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ORIGINAL ARTICLE

Changes of lipid profiles, glucose, and hemogram after administration of *Ruta graveolens* extract in diabetic rats

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Abstract Diabetes mellitus is a disease that affects millions of people in the world. Many people use therapeutic herbal medicine for many reasons. In the present study, the effects of *Ruta graveolens* extract on the level of blood glucose, lipids, and hematological parameters have been studied. For this purpose 30 adult male Wistar rats weighing (200–300 g) were divided randomly into six groups (A, B, C, D, E, and F) and housed in single cages. The control group (A) was injected with normal saline. Diabetes was induced by injection of streptozotocin (60 mg/kg, i.p.) in other five groups. Group C received glibenclamide (10 mg/kg) orally, and groups D, E, and F received hydroalcoholic extract of *R. graveolens* (10, 20, and 30 mg/kg, i.p.) for 10 days, respectively. Blood samples were taken by heart puncture, and the level of glucose and lipids were measured. Hematological parameters including complete blood count was also determined by using automated cell counter. Results showed that administration of *R. graveolens* extract caused a significant decrease in the levels of cholesterol and LDL-c ($p<0.05$) by dose-dependent manner, whereas no significant changes were seen in glucose, triglycerides, VLDL-c, and HDL-c values in diabetic rats. It appears that *R. graveolens* extract has significant effects on total cholesterol and LDL-c in diabetic rats

Keywords Diabetes · *Ruta graveolens* extract · Glucose · Lipid · Rat

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Introduction

Diabetes mellitus is a chronic metabolic disorder that is characterized by a high level of blood glucose induced by insulin deficiency or insulin resistance. Diabetes mellitus is a major cause of disability and hospitalization and increases its prevalence (Vats et al. 2002). This disorder is associated with an increased risk of thrombotic, atherosclerotic, and cardiovascular disease (Chattopadhyay and Bandyopadhyay 2005).

Hyperglycemia increases diacylglycerol levels and activates protein kinase C activity in the aorta of diabetic rats (Inoguchi et al. 1994) and dogs (Xia et al. 1994). Hyperlipidemia is a feature of drug-induced diabetes in rats (Still et al. 1964) and rabbits (Miller and Wilson 1984; Nordestgaard et al. 1988). Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes (Gandhi 2001). Low-density lipoprotein in diabetic patients leads to abnormal metabolism and is associated with increase in very low-density lipoprotein (VLDL-c) secretion (Howard 1987). Streptozotocin is a compound commonly used for the induction of type I diabetes in experimental rats. This agent causes diabetes by rapid depletion of B cells, which leads to a reduction of insulin release (Tomlinson et al. 1992). *Ruta graveolens* (Rutaceae), commonly known as rue, is known as a medicinal plant since ancient times and currently used for treatment of various disorders such as aching pain, eye problems, rheumatism, and dermatitis (Conway and Slocumb 1979; Miguel 2003). *Ruta* is a native of the Mediterranean

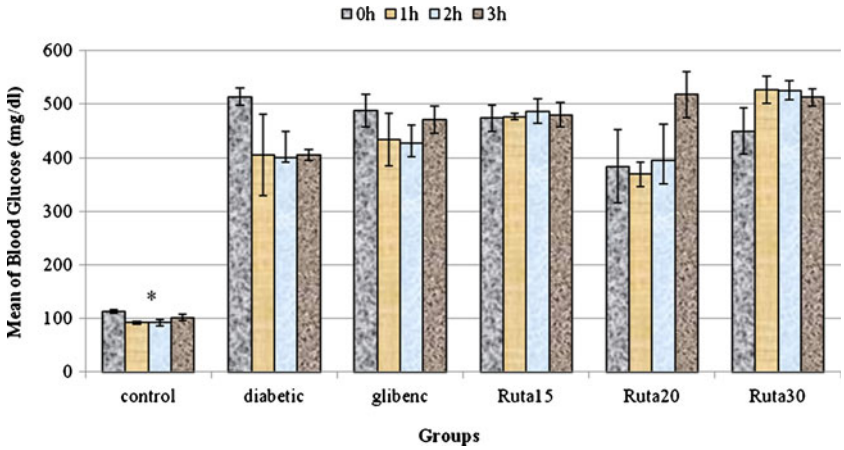

Fig. 1 Mean (±SE) blood glucose concentration with time in the different groups. *Asterisk* represents significant difference between control group to others ($p<0.05$)

region but cultivated throughout Europe and many countries of Asia (Raghav et al. 2006). The plant contains more than 120 compounds of different classes of natural products such as acridone alkaloids, coumarins, essential oils, flavonoids, and furoquinolines (Kuzovkina et al. 2004). The plant is used as a contraceptive to relieve symptoms of hangover (Chávez et al. 2003) and applied externally as a poultice against rheumatic pain (Atta and Alkofahi 1998; Chávez et al. 2003). The aim of this study was to evaluate the effects of aerial part extract of *R. graveolens* on the level of glucose, lipid profiles, and hematological parameters in diabetic rats.

Materials and methods

R. graveolens extract

In this research dried aerial parts of *R. graveolens* were used from Ilam province, Iran. The plant was taxonomically identified at the Department of Botany, School of Agriculture, Shahid Chamran University, Ahvaz, Iran. The plant was powdered, by using a grinder. One hundred grams of this powder was placed in a beaker and 1,000 ml of 70% ethanol added. The mixture was left at room temperature for 3 days. The extract was separated, and the remaining plant was extracted with more ethanol after 2 days. The extract was filtered by Whattman (No. 1) filter paper and concentrated under vacuum evaporation and then dried in oven at 40°C.

Induction of experimental diabetics

Diabetes mellitus was induced by streptozotocin (Sigma, Germany) which was administrated intraperitoneally at a single dose of 60 mg/kg in normal saline. Blood glucose was measured by a glucometer (Convergent, Germany) before streptozotocin administration and 3 days later.

Experimental animals

The study was performed in adult male Wistar rats, weighing 200–300 g, provided by the animal house of Jondishapour University of Ahvaz, Iran. They had access to

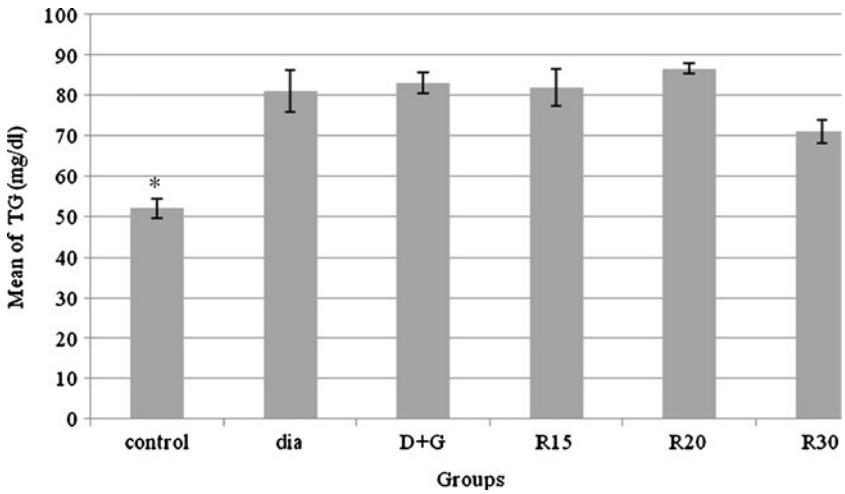

Fig. 2 The mean (±SE) plasma levels of triglyceride in the different groups. *Asterisk* represents significant difference between the control group to others ($p<0.05$)

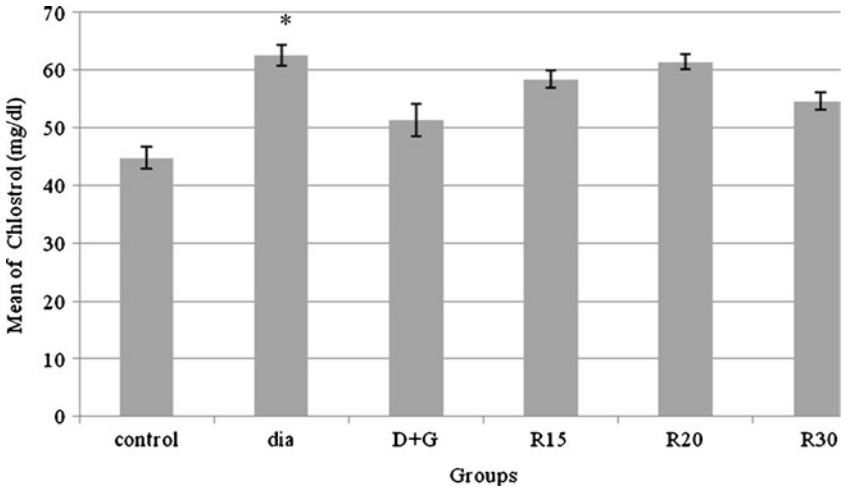

Fig. 3 The mean (±SE) plasma levels of cholesterol in the different groups. *Asterisk* represents significant difference between diabetic group to control, diabetic + glibenclamide and diabetic + *Ruta* 30 mg/kg ($p<0.05$)

food and water ad libitum and were submitted to 12-h dark/light cycle.

Experimental treatments

The animals were divided into six groups each comprising of five animals. Group A served as normal controls. Groups B, C, D, E, and F were induced by a single intraperitoneal injection of 60 mg/kg of streptozotocin. Group B (diabetic) did not receive any treatment. Group C, comprised of diabetic rats, was treated orally with glibenclamide (10 mg/kg). Group D, E, and F comprised of diabetic rats were treated with *R. graveolens* (10, 20, and 30 mg/kg, i.p.), respectively. Blood level of glucose was measured before and 1, 2, and 3 h after administration. Administration of the extract continued for 10 days. After 20 days rats were anesthetized by chloroform, blood samples were taken by heart puncture, and heparinized plasmas were separated for determination of lipid profiles, glucose, and hematological parameters.

Biochemical estimations

The plasma levels of triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-c) were determined spectrophotometrically using enzymatic colorimetric assay kits (ZistChem Diagnostics Tehran, Iran), while low-density lipoprotein cholesterol (LDL-c) and VLDL-c were calculated by the Friedewald formula. The hematological analysis was carried out for RBC, WBC, and platelet count and MCH, MCV, and MCHC were by cell counter apparatus (Mindray2800-C Vet).

Statistical analysis

All results were expressed as the mean±SE. The statistical significance of differences between groups was analyzed by

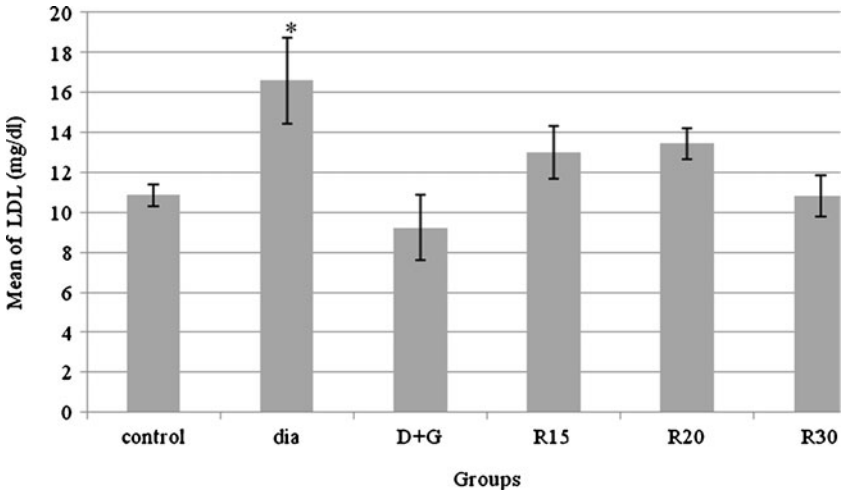

Fig. 4 The mean (±SE) plasma levels of LDL-c of the different groups. *Asterisk* represents significant difference between diabetic group to control, diabetic + glibenclamide and diabetic + *Ruta* 30 mg/kg ($p<0.05$)

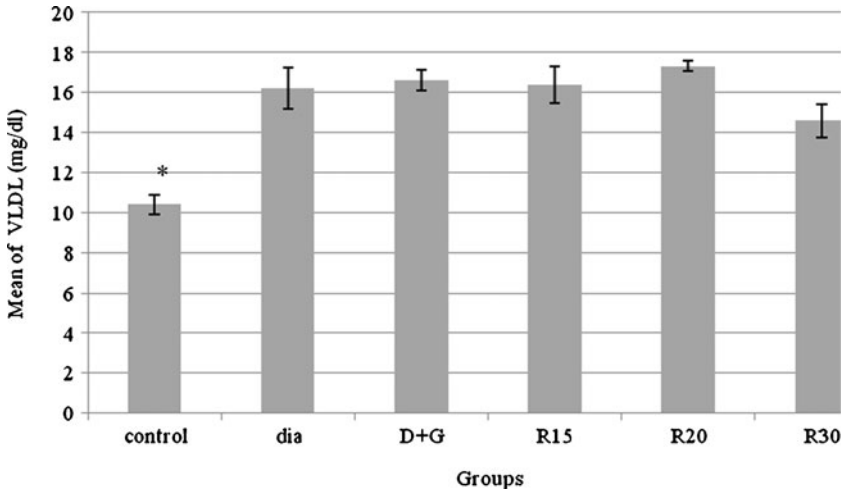

Fig. 5 The mean (±SE) plasma levels of VLDL-c of the different groups. *Asterisk* represents significant difference between control group to others ($p<0.05$)

LSD and Tukey's test. Data were considered statistically significant with p values less than 0.05.

Results

Glucose concentration increased after streptozotocin administration within 72 h. The mean of blood glucose level increased from 113±8 to 514±16 mg/dl after 72 h. Therapeutic application of *Ruta* extract and glibenclamide did not significantly decrease blood glucose concentration with time (Fig. 1).

The mean plasma levels of triglyceride was significantly increased in diabetic rats to the control group ($p=0.01$). This mean was 51.97±2.49 mg/dl in the control group and reached to 80.94±5.13 mg/dl in diabetic rats (Fig. 2). Administration of the *Ruta* extract and glibenclamide did not significantly decrease plasma levels of triglyceride.

The mean plasma levels of total cholesterol were significantly increased in diabetic rats to the control group ($p<0.05$). This mean was 44.72±1.93 mg/dl in the control group and reached to 62.57±1.79 mg/dl in the diabetic group (Fig. 3). Administration of glibenclamide and the *R. graveolens* extract (30 mg/kg) significantly decreased plasma levels of total cholesterol ($p<0.05$).

The mean plasma levels of LDL-c was significantly increased in diabetic rats to the control group ($p=0.01$). This mean was 10.86±0.53 mg/dl in the control group and reached to 16.6±2.16 mg/dl in the diabetic rats (Fig. 4). Glibenclamide significantly decreased plasma levels of LDL-c ($p<0.05$). Also *R. graveolens* extract (at 30 mg/kg) significantly decreased plasma levels of LDL-c ($p=0.01$).

The mean of plasma levels of VLDL-c was significantly increased in the diabetic rats to the control group ($p=0.01$). This mean was 10.4±0.5 mg/dl in the control group and reached to 16.2±1.02 mg/dl in the diabetic group (Fig. 5). Administration of glibenclamide and the *R. graveolens* extract did not significantly change the plasma levels of VLDL-c. The changes of HDL in different

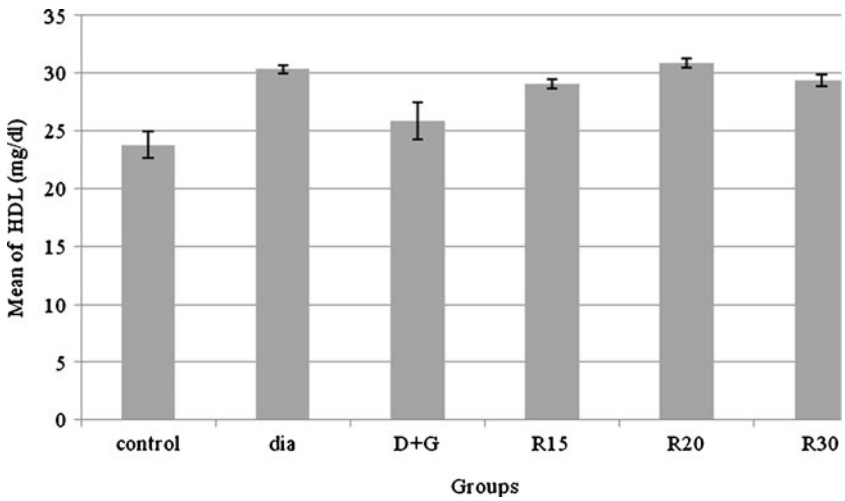

Fig. 6 The mean (±SE) plasma levels of HDL-c of the different groups. There is no significant difference between groups

Table 1 Mean (±SE) of hematological results in different groups

	Control	Diabetes	Glibenclamide	*Ruta*, 10 mg/dl	*Ruta*, 20 mg/dl	*Ruta*, 30 mg/dl
RBC ($\times 10^6/\mu l$)	7.5±0.1	7.6±0.07	7±0.26	7±0.33	7.3±0.26	7.5±0.35
HGB g/dL	12.16±0.53	13±0.26	12±0.93	11.33±1.98	12.36±0.82	12.44±1.05
HCT (%)	38.6±1.91	41.83±1.38	39.66±2.85	34.64±11.72	41±2.7	41.1±3.8
MCV (fl)	51.24±0.57	54.83±1.57	56.24±1.15	53.6±2.1	55.88±1.27	54.94±0.57
MCH (pg)	16.08±0.18	16.96±0.33	16.94±0.36	24.9±8	16.76±0.29	16.56±0.39
MCHC (g/dl)	31.46±0.39	31.03±0.56	30.2±0.3	27.92±5.77	30.12±0.5	30.24±0.74
PLT ($\times 10^3/\mu l$)	356±36	330±2	358±68	262±62	293±23	397±69
WBC ($\times 10^3/\mu l$)	8.76±1.7	9.1±0.8	11±1.6	8.1±1.1	6.5±1.3	11.28±0.9

There is no significant difference between groups

groups did not show significant changes to control group (Fig. 6).

The hematological analysis was carried for evaluation of CBC including RBC, WBC and platelet count and calculation of MCV, MCH and MCHC. Parameters did not show significant differences between different groups (Table 1).

Discussion

Diabetes mellitus is a multifactorial disease that has a significant impact on the health, quality of life, life expectancy of patients, and on the health care system. Diabetes is characterized by hyperglycemia together with biochemical alterations of lipid metabolism (Jensen et al. 1988). The therapeutic effect of *R. graveolens* extracts is generally dependent upon the degree of B cell destruction in induced diabetes. Treatment of moderate streptozotocin diabetic rats with medicinal plant extract resulted in the activation of B cells and granulation returning to normal, showing an insulinogenic effect (Kedar and Chakrabarti 1982). In streptozotocin-induced diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol, triglycerides, LDL-c, and VLDL-c and decreases in HDL-c (Mitra et al. 1995). In this research the *R. graveolens* extract (30 mg/kg) decreased total cholesterol and LDL-c in plasma of diabetic rats. According to the results, the LDL/HDL ratio in the control group was 0.45. The ratios in the groups that received 10, 20, and 30 mg/kg *R. graveolens* extract were 0.44, 0.43, and 0.36, respectively. Based on these ratios, it seems that there is a negative correlation between the dose of *R. graveolens* extract and the ratio of LDL/HDL.

Ratheesh et al. (2010) showed that oral administration of *R. graveolens* reduced the level of total cholesterol and LDL-c in hypercholesterolemic rats. Supplementation of *R. graveolens* significantly increased the plasma level of HDL-c. The atherosclerotic index, defined as the ratio of LDL to HDL, also decreased in *R. graveolens* treatment (Ratheesh et al. 2010). *R. graveolens* contains different active compounds, out of them rutin, a flavonoid, is known to have nitric oxide-scavenging activity (Van Acker et al. 1995). According to the available literature, *R. graveolens* plant contains approximately 2% of rutin. An interesting recent study reported the decrease in lipopolysaccharide-induced nitric oxide production by rutin in vivo due to inhibition of nitric oxide synthase protein expression (Pathak et al. 2003; Shen et al. 2002). Lipid peroxidation product was decreased and activities of antioxidant enzymes increased by *Ruta* (Ratheesh et al. 2009). Many of the biological actions of flavonoids have been attributed to *R. graveolens*' powerful hypolipidemic properties (Koshy and Vijayalakshmi 2001; Wang and Ng 1999). Several clinical trials have documented beneficial modifications of the LDL/HDL ratio after intake of flavonoid-containing food products (Weggemans and Trautwein 2003). Some studies have confirmed the presence of antioxidant phenolic compounds in the aerial part of *R. graveolens* (Saieed et al. 2006).

These findings were obtained in normal rats, but the effect of *R. graveolens* on cholesterol and LDL-c in diabetic rats was not observed. The reason of this difference may be related to diabetic conditions. Ratheesh et al. (2009) showed that the methanolic extract of *R. graveolens* markedly reduced cell influx, edema formation, release of mediators, and oxidative stress associated with arthritic condition. These effects may be due to the broad range of biologically active compounds present in the plant extract (Ratheesh et al. 2009).

Glibenclamide is a sulfonylurea compound which is used because of its hypoglycemic effect. In the present study, it did not change glucose but it decreased LDL-c level in diabetic rats. It appears that this drug did not affect the plasma level of glucose. In the present study, hematological parameter did not show any significant changes, but in one study, the level of total leukocytes count was decreased significantly after *R. graveolens* extract administration in rats (Ratheesh et al. 2010).

In conclusion, use of *R. graveolens* extract reduced the lipid imbalances in diabetes. Thus the plant may be useful to control lipid imbalances for the therapeutic treatment of conditions associated with diabetes. Further studies are in progress to elucidate the exact mechanism of action of the active ingredient in *R. graveolens* that mediates the protective effects.

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