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Resveratrol regulates hyperglycemia-induced modulations in experimental diabetic animal model

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is associated with variable degrees of glucose intolerance, impaired insulin secretion, insulin resistance and increased hepatic glucose production. The aim of present study was to investigate the therapeutic potentials of resveratrol (RSV) alone and/or in combination with vitamin-E (Vit-E) against hyperglycemia-induced modulations using experimentally alloxan-induced diabetic animal model. Alloxan was used to induce diabetes mellitus in white albino rats and metformin (MF) was used as standard anti-diabetic drug to compare the therapeutic potentials of RSV (alone and/or with Vit-E) by estimating the effect of treatment on glycemia, insulin resistance, liver and kidney function biomarkers, anti-oxidant status, and serum levels of calcium and magnesium. The results of present study indicate the RSV ($P < 0.001$) alone and/or in combination with Vit-E ($P < 0.001$) exhibited a highly significant therapeutic potentials by ameliorating the glycemia-induced modulations. Moreover, we also found that RSV in combination with Vit-E also exhibited a better therapeutic effects when compared with that of MF ($P < 0.001$) and Vit-E ($P < 0.05$), respectively. Hence, we conclude that RSV alone and/or in combination with Vit-E exhibit its significant therapeutic potentials against hyperglycemia-induced modulations in experimental diabetic animal model and may be one of the most exciting prospect for future treatment of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disorder that refers to the clustering of risk factors including dyslipidemia, hyperglycemia, glucose intolerance, insulin resistance, impaired insulin secretion, glucose utilization and hepatic glucose metabolism [1]. Among the various causative factors, the most important pathogenic factors are generation of oxidative stress and tissue-specific inflammation [1–6]. The clinical heterogeneity of this metabolic disorder can be explained by its significant effect on fat, protein and carbohydrate metabolism, and dysfunctioning of transcriptional-mediated cellular pathways [7]. The risk of developing T2DM is increased which is associated with impaired glucose tolerance and hyperglycemia-mediated modulations [8]. Among the various anti-diabetic drugs, a biguanide such as metformin (MF) is being widely used as standard anti-diabetic medication. It works in various approaches to decrease the glucose levels in individuals with T2DM and enhances the sensitivity of muscle cells for insulin as well [9,10]. Moreover, it delays sugar absorption from the intestines into the circulatory system subsequent to eating so that there is a lesser extent to spike in blood sugar levels after meals [11]. Due to certain unavoidable side effects exhibited by synthetic anti-diabetic drugs [12–16], several approaches are being investigated to overcome and prevent the incidences of unavoidable side effects during the treatment of T2DM [17–21].

Resveratrol (RSV), (3,5,4-trihydroxy-*trans*-stilbene), is a stilbenoid which is a naturally occurring phenol and phytoalexin produced by several plants. It is most abundantly found in red grapes, blueberries, peanuts, pistachios, and cranberries [22]. RSV exerts beneficial impact on humans and might be helpful in treating and preventing metabolic sicknesses such as obesity and diabetes mellitus. As a potent anti-oxidant, RSV is a plant-derived polyphenolic compound that possesses diverse pharmacological properties. Some studies have revealed that RSV administration enhances insulin sensitivity in patients with T2DM and diabetic rats [23]. Various investigations have exhibited that resveratrol is capable of decreasing blood glucose levels in animals with marked hyperglycemia. In pancreatic tissue, resveratrol improves anti-oxidant defense by increasing the activity of anti-oxidant enzymes such as, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase and glutathione-S-transferase and protect the pancreatic cells from free radical damage [24].

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Vitamin E (Vit-E) is a major lipid-soluble chain-breaking anti-oxidant with anti-inflammatory properties. *In vitro* studies have showed that Vit-E may enhance the insulin activity and secretion by protecting β-cells and peripheral tissues from free radical-mediated damage, prompting the hypothesis that Vit-E may help delay the development of T2DM [25]. In addition to its anti-oxidant properties, Vit-E also decreases the cytotoxic impact of oxidized lipoprotein, enhances endothelial function [26]. In addition, observational studies have proposed that supplemental Vit-E users have lower risks of coronary events [27]. For the prevention of microvascular complications of diabetes, Vit-E is also proposed. In fact, in animal models, it diminishes the hyperglycemia-induced protein kinase C (PKC) activation and D-acetylglycerol (DAG) levels, which have been related with abnormalities in the renal, and vascular tissues in diabetes [28].

In present study, we aimed to comparatively investigate the therapeutic potentials of RSV alone and/or in combination with Vit-E on hyperglycemia-induced modulations in experimentally-induced diabetes mellitus in animal model. To compare the therapeutic effects of RSV and Vit-E, we used MF as standard anti-diabetic drug and alloxan was used to induce the diabetes mellitus in experimental animal model.

2. Material and methods

2.1. Chemicals required

RSV and alloxan were purchased from Bristol Mayer Biotech Pakistan. Vit-E and MF were purchased from Merck Pharmaceuticals Pakistan. All other chemicals used were of analytical grade.

2.2. Experimental animals

36 albino rats of same sex (180–250 g) were purchased from the animal house of University of Agriculture, Faisalabad, Pakistan and were kept at room temperature (25 ± 2 °C). Rats were fed with normal diet with water *ad libitum*. The study protocol was approved from the "Institutional Biosafety Committee" of University of Agriculture, Faisalabad, Pakistan. Alloxan (120 mg/kg body weight of rat) was injected intraperitoneally into the rats (n = 30) and after 3–4 days (when diabetes confirmed), all rats were divided into 5 equal groups each including 6 rats for 30 days study period. Group 1 (n = 6) was marked as non-diabetic control (NDC). Group 2 was marked as diabetic control (DC) in which diabetes was induced by IP administration of alloxan, but this group did not receive any treatment throughout the study period. Group 3 was treated with MF (500 mg/kg/day) and marked as MF group. Group 4 was treated with RSV (30 mg/kg/day) and was marked as RSV group. Group 5 was treated with Vit-E (50 mg/kg/day) and was marked as Vit-E group. Group 6 was treated with the combination of RSV (30 mg/kg/day) and Vit-E (50 mg/kg/day) and was marked as RSV + Vit-E group.

2.3. Blood sampling

Blood samples were collected at 1st (before), 15th (during) and 30th (last) day of treatment and centrifuged at 3000 × *g* for 20 min and serum was separated and stored at freezing temperature (−20 °C) for further analysis.

2.4. Biochemical analysis

2.4.1. Estimation of glycemic control biomarker

To investigate the impact of treatment on glycemia, we measured the blood glucose 2–3 times a week throughout the study period with the help of Accu-Check glucometer.

2.4.2. Estimation of glucose tolerance

Before the end of study protocol, oral glucose tolerance test (OGTT) was performed. Blood samples were collected afterwards at specified time intervals. Rats were fasted overnight prior to perform OGTT procedure. Fasting blood sugar (FBS) was determined using glucometer. Later, the glucose (2 gm/kg body weight of rats) was administered into the rats *via* gavage and blood glucose concentrations were measured from the fresh blood samples at 30, 60, 90 and 120 minutes.

2.4.3. Estimation of insulin resistance

Homeostatic model assessment for insulin resistance (HOMA-IR) is the formula for predicting the insulin resistance in peripheral tissues. For HOMA-IR, Fasting insulin (μU) and glucose (mM) levels were measured and insulin resistance was calculated by using the following formula:

$$\text{HOMA}-\text{IR} = \text{Fasting insulin } (\mu\text{U}) \text{ X Fasting glucose (mM)}/22.5$$

2.4.4. Estimation of liver function biomarkers

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the enzymes that predict the normal functioning of liver. The serum levels of AST and ALT were estimated using their corresponding assay reagent kits at 1st, 15th and 30th day of study protocol.

2.4.5. Estimation of anti-oxidant status

Glutathione (GSH), Superoxide dismutase (SOD) and catalase (CAT) are the enzymes that reflect the anti-oxidant status of the body. The serum levels of these enzymes were measured using colorimetric method before, during and at the end of treatment period.

2.4.6. Estimation of serum levels of calcium and magnesium

Serum levels of calcium and magnesium was measured by photometric test before, during and at the end treatment and following formula was used to determine the serum level of calcium and/or magnesium:

$$\text{Calcium or magnesium (mg/dl)} = \Delta\text{A sample}/\Delta\text{A standard} \times \text{conc. standard (mg/dl)}$$

2.4.7. Estimation of kidney function biomarkers

Blood urea nitrogen (BUN) and creatinine are the kidney function biomarkers which were measured before, during and after the treatment period by using their commercial assay kits.

3. Results

3.1. Effect of treatment on glycemia

To investigate the effect of treatment (RSV alone and/or in combination with Vit-E) on hyperglycemia, we measured the blood glucose level (mg/dL) 2–3 times/week. We found that before the start of treatment, alloxan significantly increased the blood glucose level ($P < 0.001$) in all experimental rats as compared to that of NDC rats (Fig. 1). When treatment started, we found that MF, RSV, Vit-E and RSV + Vit-E started to exhibit significant hypoglycemic effects at 15th day ($P < 0.05$), 9th day ($P < 0.05$), 12th day ($P < 0.01$) and 9th day ($P < 0.05$), respectively (Fig. 1). At the end of treatment period, we found that RSV, and RSV + Vit-E exhibited highly significant hypoglycemic effects as compared to that of MF ($P < 0.01$) and Vit-E ($P < 0.001$) treated experimental rats (Fig. 1).

3.2. Effect of treatment on glucose tolerance and insulin resistance

Before the end of treatment period, we performed OGTT to estimate the impact of RSV alone and/or in combination with Vit-E on glucose tolerance. OGTT was performed after an overnight fasting as described previously [29]. Before administering the calculated amount of glucose