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Insulin sensitization via partial agonism of PPARγ and glucose uptake through translocation and activation of GLUT4 in PI3K/p-Akt signaling pathway by embelin in type 2 diabetic rats

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ABSTRACT

Background: The present study was aimed at isolating an antidiabetic molecule from a herbal source and assessing its mechanism of action.
Methods: Embelin, isolated from *Embelia ribes* Burm. (Myrsinaceae) fruit, was evaluated for its potential to regulate insulin resistance, alter β-cell dysfunction and modulate key markers involved in insulin sensitivity and glucose transport using high-fat diet (HFD) fed-streptozotocin (STZ) (40 mg/kg)-induced type 2 diabetic rats. Molecular-dockings were performed to investigate the binding modes of embelin into PPARγ, PI3K, p-Akt and GLUT4 active sites.
Results: Embelin (50 mg/kg b wt.) reduced body weight gain, blood glucose and plasma insulin in treated diabetic rats. It further modulated the altered lipid profiles and antioxidant enzymes with cytoprotective action on β-cell. Embelin significantly increased the PPARγ expression in epididymal adipose tissue compared to diabetic control group; it also inhibited adipogenic activity; it mildly activated PPARγ levels in the liver and skeletal muscle. It also regulated insulin mediated glucose uptake in epididymal adipose tissue through translocation and activation of GLUT4 in PI3K/p-Akt signaling cascade. Embelin bound to PPARγ; it disclosed stable binding affinities to the active sites of PI3K, p-Akt and GLUT4.
Conclusions: These findings show that embelin could improve adipose tissue insulin sensitivity without increasing weight gain, enhance glycemic control, protect β-cell from damage and maintain glucose homeostasis in adipose tissue.
General significance: Embelin can be used in the prevention and treatment of type 2 diabetes mellitus caused due to obesity.

1. Introduction

Type 2 diabetes mellitus (T2DM), also known as non insulin-dependent diabetes mellitus, is characterized by insulin resistance and impaired insulin secretion due to β-cell dysfunction [1]. Increase in epididymal adipose tissue mass leads to obesity, thereby mounting the risk of insulin resistance involving impaired insulin-stimulated glucose uptake in the peripheral tissues [2]. Moreover insulin resistance can affect insulin secretion, predominantly due to depressed β-cell function including destruction in the β-cell mass [3]. Synthetic drugs cause some side effects. Therefore, medicinal herbs are emerging as good alternatives.

Thiazolidinediones (TZDs), a class of oral antidiabetic synthetic drugs including rosiglitazone and pioglitazone, are specific agonists of peroxisome proliferator-activator receptor gamma (PPARγ), and function as insulin sensitizers to treat insulin resistance in T2DM [4]. TZDs are reported to have adverse side effects including body weight gain, peripheral edema, and increased risk of cardiac hypertrophy [5]. Drugs retaining partial PPARγ agonism with tissue-specific receptor modulation could be wisely designed for secure diabetic therapy [6]. Glucose transporter proteins, GLUT1 and GLUT4, the two integral isoforms present in adipose tissue, play the role of tissue glucose uptake and regulate body glucose homeostasis [7]. GLUT1 is involved in a low intensity of glucose uptake. GLUT4 plays an explicit role in regulation of glucose homeostasis through translocation and activation, subsequently triggered

Abbreviations: T2DM, Type 2 diabetes mellitus; HFD, high fat diet; STZ, streptozotocin; TZDs, Thiazolidinediones; FBG, fasting blood glucose; b wt., body weight; TC, total cholesterol; TG, triglycerides; FFA, free fatty acids; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; OGTT, oral glucose tolerance test; PBS, phosphate buffer saline; MW, Molecular weight

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by insulin dependent phosphatidylinositol 3-kinase (PI3K)/phosphorylated protein kinase B (p-Akt) pathway [8]. Hence therapeutic approaches that target these specific metabolic markers in the management of T2DM are advocated.

Embelia ribes Burm. (Myrsinaceae) is widely distributed in India and has been documented in Indian traditional medicine system in the treatment of diabetes [9]. *E. ribes* fruit is well known for its antidiabetic effect [10]. Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone), a major constituent of *E. ribes*, has been established for its antidiabetic effect in alloxan induced insulin-deficient animal model [11]. Pharmacological evidences demonstrated that embelin possessed antifertility, antitumor, anti-inflammatory, analgesic, antioxidant, hepatoprotective, wound healing, antibacterial and anticonvulsant properties [11]. However, the antidiabetic property of embelin in high-fat diet (HFD) fed-streptozotocin (STZ)-induced insulin resistant animal model has not been explored so far. This work was aimed to evaluate the insulin sensitization via agonism of PPARγ and glucose uptake through translocation and activation of GLUT4 in PI3K/p-Akt signaling pathway by embelin from *E. ribes*; we also aimed to assess the docking of embelin into PPARγ, PI3K, p-Akt and GLUT4 active sites.

2. Materials and methods

2.1. Chemicals and reagents

STZ and all fine chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). MMLV Reverse Transcriptase, dNTPs, Taq polymerase, primers for PCR were purchased from GIBCO BRL (USA). Antibodies PPARγ, PI3K, p-Akt, GLUT1, GLUT4, β-actin and anti insulin were obtained from Calbiochem (Germany). TRIzol reagent was purchased from Invitrogen (USA). Ultrasensitive rat insulin ELISA kit was purchased from Crystal Chem, Inc. (USA). Organic solvents were obtained from Merck (Germany). Rosiglitazone and all other laboratory chemicals were obtained from local firms (India) and were of the analytical grade.

2.2. Plant material

Fresh matured fruits of *Embelia ribes* Burm. (Myrsinaceae) were collected during December 2010 from medicinal farm at Koyambedu, Chennai, India and was authenticated by Dr. M. Ayyanar, plant taxonomist, Department of Botany, Pachaiyappa's College, Chennai, India. A voucher specimen (no. ER/ERI/589) was preserved at the herbarium of the institute for future reference.

2.3. Extraction and isolation of embelin

E. ribes fruits were air dried at 38 °C and coarsely powdered. The powdered material (5 kg) was extracted with 15 l (1:3 w/v) of chloroform by cold percolation for 48 h with intermittent shaking. The extract was filtered through a Buchner funnel with Whatman No. 1 filter paper and the filtrate was concentrated to dryness under reduced pressure using vacuum rotary flash evaporator at 35–40 °C. The crude extract (25.8 g) was chromatographed on a silica gel column (100–200 mesh) and eluted with benzene; it yielded yellow orange crystalline powder which was then washed with diethyl ether to give embelin. The purity of embelin was confirmed to be 98.08% by HPLC with mobile phase, acetonitrile:water (3:1) (UV detection at 290 nm). It gave positive ferric reaction for phenol and pink color with alcoholic NaOH for quinone. The structural identity of the molecule was characterized by comparison of UV, IR and ^{1}H NMR spectra with literature values [12]. The structure of embelin is shown in Fig. 1. The chemical characteristics of embelin were retrieved from pubchem data base, (http://pubchem.ncbi.nlm.nih.gov/search/search.cgi).

2.4. Experimental animals

Male Wistar rats weighing 180 ± 10 g procured from the animal house of Entomology Research Institute were conditioned at temperature 22 ± 2 °C, a relative humidity of 60 ± 5%, and 12/12 h day/night cycle (lights on 6.00 A.M.), for 7 days in polypropylene cages during which they were given free access to commercial standard pellet diet (69% carbohydrate, 21% protein, 5% fat, fibers, vitamin and minerals) and water. The animal facilities and all experimental procedures were carried out according to National Institutes of Health (NIH) guidelines after getting approval of the Institute's Animal Ethics Committee (IAEC-ERI-LC-43).

2.5. Dose fixation study

Embelin 50 mg/kg b wt. was fixed to be an optimal dose for this experiment (based on signs of behavioural patterns, physiological alterations, body weight changes and gross pathological observations in experimental normal rats; data not shown).

2.6. Development of T2DM

After acclimatization, experimental rats excluding the normal control and normal treated groups were given free access to standardized HFD consisting of 25% coconut oil, 2% cholesterol, and 73% commercial standard pellet diet [1]. In addition, a solution of cholesterol in coconut oil (100 mg/ml) was also given (5 ml/kg) by intragastric tube for 2 weeks as described previously [1] prior to single intraperitoneal injection with streptozotocin (STZ) (40 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5). Citrate buffer (vehicle) alone was injected to normal control and normal treated rats. Blood samples

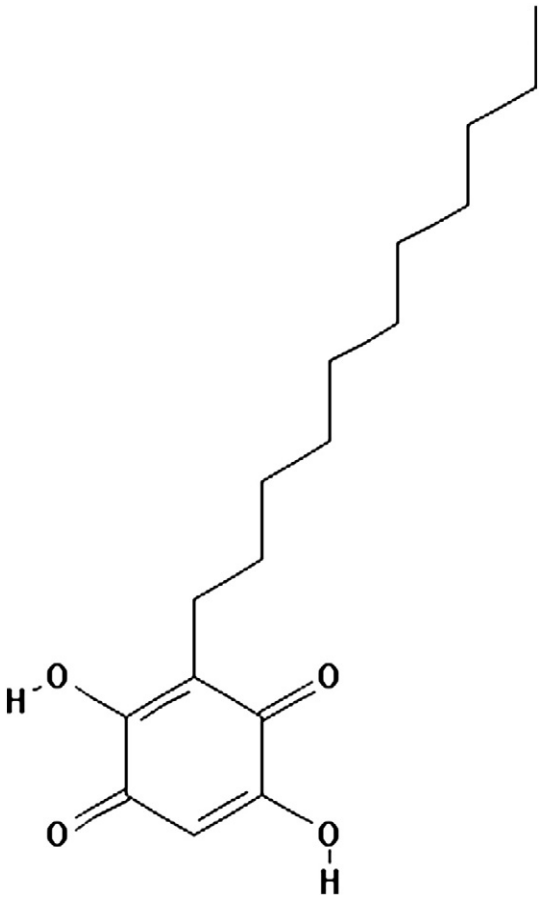

Fig. 1. Structure of embelin.