administration of glucose solution (3 g/kg b.w.) through gastric intubation. Blood samples were left to coagulate, centrifuged, and clear non-hemolyzed serum was obtained for determination of glucose concentration according to the method of Trinder (32), using reagent kit purchased from Spinreact Company (Spain).

Serum fructosamine was determined according to the method of Baker *et al.* (33) using reagent kit purchased from Reactivos Spinreact Company (Spain). Serum insulin and C-peptide were assayed in the Radioactive Isotopes Unit, Middle Eastern Regional Radioisotope
Center (Dokki, Giza) by radioimmunoassay kits of DPC (Diagnostic Products Corporation, Los Angeles, USA) [coat-A-count] according to the methods of Marschner et al. (34) and Bonser and Garcia-Webb (35), respectively.

Liver glycogen content was determined according to the method of Seifter et al. (36). Liver hexokinase activity was determined according to the method of Branstrup et al. (37). Liver glucose-6-phosphatase and glycogen phosphorylase activities were determined according to the methods of Begum et al. (38) and Stallman and Hers (39), respectively. The liberated inorganic phosphate by glucose-6-phosphatase and glycogen phosphorylase was estimated according to the method of Munoz et al. (40) using reagent kits obtained from Biosystems, S.A (Spain). Serum total lipids (41), triglycerides (42), cholesterol (43), HDL-cholesterol (44) and liver HMG-CoA reductase (45) were also estimated. Serum LDL-cholesterol level was calculated from Friedewald (46) formula (LDL-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol). Serum vLDL-cholesterol concentration was calculated according to Nobert (47) formula (vLDL-cholesterol = triglycerides/5).

Liver lipid peroxidation, reduced glutathione, total thiols and catalase and peroxidase activities were also measured according to the methods of Preuss et al. (48), Beutler et al. (49), Koster et al. (50), Cohen et al. (51) and Kar and Mishra (52), respectively. Humalyzer 2000 Chemical Analyzer (Germany) was used for spectrophotometric measurements.

Physiologic techniques for the mechanism of action

Isolation of islets of Langerhans and incubation technique

Pancreatic islets were isolated from adult normal male albino rats (180-200 g) using the collagenase digestion technique of Howell and Taylor (53). Ten equal medium sized islets were picked in 0.35 mL Gey & Gey buffer (54) containing 2% bovine serum albumin using fine Pasteur pipette and stereo binocular microscope (American Optics, USA). Then, R. graveolens infusion or rutin was added to the isolated islets to reach final concentrations of 0.2 and 1.0 mg/mL for each and incubation was carried out for 1 h at 37 °C. These two doses of R. graveolens infusion and rutin (0.2 and 1.0 mg/mL) were tested at two different concentrations of glucose (2mM and 8mM). Blanks were prepared by adding an equivalent volume of the vehicle. At the end of the incubation period, mixtures at a final volume 0.7 mL were centrifuged for 5 min at 3000 rpm and 0.2 mL of the supernatant was assayed for insulin concentration.

Intestinal glucose and cholesterol absorptions (in situ)

An intestinal perfusion technique (55) using variable flow mini-pump (Control Company, Texas, USA) was adopted to study the effect of R. graveolens infusion and rutin on intestinal glucose and cholesterol absorptions in normal rats fasted for 24 h and anesthetized with intraperitoneal sodium thiopental solution (50 mg/kg). Perfusion rate was 20 mL/h to a total volume of 20 mL via a thermoregulator set at 37 °C through 20-cm intestinal segment in situ of the anesthetized rats [with the addition of goat bile (1 mL/20 mL perfusion solution), in case of testing cholesterol absorption only]. The perfusing solution was composed of the following (in g/L): 7.37 g NaCl, 0.2 g KCl, 0.065 g NaH2PO4 · 2H2O, 0.213 g MgCl2,6H2O, 1.02 g CaCl2, 0.6 g NaHCO3 and 1.0 g glucose or cholesterol, at pH 7.5. The results were expressed as percentage glucose or cholesterol absorption calculated from the amount of glucose or cholesterol in solution before and after perfusion with 62.5, 125, 250 mg/mL of R. graveolens and 25, 50, 100 mg/mL rutin in the solution.

Peripheral glucose consumption and insulin binding affinity (in vitro)

The peripheral glucose consumption was studied in rat diaphragm preparations from diabetic rats fasted for 24 h previous to sacrifice. Diaphragms were divided into two halves and incubated according to Zarzuelo et al. (55) at 37 °C, with constant oxygenation and shaking for 1 h. The nutrient solution was prepared with the following formula: 725 mL
1.3% NaHCO₃ was aerated for 3 min with carbogen, then added to 750 mL of saline solution. This saline solution was composed of the following (in g/L): 9.5 g NaCl, 0.4 g KCl, 0.3 g CaCl₂, 0.35 g NaHCO₃, 0.35 g MgCl₂\_7H₂O, 0.2 g KH₂PO₄ and 1.0 g glucose. The resultant mixture was aerated with carbogen (95% O₂ : 5% CO₂) for 10 min before the incubation and at time intervals of 15 min during the incubation. The results were expressed as glucose consumption per 1 g diaphragm wet weight. The concentrations used were 0.2 and 1 mg/mL for *R. graveolens* and rutin in the presence and absence of insulin (25 µIU/mL). Stocks from samples incubated in the presence of insulin were taken in separate vials to estimate the non-binding insulin in the perfusion solution and the binding insulin was calculated from the formula (insulin binding affinity = insulin conc. before incubation – non-binding insulin in the perfusing solution after incubation).

**RNA extraction and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)**

Visceral adipose tissue RNA was isolated according to the method of Otto *et al.* (56) by SV Total RNA Isolation System using reagent kit purchased from Promega Company (USA). RT-PCR for resistin and PPARγ was done by using Accupower RT/PCR PreMix (provided from Bioneer, Reno, USA). The kit contained 0.5 mL thin wall microtubes, each containing lyophilized reaction mixture of 10 mM dNTP, 10 U RNasin, 20 U M-MLV reverse transcriptase, IU thermostable DNA polymerase, precipitant and loading dye. The primers (obtained through Clinilab Company, Cairo, Egypt) used were as follows: PPARγ; sense, 5’GGGTGAAA ACTCTG GAGATT C3’, antisense, 5’TCA GCA ACCAT TGGTG CAGCT CT3’ and resistin; sense, 5’GCTC AGTT TCTC AATCAA CCGT C3’, antisense, 5’CTGAGCTC TCTGCCAC GTACT3’.

To each reaction tube, 1 µg of RNA and 20 pmol of primer were added and the volume was made up to 50 µL with distilled water. The lyophilized reaction mixture of each tube was dissolved by vortexing and span-down briefly. The tubes were placed on a double heated led thermal cycler and the reaction series was performed as follows: 57 °C for 10 min, 42 °C for 60 min, 94 °C for 5 min, 35 cycles of 94 °C for 30 sec, 57 °C for 30 sec, 72 °C for 1 min.

RT-PCR samples were loaded in 1% agarose gel wells (Sigma, USA); 50V electrical power was applied (5 volts per 1 cm), then the samples were left to migrate for appropriate time. After migration, the cDNA bands were observed in the gel using UV illuminator. Gel images were scanned using a conventional computer scanner and then the images were analyzed using the computer software Pro-gel analysis v. 3.1.

**Statistical analysis**

The data were analyzed using the one-way analysis of variance (ANOVA) (57), followed by least significant difference (LSD) test to compare various groups with each other. Results were expressed as mean ± standard error (SE) and values of $P>0.05$ were considered non-significantly different, while those of $P<0.05$ and $P<0.01$ were considered significant and highly significant, respectively. F-probability expresses the general effect between groups. The means that were not significantly different are followed by the same superscript symbol(s).

**RESULTS**

Qualitative phytochemical screening revealed the presence of carbohydrates and/or glycosides, tannins, alkaloids and/or nitrogenous bases, flavonoids, saponins, unsaturated sterols and/or triterpenes and resins in the tested plant (Table 1).

The oral glucose tolerance curve of diabetic rats showed a highly significant elevation at fasting state and 30, 60, 90 and 120 min after oral glucose loading as compared to normal animals. The treatment of diabetic animals with *R. graveolens* infusion and rutin induced a potential improvement of elevated values at all points of OGTT curve. However, while rutin seemed to be more potent at fasting state, the plant infusion appeared to be more effective at all other tested points after oral glucose loading (Fig. 1).
Table 1. Preliminary phytochemical screening of *R. graveolens*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates and/or glycosides</td>
<td>Molish's test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Fehling's test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Benedict's test</td>
<td>++ ve</td>
</tr>
<tr>
<td>Alkaloids and/or nitrogenous bases</td>
<td>Mayer's test</td>
<td>+++ ve</td>
</tr>
<tr>
<td></td>
<td>Drageendorff's test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Wagner's test</td>
<td>++ ve</td>
</tr>
<tr>
<td>Unsaturated sterols and/or triterpenes</td>
<td>Libermann-Burchard test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Salkwiski's test</td>
<td>+ ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+++ ve</td>
</tr>
<tr>
<td></td>
<td>NaOH test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Amyl alcohol test</td>
<td>++ ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth test</td>
<td>+ ve</td>
</tr>
<tr>
<td></td>
<td>Hemolysis test</td>
<td>+ ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Match stick test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Vanillin HCl test</td>
<td>++ ve</td>
</tr>
<tr>
<td></td>
<td>Phenazine test</td>
<td>++ ve</td>
</tr>
<tr>
<td>Resins</td>
<td></td>
<td>++ ve</td>
</tr>
</tbody>
</table>

++ve, +++ve and ++++ve = presence of active principles in low, moderate and high quantities, respectively

Table 2. Serum fructosamine, insulin, C-peptide and liver glycogen contents of normal, diabetic control and diabetic treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Fructosamine (µmol/L)</th>
<th>Liver glycogen (mg/g tissue)</th>
<th>Insulin (µIU/mL)</th>
<th>C-peptide (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>163.046±2.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.64±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.17±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.87±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>233.046±3.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.80±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.26±0.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.85±0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic treated with <em>R. graveolens</em></td>
<td></td>
<td>194.37±4.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.17±0.87&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.43±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.69±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic treated with rutin</td>
<td></td>
<td>187.146±3.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.05±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.64±1.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.38±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F-probability</td>
<td></td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>LSD at 5% level</td>
<td></td>
<td>11.44</td>
<td>2.15</td>
<td>2.13</td>
<td>0.57</td>
</tr>
<tr>
<td>LSD at 1% level</td>
<td></td>
<td>15.59</td>
<td>2.93</td>
<td>2.90</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE; there were six animals in each group; the means marked with the same superscript symbol(s) were not significantly different; the means with difference higher than the value of LSD at 5% level were significantly different (P<0.05) and those with difference higher than the value of LSD at 1% level were highly significantly different (P<0.01)

Table 3. Insulin release from isolated islets incubated for 1 h with two different doses of *R. graveolens* infusion and rutin in the presence of 2mM and 8mM glucose in vitro

<table>
<thead>
<tr>
<th>Glucose concentration</th>
<th>Insulin release (µU/islet/h)</th>
<th>Control</th>
<th>0.2 mg/mL</th>
<th>1.0 mg/mL</th>
<th>0.2 mg/mL</th>
<th>1.0 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R.</td>
<td>graveolens</td>
<td>Rutin</td>
<td>R.</td>
<td>Rutin</td>
</tr>
<tr>
<td>2mM</td>
<td></td>
<td>3.27±1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.50±0.79&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.50±1.59&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.91±1.13&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.29±2.76&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>8mM</td>
<td></td>
<td>17.48±1.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.31±0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.28±0.45&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29.10±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.11±2.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-probability: P<0.001; LSD at 5% level: 4.40; LSD at 1% level: 6.26
As indicated in Table 2, the liver glycogen content and serum insulin and C-peptide exhibited significant decrease in diabetic rats. In contrast, serum fructosamine was remarkably elevated ($P<0.01$; LSD) in diabetic rats as compared to normal. Treatment of diabetic animals with the plant infusion and rutin induced profound amendment of these deteriorated changes. Rutin seemed to be more effective in improving these altered variables.

Both concentrations of both agents tested (Table 3) induced significant insulinitropic effects at 2 mM and 8 mM glucose as compared with the corresponding blanks; rutin appeared to be more potent than *R. graveolens* infusion.

Data shown in Table 4 indicated that the addition of *R. graveolens* extract as well as rutin to the incubation media caused a significant increase in glucose uptake by the rat diaphragm, in a dose dependent manner. The present data showed the effect of both agents to be potentiated in the presence of insulin. Moreover, rutin seemed to be more effective in enhancing peripheral glucose uptake in the presence and absence of insulin.

The addition of both rue infusion and rutin to the incubation media caused an increase of insulin binding to the rat diaphragm in a dose dependent manner. Both agents produced a significant increase in insulin binding with increasing concentrations as compared with the blank. Rutin seemed to be more effective in enhancing insulin binding to the rat diaphragm (Table 5).

Data on the effects of *R. graveolens* and rutin at varying concentrations on intestinal glucose absorption are given in Table 6. All the concentrations of *R. graveolens* and rutin tested produced marked decrease in the intestinal glucose absorption. The increasing concentration of both agents was found to be accompanied by detectable decreases in the intestinal glucose absorption. In addition, the concentrations of rutin tested seemed to be more effective than those of *R. graveolens* infusion in reducing intestinal glucose absorption. On the other hand, both *R. graveolens* infusion and rutin induced

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**Table 4.** Changes in peripheral glucose uptake (mg/g fresh diaphragm) of diabetic rat diaphragm with increasing doses of *R. graveolens* infusion and rutin in the presence and absence of insulin in vitro

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th><em>R. graveolens</em> Infusion</th>
<th>Rutin</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the absence of insulin</td>
<td>1.13±0.08$^i$</td>
<td>3.79±0.07$^g$</td>
<td>4.13±0.04$^h$</td>
</tr>
<tr>
<td>In the presence of insulin (25 μIU/mL)</td>
<td>5.75±0.07$^a$</td>
<td>6.00±0.07$^d$</td>
<td>6.33±0.17$^c$</td>
</tr>
</tbody>
</table>

F-probability: $P<0.001$; LSD at 5% level: 0.235; LSD at 1% level: 0.316

**Table 5.** Effect of *R. graveolens* infusion and rutin on insulin binding affinity to rat diaphragm in vitro

<table>
<thead>
<tr>
<th>Binding insulin concentration (μIU/100 mg fresh diaphragm/h)</th>
<th>Blank</th>
<th><em>R. graveolens</em></th>
<th>Rutin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 mg/mL</td>
<td>0.2 mg/mL</td>
<td>1.0 mg/mL</td>
<td>0.2 mg/mL</td>
</tr>
<tr>
<td>56.78±0.45$^a$</td>
<td>79.72±0.06$^d$</td>
<td>112.17±0.79$^b$</td>
<td>89.25±0.95$^c$</td>
</tr>
</tbody>
</table>

F-probability: $P<0.001$; LSD at 5% level: 1.78; LSD at 1% level: 2.41
Table 6. Changes in intestinal glucose absorption with increasing dose of *R. graveolens* and rutin

<table>
<thead>
<tr>
<th>Blank</th>
<th>Dose (mg/mL)</th>
<th>Absorption %</th>
<th>Dose (mg/mL)</th>
<th>Absorption %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>62.5</td>
<td>28.97±0.56b</td>
<td>25</td>
<td>16.40±0.94a</td>
</tr>
<tr>
<td></td>
<td>45.55±1.42a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>27.23±0.31b</td>
<td>50</td>
<td>14.30±0.34a</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>24.82±0.56c</td>
<td>100</td>
<td>12.97±0.19d</td>
</tr>
</tbody>
</table>

F-probability: *P*<0.001; LSD at 5% level: 2.89; LSD at 1% level: 3.894

Table 7. Changes in intestinal cholesterol absorption with increasing dose of *R. graveolens* infusion and rutin

<table>
<thead>
<tr>
<th>Blank</th>
<th>Dose (mg/mL)</th>
<th>Absorption %</th>
<th>Dose (mg/mL)</th>
<th>Absorption %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>62.5</td>
<td>104.67±0.76c</td>
<td>25</td>
<td>110.00±1.21b</td>
</tr>
<tr>
<td></td>
<td>131.50±1.75a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>95.33±0.76e</td>
<td>50</td>
<td>102.00±0.52c</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>94.50±0.88e</td>
<td>100</td>
<td>98.83±0.54d</td>
</tr>
</tbody>
</table>

F-probability: *P*<0.001; LSD at 5% level 2.89; LSD at 1% level 3.89

Table 8. Liver glucose-6-phosphatase, glycogen phosphorylase and hexokinase activities of normal, diabetic control and diabetic rats treated with *R. graveolens* infusion and rutin

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Glucose-6-phosphatase (µg Pi liberated/100 mg tissue/h)</th>
<th>Glycogen phosphorylase (µg Pi liberated/100 mg tissue/h)</th>
<th>Hexokinase (µg glucose phosphorylated/100 mg tissue/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Glucose-6-phosphatase</td>
<td>2.00±0.08c</td>
<td>1.73±0.99d</td>
<td>26.18±0.07a</td>
</tr>
<tr>
<td></td>
<td>Glycogen phosphorylase</td>
<td>15.95±0.21a</td>
<td>12.65±0.02a</td>
<td>3.82±2.04c</td>
</tr>
<tr>
<td></td>
<td>Hexokinase</td>
<td>4.79±0.27b</td>
<td>7.02±3.42b</td>
<td>9.07±0.96b</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Glucose-6-phosphatase</td>
<td>4.35±0.23b</td>
<td>5.85±2.77c</td>
<td>8.17±3.89b</td>
</tr>
<tr>
<td></td>
<td>Glycogen phosphorylase</td>
<td>F-probability: <em>P</em>&lt;0.001</td>
<td>F-probability: <em>P</em>&lt;0.001</td>
<td>F-probability: <em>P</em>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Hexokinase</td>
<td>LSD at 5% level: 0.62</td>
<td>LSD at 5% level: 0.75</td>
<td>LSD at 1% level: 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LSD at 1% level: 0.84</td>
<td>LSD at 1% level: 1.02</td>
<td>LSD at 1% level: 1.25</td>
</tr>
</tbody>
</table>

Table 9. Serum lipid profile and liver HMG-CoA reductase activity of normal, diabetic control and diabetic rats treated with *R. graveolens* infusion and rutin

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipids (g/L)</th>
<th>CH (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL-CH (mg/dL)</th>
<th>LDL-CH (mg/dL)</th>
<th>VLDL-CH (mg/dL)</th>
<th>HMG-CoA reductase (HMG-CoA/ mevalonate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.44±0.11c</td>
<td>57.00±0.56d</td>
<td>45.85±0.71d</td>
<td>13.60±0.39d</td>
<td>30.29±0.50a</td>
<td>2.29±0.50d</td>
<td>1.12±0.54d</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>4.81±0.27a</td>
<td>89.58±0.18a</td>
<td>151.36±2.45a</td>
<td>15.43±0.45d</td>
<td>15.43±0.45d</td>
<td>12.66±0.29b</td>
<td>1.31±0.73b</td>
</tr>
<tr>
<td>Diabetic treated with <em>R. graveolens</em></td>
<td>3.07±0.16b</td>
<td>69.56±0.60b</td>
<td>63.29±1.47b</td>
<td>24.38±0.22c</td>
<td>32.52±0.70b</td>
<td>11.61±0.28c</td>
<td>1.25±0.01c</td>
</tr>
<tr>
<td>Diabetic treated with rutin</td>
<td>3.05±0.15b</td>
<td>66.69±0.49d</td>
<td>58.03±1.42c</td>
<td>26.60±0.18b</td>
<td>28.66±0.34c</td>
<td>11.61±0.28c</td>
<td>1.25±0.01c</td>
</tr>
</tbody>
</table>

F-probability: *P*<0.001; LSD at 5% level: 0.47; LSD at 1% level: 0.642

CH: cholesterol; TG: triglycerides; LDL-CH: low density lipoprotein cholesterol; HDL-CH: high density lipoprotein cholesterol; VLDL-CH: very low density lipoprotein cholesterol

CH: cholesterol; TG: triglycerides; LDL-CH: low density lipoprotein cholesterol; HDL-CH: high density lipoprotein cholesterol; VLDL-CH: very low density lipoprotein cholesterol
potential decreases in intestinal cholesterol absorption in a dose dependent manner, with a more potent effect of *R. graveolens* (Table 7).

Regarding liver glucose-6-phosphatase and glycogen phosphorylase activities, they were increased in diabetic rats, as illustrated in Table 8. The administration of both *R. graveolens* and rutin produced a profound improvement of these altered enzyme activities. In contrast, liver hexokinase activity (Table 8) showed a different pattern. The enzyme activity was enormously suppressed in diabetic control rats and profoundly increased in diabetic rats treated with *R. graveolens* and rutin. Furthermore, rutin appeared to be more potent in decreasing the activities of glucose-6-phosphatase and glycogen phosphorylase. On the other hand, treatment with *R. graveolens* produced a more potent effect than treatment with rutin in increasing hexokinase activity.

Data on the effect of *R. graveolens* and rutin on lipid profile of diabetic rats are presented in Table 9. Diabetic rats exhibited a highly significant increase (*P*<0.01; LSD) in serum total lipids, cholesterol, triglycerides, LDL- and vLDL-cholesterol and liver HMG-CoA reductase activity as compared with the non-diabetic group. Moreover, HDL-cholesterol was affected in an opposite manner, as it was decreased (*P*<0.01; LSD) in diabetic rats and significantly increased (*P*<0.01; LSD) in response to both treatment agents. The administration of both *R. graveolens* infusion and rutin led to marked amelioration of all parameters of the altered lipid profile.

Liver lipid peroxidation, total thiols, reduced glutathione content and liver antioxidant enzymes variation as the result of treatment of diabetic rats with *R. graveolens* and rutin are presented in Table 10.

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**Table 10. Liver lipid peroxidation, total thiols, reduced glutathione content, catalase and glutathione peroxidase activity in normal, diabetic control and diabetic rats treated with *R. graveolens* infusion and rutin**

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Lipid peroxidation (nmol MDA/gm/h)</th>
<th>Total thiols (nmol/100 mg)</th>
<th>Reduced glutathione (nmol/100 mg)</th>
<th>Catalase (k x 10²)</th>
<th>Glutathione peroxidase (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>24.4±0.85c</td>
<td>196.97±6.61a</td>
<td>74.93±3.74a</td>
<td>52.68±1.38a</td>
<td>75.96±1.17a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>41.2±2.15a</td>
<td>89.93±2.46c</td>
<td>57.52±1.47b</td>
<td>22.86±0.49d</td>
<td>47.21±2.52b</td>
</tr>
<tr>
<td>Diabetic treated with</td>
<td></td>
<td>29.9±1.85b</td>
<td>134.53±9.12b</td>
<td>70.83±4.27b</td>
<td>39.95±0.47c</td>
<td>62.97±3.86c</td>
</tr>
<tr>
<td><em>R. graveolens</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic treated with rutin</td>
<td></td>
<td>29.3±0.90b</td>
<td>145.02±12.01b</td>
<td>72.88±4.43a</td>
<td>44.10±1.84b</td>
<td>68.66±2.97ab</td>
</tr>
<tr>
<td>F-probability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P</em>&lt;0.001</td>
<td><em>P</em>&lt;0.001</td>
<td><em>P</em>&lt;0.001</td>
<td><em>P</em>&lt;0.001</td>
<td><em>P</em>&lt;0.001</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td></td>
<td>4.59</td>
<td>24.58</td>
<td>10.83</td>
<td>3.55</td>
<td>8.27</td>
</tr>
<tr>
<td>LSD at 1%</td>
<td></td>
<td>6.26</td>
<td>33.48</td>
<td>14.78</td>
<td>4.84</td>
<td>11.28</td>
</tr>
</tbody>
</table>

---

**Figure 2.** PPARγ cDNA pattern in adipose tissue of normal control (Nc), diabetic control (Dc), diabetic treated with rutin (Rn) and diabetic treated with infusion of *R. graveolens* (Er).

**Figure 3.** Semiquantitative analysis of PPARγ cDNA using scanning densitometry.
Liver malondialdehyde (MDA) content, which is an indicator of lipid peroxidation, was significantly increased in diabetic rats when compared to normal rats. On the other hand, treatment with both tested agents markedly ameliorated the increased liver MDA content. Meanwhile, the depleted liver total thiols and reduced glutathione contents of diabetic rats were significantly increased as the result of treatment with both *R. graveolens* and rutin.

Concerning the effect of *R. graveolens* infusion and rutin on PPARγ expression in visceral adipose tissue (Figures 2 and 3), densitometric analysis revealed a significant decrease of adipose tissue PPARγ cDNA in diabetic rats as compared with the normal control group. The treatment of diabetic animals with rutin produced a significant amelioration of PPARγ expression while treatment of diabetic rats with *R. graveolens* infusion did not show an increase. On the other hand, there was a significant increase in visceral adipose tissue resistin cDNA of diabetic rats as compared with the normal control ones, as illustrated in Figures 4 and 5. The administration of both *R. graveolens* and rutin produced a significant decrease in the visceral adipose tissue resistin expression.

**Figure 4. Resistin cDNA pattern in adipose tissue of normal control (Nc), diabetic control (Dc), diabetic treated with rutin (Rn) and diabetic treated with infusion of *R. graveolens* (Er).**

**Figure 5. Semiquantitative analysis of resistin cDNA using scanning densitometry.**

**Figure 6. Pancreatic tissues of normal male albino rats.** The pancreas is subdivided by septa (s) into pancreatic lobules. The exocrine portion of the pancreas consists of pancreatic acini (pa), while endocrine portion consists of the islets of Langerhans (IL) which are scattered throughout the pancreas and contain alpha cells (a) at the periphery of islets, beta cells (b) in the core of islets and delta cells (d) of a relatively larger size. (X400)
As for the histologic study, the islets of Langerhans in the pancreas of diabetic rats exhibited marked degeneration of cells (Figure 7A and 7B) as compared with normal pancreata (Figure 6A and 6B). The islets of diabetic rats had vacuolations, many hydropic and necrotic cells, and pyknotic or irregular hyperchromatic nuclei. The treatment with either plant aqueous extract or rutin markedly succeeded to amend the disrupted islets of Langerhans of diabetic rats; the islet architecture and integrity were improved (Figures 8A, 8B, 9A and 9B). In spite of these ameliorations, there were still a few necrotic areas (n), pyknotic nuclei (pk) and vacuolations (v) in the islets of diabetic rats treated with the plant aqueous extract and rutin.

Figure 7. Pancreata of diabetic rats. Normal architecture of the islets is disrupted. Islets showing many hydropic cells, necrotic cells (n), pyknotic nuclei (pk), vacuolations (v) and irregular hyperchromatic nuclei (hcn). (X400)

Figure 8. Pancreata of diabetic rats treated with *R. graveolens* infusion. There are still few necrotic areas (n), pyknotic nuclei (pk) and vacuolations (v). The islet architecture is more organized and less disrupted as compared with that of diabetic control. Alpha cells (a) were noticed at the periphery of islets and many beta cells (b) in the core of the islets appeared intact. (X400)
DISCUSSION

Study results revealed the treatment of diabetic rats with either *R. graveolens* infusion or its flavonoid, rutin, to lead to significant amelioration of glucose tolerance. A decrease in elevated serum glucose levels is in agreement with the results of Chakravarthy et al. (58,59), who demonstrated similar effects of the flavonoid, (-)-epicatechin, in alloxan diabetic rats, and also with those reported by Nuraliev and Avezov (60) on a hypoglycemic effect of quercetin in alloxan diabetic animals. Furthermore, the hypoglycemic effect of rutin in STZ diabetic animals was recorded by Vessal *et al.* (61) and Kamalakkannan and Prince (23). Thus, the hypoglycemic effect of *R. graveolens* may be due to the presence of flavonoids such as rutin. Moreover, the antihyperglycemic effect may also be attributed to the presence of substances other than flavonoids, i.e. glycosides, alkaloids, saponin, tannins, resins and triterpenes. Such compounds have been reported to be responsible for hypoglycemic action by several authors (61-65). The present study indicated these groups of compounds to be found in the tested plant.

In comparison with normal control rats, the present study revealed a profound decrease in fasting insulin level of STZ diabetic rats. This finding agrees with Akhani *et al.* (66) and may be ascribed to the diabetogenic effect of STZ, which leads to marked degenerative changes in β-cells, as indicated in the present study. Serum insulin concentration was increased markedly as a result of treating diabetic rats with both *R. graveolens* and rutin.

By its ability to scavenge free radicals and to inhibit lipid peroxidation (67), rutin prevents STZ-induced oxidative stress, protects β-cells resulting in increased insulin secretion, and decreases elevated blood glucose levels. In this context, a study by Vessal *et al.* (61) showed that quercetin, an aglycone of rutin, decreased elevated blood glucose concentration and increased insulin release in STZ-induced diabetic rats. Also, Coskun *et al.* (68) report that in STZ-induced diabetic rats, quercetin protected pancreatic β-cells by decreasing oxidative stress and preserving β-cell integrity. According to the work of Kamalakkannan and Prince (23), the increased insulin levels could also be due to the stimulatory effect of rutin, thereby

Figure 9. Pancreata of diabetic rats treated with rutin showing its ameliorative effect on the islets of Langerhans. There are still few necrotic areas (n), pyknotic nuclei (pk) and vacuolations (v). Beta cells (b) in the core of islets appeared with improved integrity and were obviously increased in number. Alpha cells (a) and delta cells (d) at the periphery of islets were also noticed. (X400)
potentiating insulin secretion from the existing β-cells of the islets of Langerhans in diabetic treated rats. Moreover, Hii and Howell (69) showed that exposure of isolated rat islets to certain flavonoids such as (-)-epicatechin or quercetin enhanced insulin release. They suggest that such flavonoids may act on islet function, at least in part, via alteration in Ca²⁺ fluxes and in cyclic nucleotide metabolism.

In the present study, serum C-peptide was profoundly decreased in diabetic rats. The treatment with both R. graveolens infusion and rutin produced a marked increase in serum C-peptide level of diabetic rats. According to the results of Kamalakkannan and Prince (23), the increase in serum C-peptide levels concomitant with the increase of serum insulin levels in diabetic rats treated with rutin reflects the increase in insulin secretion.

According to our results, the marked increase in serum insulin and C-peptide levels after treatment of diabetic rats with R. graveolens infusion and rutin was due to the stimulatory effects of these agents on the insulin secretory response of the islets of Langerhans on the one hand, as indicated by the present in vitro study, in addition to the ameliorative effects of these agents on the integrity of β-cells as revealed by histologic study on the other hand.

While serum glucose concentration measurement is usually used to detect glycemic regulation in diabetes after treatment, fructosamine appeared more useful because it detects deterioration or improvement over a period of several days (1-3 weeks) after diabetes management. Serum fructosamine, a putative measure of glycosylated proteins, has been suggested by many authors to be of value as a screening test for diabetes mellitus (70,71). In the present study, serum fructosamine level was profoundly increased in diabetic rats at fasting state as compared with normal ones. On the other hand, treatment of diabetic rats with R. graveolens aqueous extract and rutin induced an obvious decrease of elevated fructosamine level. Elgawish et al. (72) report that agents with antioxidant or free radical scavenging power may inhibit oxidative reactions associated with protein glycation. Therefore, rutin with its free radical scavenging capability effectively reduced the formation of glycated proteins.

This explanation was supported by the present study, indicating that R. graveolens infusion and rutin have a strong antioxidant activity. A decrease in blood glucose levels may have also contributed to decreased levels of glycated proteins in R. graveolens and rutin treated diabetic rats. This finding is in accordance with the study by Kamalakkannan and Prince (23), which showed decreased levels of glycated hemoglobin in rutin-treated diabetic rats.

The liver glycogen level may be considered as the best marker to assess the anti-hyperglycemic activity of any drug (73). The increased hepatic glucose output in diabetes may be derived from glycogenolysis and/or gluconeogenesis, as reported by Raju et al. (74). Our results revealed an enormous depletion in hepatic glycogen content accompanied by a decreased hexokinase activity and profound elevation of hepatic glycogen phosphorylase activity and the gluconeogenic enzyme, glucose-6-phosphatase, as compared to that of normal control ones. These results are in accordance with those of Lavoie and Van de Werve (75), Ahmed (76) and Abdel-Moneim et al. (77), who found that STZ-induced diabetes reduced hepatic glycogen content and increased glucose-6-phosphatase activity in diabetic rats. These results are also in agreement with the work of Grover et al. (73), who demonstrated a decreased enzymatic activity of hexokinase in diabetic animals, resulting in depletion of liver glycogen. These changes may be due to insulin deficiency and/or insulin resistance, which in turn results in the activation of glycogenolytic and gluconeogenic pathways (77,78). Moreover, deficiency of insulin secretion decreases hepatic tyrosine kinase responsible for the activation of glycogen synthase and as a result, glycogen breakdown prevails in diabetic animals (79). In the present study, the elevation of liver glycogen content after treatment with R. graveolens extract and rutin was due to amelioration of these altered enzyme activities secondary to the increase of insulin levels in the blood as well as improvement of insulin action. In addition, the enhanced peripheral glucose uptake and increased hepatic hexokinase activity as well as decreased glucose-6-phosphatase activity after treatment with the tested agents, as indicated in the
present *in vitro* and *in vivo* studies, lead to more intense formation of glucose-6-phosphate, which in turn inactivates glycogen phosphorylase and activates glycogen synthase in the liver and muscle (79,80). The increase in hexokinase activity and the decrease in glucose-6-phosphatase, in the present study, may have also reflected a decrease in hepatic glucose output and enhanced peripheral glucose uptake as the result of treatment.

The data obtained in the present study indicated that *R. graveolens* infusion and rutin produced a marked increase of peripheral glucose consumption in the presence and absence of insulin as compared with the corresponding controls. Both agents acted in a dose dependent manner with a more potent effect for rutin than rue infusion. The marked increase in the peripheral glucose uptake in the absence of insulin as a result of the tested agents suggests that they may have insulin mimetic action or non-insulin mediated effect. The enhanced rate of peripheral glucose uptake in the absence of insulin may involve an insulin independent increased protein expression of both GLUT1 and GLUT4 (81-83). Furthermore, all doses of both agents were able to potentiate the enhanced effect of insulin on peripheral glucose uptake in the presence of insulin. Such findings suggest that both an insulin-independent rise in glucose uptake *per se* as a direct result of the tested agents would possibly synergize with insulin to formulate concrete mechanisms contributing to the hypoglycemic activity observed herein. In addition, both *R. graveolens* and rutin increased the *in vitro* insulin binding affinity of rat diaphragm in a dose dependent manner. Thus, the insulin mediated effects of the tested agents in the present study may have included the increase in insulin binding affinity by these agents. In addition to the effect on hepatic glucose output and peripheral glucose uptake, the plant infusion and rutin induced a profound decrease in intestinal glucose absorption in a dose dependent manner.

Our results proved that both *R. graveolens* and rutin improved glucose tolerance and this amelioration seemed to be mediated *via* alleviation of the islet architecture, enhancement of insulin release, insulin binding affinity and peripheral glucose uptake and decreasing intestinal glucose absorption in addition to decreasing the activity of gluconeogenic and glycogenolytic enzymes.

In view of the lipid profile, diabetic rats exhibited marked elevation of serum total lipids, triglycerides, total cholesterol, LDL-cholesterol, VLDL-cholesterol concentrations and hepatic HMG-CoA reductase activity.

The treatment of STZ diabetic rats with *R. graveolens* extract or rutin produced potential improvement of these altered serum lipid variables. These results are in agreement with the work of Nuraliev and Avezov (60), who demonstrated the decreasing effects of quercetin on decreasing the level of cholesterol and LDL-cholesterol.

The ability of quercetin to reduce plasma cholesterol and triglycerides in diabetic animals could be explained by the insulin releasing capacity of quercetin, which is a metabolite of rutin in isolated rat islets of Langerhans (69). The reduction of intestinal cholesterol absorption might have a role in the mechanism of action to augment the hypolipidemic activity of the tested materials. This assumption is supported by the present results which indicated that both plant extract and rutin successfully decreased the intestinal cholesterol absorption *in situ*. Furthermore, as evidenced in this study, the hypolipidemic activity of both treatments may also be mediated, at least in part, *via* inactivation of hepatic HMG-CoA reductase, a key enzyme, in cholesterol synthesis. In concurrence with this attribution, Raz et al. (84) state that inhibitors of hepatic HMG-CoA reductase are well established drugs for the treatment of hypercholesterolemia and decrease the incidence of dyslipidemia in diabetic subjects. This also coincides well with the work of Jung et al. (85), who state that flavonoids decrease liver HMG-CoA reductase activity in type 2 diabetic mice. Moreover, rutin has been reported to lower hepatic and blood cholesterol levels, as stated by Park et al. (86).

Taken together, it can be concluded that the ameliorative effect of *R. graveolens* extract or rutin on serum lipid variables may be attributed to their insulin
releasing capacity and insulin binding affinity and decreasing intestinal cholesterol absorption and activity of hepatic HMG-CoA reductase.

In the present study, amelioration of the glycemic and lipidemic states of diabetic rats in response to treatment with rutin may also be attributed to the increased expression of PPAR\(\gamma\). Rutin showed a potential effect on the expression of PPAR\(\gamma\), but \textit{R. graveolens} infusion had no effect on PPAR\(\gamma\) expression; this may explain why rutin had more effective hypoglycemic and hypolipidemic effects than \textit{R. graveolens} infusion. The effect of PPAR\(\gamma\) on lipid and glucose control may be explained according to Lee \textit{et al}. (87), Staels and Fruchart (88), Feige \textit{et al}. (89) and Lefebvre \textit{et al}. (90), who state that PPAR\(\gamma\) promotes pre-adipocyte differentiation, stimulates the storage of fatty acids (FAs) in adipocytes and enhances insulin sensitivity. The action of PPAR\(\gamma\) on insulin sensitivity results from its ability to channel FAs into adipose tissue, thus decreasing plasma FA concentration and alleviating lipotoxicity in skeletal muscle, liver and pancreas. Also, PPAR\(\gamma\) activation has been reported to improve insulin resistance by lowering the hepatic triglyceride content (91,92), activating hepatic glucokinase expression (93) and exhibiting an antiatherogenic effect synergistic with an HMG-CoA reductase inhibitory effect in rabbits (94). In addition, PPAR\(\gamma\) can affect insulin sensitivity by regulating adipocyte hormones, cytokines and proteins that are involved in insulin resistance. Indeed, PPAR\(\gamma\) downregulates the expression of genes encoding resistin and TNF\(\alpha\), whereas it induces adiponectin expression, which increases FA oxidation by activation of the AMP-activated protein kinase pathway (89,90). Moreover, Willson \textit{et al}. (95) demonstrated the activation of PPAR\(\gamma\) to improve insulin sensitivity and lower circulating levels of glucose, triglycerides and FFAs without stimulating insulin secretion in rodent models of type 2 diabetes. Also, Hevener \textit{et al}. (96) state that PPAR\(\gamma\) agonists increase glucose uptake in adipose tissue and skeletal muscle. Our results coincide with the work of Jung \textit{et al}. (85), who found an increased expression of PPAR\(\gamma\) by the flavonoids hesperidin and naringin, and that of Anandharajan \textit{et al}. (97), who showed an increased PPAR\(\gamma\) expression by \textit{Pterocarpus marsupium} isoflavone on L6 myoblasts and myotubes.

The data obtained demonstrated that the treatment of diabetic rats with either \textit{R. graveolens} extract or rutin caused a marked decrease of resistin mRNA transcripts that greatly increased in STZ diabetic rats. According to Rajala \textit{et al}. (13), the insulin resistant effects of resistin are thought to account for the activation of glucose-6-phosphatase, which subsequently prevents glycogen synthesis and increases the rate of glucose production. The present results supported this suggestion since the increase in resistin expression was associated with profound elevation of glucose-6-phosphatase activity. On the other hand, Antuna-Puente \textit{et al}. (98) postulated that the absence of resistin could allow for the activation of activated mitogen pyruvate kinase (AMPK) and reduce gene expression encoding for hepatic gluconeogenesis enzymes. However, little is known concerning the control of resistin gene expression. Steppan \textit{et al}. (99) demonstrated that resistin mRNA and protein were downregulated during fasting and upregulated upon refeeding. Thus, these results reflect the ameliorative effect of \textit{R. graveolens} and rutin on the glycemic and lipidemic states of diabetic rats.

This study revealed that both study agents had antihyperglycemic and antihyperlipidemic efficacy which may be mediated \textit{via} pancreatic and extrapancreatic effects. However, further clinical studies are required to assess the efficacy and safety of \textit{R. graveolens} and rutin in diabetic humans.
REFERENCES


5. Rosenson RS. Effects of peroxisome proliferator activated receptors on lipoprotein metabolism and glucose control in type 2 diabetes mellitus. Am J Cardiol 2007;99(Suppl):96B-104B.


89. Feige JN, Gelman L, Michalik L, Desvergne B, Wahl W. From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. Prog Lipid Res 2006;45:120-159.


94. Shiomi M, Ito T, Tsukada T, Tsujita Y, Horikoshi H. Combination treatment with troglitazone, an insulin action enhancer, and pravastatin, an inhibitor of HMG-CoA reductase, shows a synergistic effect on atherosclerosis in WHHL rabbits. Atherosclerosis 1999;142:345-353.


