Research Article

Lupeol regulates glucose transporter-4 and AS160 in adipose tissue of high-fat diet-fed diabetic rats

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ABSTRACT

Background: Lupeol, a natural herbal drug, is abundant in edible vegetables, fruits, and also in medicinal plants. There are reports showing that lupeol can reduce hyperglycemia and hyperinsulinemia in experimental mice model. **Objective:** The objective of this study was to identify the effect of lupeol on glucose transporter (GLUT)-4 and AS160 protein expression in adipose tissue of high-fat diet and sucrose-induced Type-2 diabetic (T2D) rats. **Materials and Methods:** Lupeol was administered orally to T2D rats (25 mg/kg body weight) for a period of 30 days. After the treatment period, animals were anesthetized and liver from control and experimental animals was dissected out and used for the protein expression of GLUT-4 and AS160 in adipose tissue. **Results:** Lupeol treatment increased GLUT-4 protein level in the T2D animals. **Conclusion:** It is concluded from the present findings that lupeol has antihyperglycemic activity through the regulation of insulin signaling molecules (GLUT-4 and AS160). Hence, lupeol can be used as one of the therapeutic agents for the management of T2D.

KEY WORDS: AS160, Glucose transporter-4, High-fat diet rat, Lupeol, Type-2 diabetes

INTRODUCTION

Adipose tissue is a crucial organ and major site to store of excess energy as triglyceride. It serves as an endocrine organ and modulates metabolic homeostasis by synthesizing a number of biologically active compounds.[1] Consumption of high dietary fat increases stored fat mass which is an established risk factor for metabolic diseases such as Type-2 diabetes (T2D), hypertension, coronary artery disease, non-alcoholic fatty liver disease, and polycystic ovarian diseases.[2] Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. Many recent reports showed that there are 425 million people in the world suffering from diabetes and also it has the chance to increase 629 million or more in 2045 (International Diabetes Federation 2017).[3] Synthetic drugs used in the management of diabetes had many deleterious side effects. In this context, herbal drugs are attaining the attractiveness for the treatment of diabetes mellitus by reason of their efficacy, low occurrence of side effects, low cost, and easy availability.[4] Lupeol (triterpenes) is one of the natural herbal drugs abundant in edible vegetables, fruits, and also in medicinal plants. It effectively reduced the hyperinsulinemia, blood glucose level in high cholesterol/high-fat diet and sucrose-induced mice.[5] However, the molecular mechanism which it exhibits antidiabetic property is not known.

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MATERIALS AND METHODS

Animals

Healthy adult male albino rats of Wistar strain (*Rattus norvegicus*) (150–180 days old weighing 180–200 g) were used in this study and maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethics committee (No: 011/2016 dated July 4, 2016) under specific humidity (65 ± 5%) and temperature (21 ± 2°C) with constant 12 h light and 12 h dark schedule. They were fed with standard rat pelleted diet (Lipton India, Mumbai, India), and clean drinking water was made available *ad libitum*. Isotonic sodium chloride solution was perfused through the left ventricle to clear blood from the organs. Adipose tissue was immediately dissected and used for the further study.

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Induction of T2D

Rats were subjected to 60 days of a high-fat diet containing cholesterol 3%, cholic acid 1%, coconut oil 30%, standard rat feed 66%, and 30% sucrose through drinking water. On the 58th day of treatment, after overnight fasting, blood glucose was checked and the rats that had blood glucose >120 mg/dl were chosen as T2D rats. Sucrose feeding through drinking water with a high-fat diet was continued until end of the study.

Experimental Design

Adult male albino rats of Wistar 150–180 days old with 180–200 g body weight were randomly divided into four groups of six rats each: Group I – control (vehicle treated); Group II – T2D rats; Group III – T2D rat treated with lupeol (25 mg/kg body weight/day) orally for 30 days; and Group IV – T2D rats treated with metformin (50 mg/kg b.wt/day) orally for 30 days. 2 days before killing, control and experimental animals were subjected to oral glucose tolerance test and insulin tolerance test. At the end of the treatment, animals were anesthetized with sodium thiopentone (40 mg/kg body weight), blood was collected through cardiac puncture, sera were separated and stored at −80°C, and 20 ml of isotonic sodium chloride solution was perfused through the left ventricle to clear blood from the organs. Liver from control and experimental animals was immediately dissected out and used for assessing the various parameters.

Protein Expression Analysis

Protein isolation

The tissues were homogenized in buffer-A containing 10 mM NaHCO3, 0.25 M sucrose, and 5 mM NaN_3 (1 ml for 100 mg) and the homogenate was centrifuged at 1300 × g for 10 min at 4°C. The supernatant was centrifuged at 12,000 × g for 15 min at 4°C. The resultant supernatant was sampled as a total protein for analysis SERB-1c. The protein concentration was estimated by the standard method of Lowry *et al*. (1951)[6] using bovine serum albumin as a standard.

Western Blot Analysis

The lysate proteins (50 µg/lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% gel) and transferred by electroblotting to polyvinylidene difluoride membrane (Bio-Rad Laboratories Inc.). The membranes were blocked with 5% non-fat dry milk and probed with the primary antibodies (1:1000 dilutions). Then, the membrane was washed and incubated for 1 h with horseradish peroxidase-conjugated rabbit anti-mouse or goat anti-rabbit secondary antibodies (1:10,000 dilution) (Genei, Bangalore, India). The specific signals were detected with an enhanced chemiluminescence detection system (Thermo Fisher scientific Inc., USA). The protein bands were captured using ChemiDoc and quantified by Quantity One image analysis system Bio-Rad Laboratories, CA. Later, the membranes were incubated in stripping buffer (Thermo Scientific, USA) and the membrane was reprobed using a β-actin antibody (1:5000). As the invariant control, the present study used rat β-actin. Primary antibodies were purchased from Santa Cruz Biotechnology, USA.

RESULTS

Effect of Lupeol on Glucose transporter (GLUT)-4 Protein Expression in T2D Rats

A significant decrease ($P < 0.05$) in glucose transporter (GLUT)-4 protein levels [Figure 1] in adipose tissue was observed in T2D animals, whereas lupeol treatment increased GLUT-4 protein level in the T2D animals.

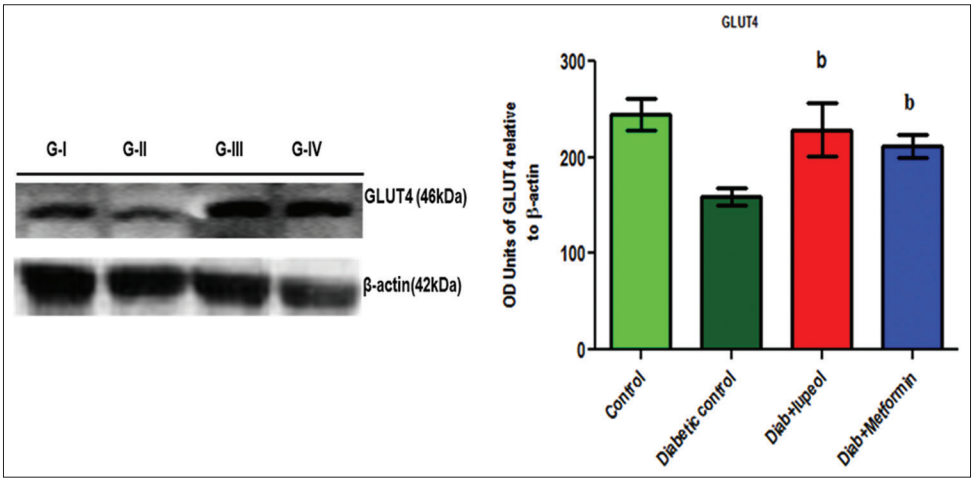

Figure 1: Effect of lupeol on glucose transporter-4 protein expression in adipose tissue of Type-2 diabetic rats. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$, a – compared with control; b – compared with diabetic control

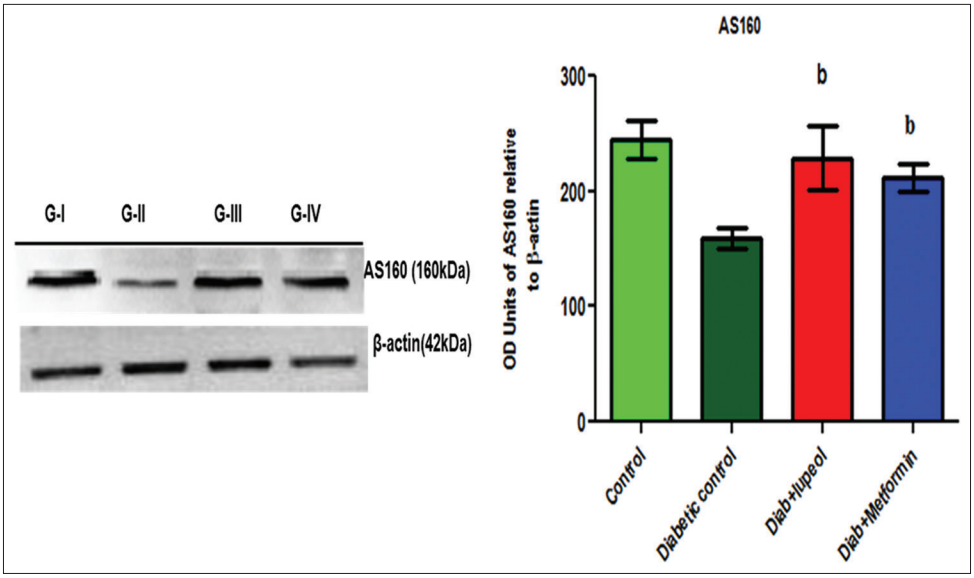

Figure 2: Effect of lupeol on AS160 protein expression in adipose tissue of Type-2 diabetic rats. Each bar represents mean ± SEM of six animals. Significance at $P < 0.05$, a – compared with control; b – compared with diabetic control

Effect of Lupeol on AS160 Protein Expression in T2D Rats

A significant decrease ($P < 0.05$) in AS160 protein levels [Figure 2] in adipose tissue was observed in T2D animals, whereas lupeol treatment increased AS160 protein level in the T2D animals as that of the standard drug metformin.[15]

DISCUSSION

Glucose transport, the rate-limiting step in carbohydrate metabolism, is facilitated by GLUT across the cell membranes.[7] GLUT-4, a major GLUT, stimulates glucose uptake into fat and muscle cells. In our study, a significant decrease in GLUT-4 protein expression was observed in high-fat and sucrose-fed T2D rats.[8] This may be due to oxidative stress-induced defect in GLUT-4 gene transcription or increased free fatty acid and lipotoxicity-mediated impairment in activation and phosphorylation of AS160 resulting from high-fat and sucrose diet.[9] Lupeol treatment restored the GLUT-4 expression in both cytosol and plasma membrane in adipose tissue of diabetic rats.[10] This may be due to the lupeol mediated increase in the signaling molecules, thereby increased the protein levels of GLUT-4 and AS160.

CONCLUSION

It is concluded from the present findings that lupeol has antihyperglycemic activity through the regulation of insulin signaling molecules (GLUT-4 and AS160). Hence, lupeol can be used as one of the therapeutic agents for the management of T2D. Further studies on the effect of lupeol on insulin downstream signaling molecules need to be carried out to ascertain its potential.

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