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Hypoglycemic activity of 6-bromoembelin and vilangin in high-fat diet fed-streptozotocin-induced type 2 diabetic rats and molecular docking studies

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ABSTRACT

Aims: This paper investigates the hypoglycemic activity of two derivatives of embelin (1) viz. 6-bromoembelin (2) and vilangin (3), in high-fat diet - STZ induced diabetic rats.
Main methods: The effects of 6-bromoembelin (2) and vilangin (3) on insulin resistance, β-cell dysfunction and glucose transport in high-fat diet (HFD) fed-streptozotocin (STZ) (40 mg/kg) induced type 2 diabetic rats were evaluated. The binding modes of 6-bromoembelin (2) and vilangin (3) into PPARγ, PI3K, Akt, and GLUT4 were also studied using Autodock 4.2 and ADT 1.5.6 programs.
Key findings: At the dose of 30 mg/kg, the plasma glucose, plasma insulin and body weight were reduced by both embelin derivatives in diabetic rats. Additionally the altered lipid profiles and hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase levels were brought to normal. Compared to diabetic control group, there was a significant increase in the expression of PPARγ in epididymal adipose tissue. Inhibition of adipogenic activity and mild activation of PPARγ levels in the skeletal muscle and liver were observed. In epididymal adipose tissue, the compounds increased the insulin-mediated glucose uptake through the activation and translocation of GLUT4 in PI3K/p-Akt signaling cascade.
Significance: The derivatives of embelin such as 6-bromoembelin (2) and vilangin (3) may be useful in the prevention and treatment of obesity-linked type 2 diabetes mellitus.

1. Introduction

Peroxisome proliferator-activated receptor gamma (PPARγ), a ligand-activated nuclear hormone receptor, is a significant drug target for regulating glucose metabolism [7,8,42]. Insulin sensitivity in the adipose, muscle, and hepatic tissues is upregulated by PPARγ [43,51]. Insulin sensitivity is modified by the production of adipokines induced by activation of PPARγ in adipose tissue. Therefore, PPARγ agonists are suitable agents to treat a group of metabolic abnormalities like blood pressure, insulin resistance, inflammation and abdominal obesity [36]. Hence, clinically the activators of PPARγ such as thiazolidinediones (TZDs) (e.g. rosiglitazone, pioglitazone) are used for the treatment of type 2 diabetes (T2DM) [32]. Several side effects such as weight gain, edema and increased plasma lipoproteins are found to be associated with PPARγ ligands. Partial or low-affinity PPARγ agonists are presently being investigated in the hope of reducing these side effects [19].

Embelin (1) (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) (Fig. 1) is the major benzoquinone from *Embelia ribes* Burm, fruits (Myrsinaceae) with several biological activities [18,25,33,41]. Utilization of glucose in peripheral tissues has been proved to be increased by embelin as an insulin sensitizer [18]. With a view to search for new hypoglycemic agents, we investigated two embelin derivatives viz. 6-bromoembelin (2) (Fig. 1) and vilangin (3) (Fig. 2).

In this study, we assessed the antidiabetic potential of the two derivatives by molecular docking with PPARγ key insulin signaling markers and evaluated their effect on PPARγ gene/protein expression via glucose transporter proteins like GLUT4 by insulin dependent phosphatidylinositol 3-kinase (PI3K)/phosphorylated protein kinase B (p-Akt) pathway to treat obesity linked T2DM. In this article, we are reporting for the first time the hypoglycemic activity of derivatives 2 and 3 with significant effect than embelin (1).

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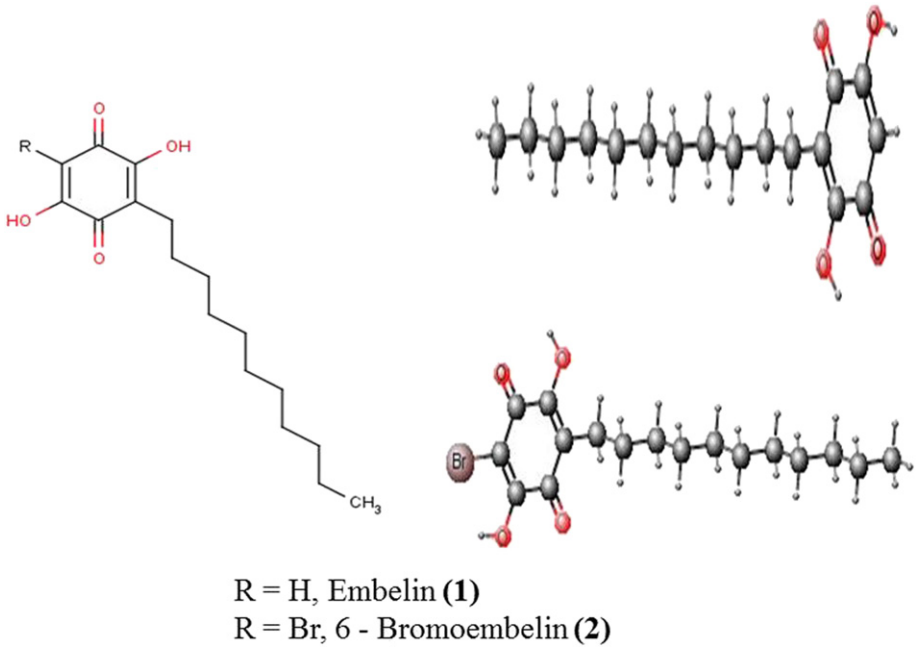

Fig. 1. Structures of embelin (**1**) and 6-bromoembelin (**2**) (2D & 3D).

2. Materials and methods

2.1. Chemistry and instruments

All solvents and materials used in this study were of analytical grade. For column chromatography, silica gel (100–200 mesh, SRL) was used. Silica gel 60F254 (Merck) pre-coated aluminium plates were used for TLC; after elution the plates were sprayed with 10% alcoholic sulfuric acid and heated at 110 °C for 5 min. UV spectra were taken on Shimazu UV–Vis spectrophotometer. FT-IR Perkin-Elmer grating spectrophotometer was used for taking IR spectra, in KBr disc. Bruker NMR instrument was used to record ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) with the solvent CDCL3 or DMSO-d_6.

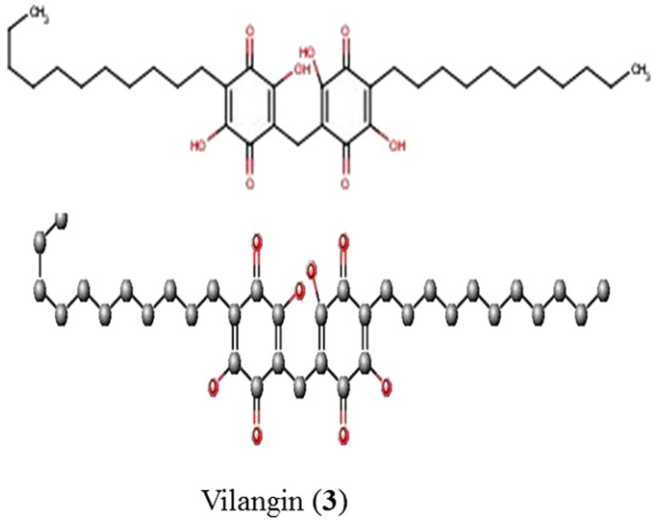

Fig. 2. Structure of vilangin (**3**) (2D & 3D).

2.2. Preparation of 6-bromoembelin (2) and vilangin (3)

Embelin (1) was obtained from the chloroform extract of *E. ribes* fruits as reported earlier [18]. 6-Bromoembelin (2) was prepared by the method of Venkata Rao et al. [49] and Vilangin was prepared by the method of Bheemasankara Rao and Venkateswarlu, [5] and Narayanaswamy et al. [38]. Briefly, embelin (1) was concentrated in acetic acid solution with formaldehyde. The identities of both derivatives were confirmed by UV, IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectroscopic data (see Supplementary data).

2.3. Biological assays

2.3.1. Chemicals and reagents

Streptozotocin (STZ) and other fine chemicals were procured from Sigma-Aldrich (St. Louis, MO, USA). dNTPs, Taq polymerase, MMLV Reverse Transcriptase and primers for PCR were obtained from GIBCO BRL (Rockville, MD). PPARγ, PI3K, p-Akt, GLUT4, β-actin and anti-insulin antibodies were bought from Calbiochem (Darmstadt, Germany). ELISA kit and RNAiso Plus were obtained from Crystal Chem, Inc. (Downers Grove, IL) and Takara (Kusatsu, Japan), respectively. Organic solvents were purchased from Merck (Darmstadt, Germany). Pioglitazone and all other chemicals of reagent grade were received from local suppliers in India.

2.3.2. Experimental animals and development of T2DM

Albino Wistar strain rats (five-week-old adult male rats) weighing 180 ± 10 g were reared in the animal house (Entomology Research Institute, Loyola College, Chennai) were used in this study. All the animals were air conditioned at temperature 22 ± 2 °C, humidity of 60 ± 5% and 12/12 h day/night cycle and fed with commercial standard pellet diet (carbohydrate 69%, protein 21%, fat 5%, fibers, vitamin, and minerals) and water [18]. All the experiments were carried out as per the guidelines of the Institutional Animal Ethics Committee (IAEC) following the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA), India (Approval No: IAEC-ERI-LC-01/13).