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Type 2 antidiabetic activity of bergenin from the roots of *Caesalpinia digyna* Rottler

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ABSTRACT

Bergenin, a major constituent of *Caesalpinia digyna* Rottler (Leguminosae) was isolated from its roots and was characterized by comparing its melting point and spectroscopic data (IR, ^{1}H, ^{13}C, Mass Spectra) with standard bergenin. Isolated bergenin was then evaluated for antidiabetic (Type 2) activity in streptozotocin (STZ)-nicotinamide induced diabetic rats. Bergenin was administered at doses of 2.5, 5, and 10 mg/kg; p.o. to normal rats which were subjected to oral glucose tolerance test (OGTT). Bergenin at same dose level was given to diabetic rats and fasting blood glucose level was estimated on 0th, 7th and 14th day of treatment while plasma lipids, antioxidant enzymes and liver glycogen level in diabetic rats were estimated on 14th day of treatment followed by histopathological studies of pancreas. Bergenin at 10 mg/kg; p.o. was found to reduce blood glucose level significantly in OGTT ($P<0.01$) while it showed a significant reduction in fasting blood glucose level in diabetic rats at same dose level only on 14th day of treatment. Bergenin in all dose levels reversed plasma lipid (reduced elevated TC, LDL-C and increased HDL-C level) profile to normal values except TG. However, bergenin showed no significant effect on liver glycogen at all dose level. The decrease in lipid peroxides and increase in superoxide dismutase (SOD) and catalase (CAT) in liver illustrated the antioxidant potential of bergenin. Histopathological studies demonstrated the regenerative effect of bergenin on pancreatic β cells. Hence, bergenin isolated from *C. digyna* possesses significant antidiabetic, hypolipidemic and antioxidant activity in Type 2 diabetic rats.

1. Introduction

Caesalpinia digyna Rottler (Leguminosae) is a large, scandent, prickly shrub or climber, up to 10 m in height, growing wild in the scrub forests of Eastern Himalayas like Assam and West Bengal while in Eastern Ghats it is found in Andhra Pradesh, Madhya Pradesh and is also grown in Ceylon and Malay Islands. The Himalaya Drug Co. (India) uses this plant as one of the ingredients of an indigenous drug preparation known as "Geriforte", which has been used for curing senile prurites. The drug also exhibits antifatigue effect in rats [1]. The root has marked astringent properties. It is given internally in pthisis and scrofulous affections; when sores exist, it is applied externally as well and also used in diabetes. In some parts of the Burma the pounded root is mixed with water and is drunk as a febrifuge which is said to have intoxicating effect [2]. The ethanol water extract of roots inhibits the growth of *Mycobacterium tuberculosis*. Chemical investigations of the plant have shown the presence of caesalpinine A, cellallocinnine, ellagic acid, gallic acid, bergenin, bonducellin, intricatinol and tannins [3–8]. Root extract and bergenin isolated from *C. digyna,* have shown significant anti-oxidant activity [9]. Bergenin has also been shown to posses antiulcerogenic, hepatoprotective, antiviral, antidiabetic/antiobesity (by in-vitro inhibition of protein tyrosine phosphatase 1B (PTP1B)),

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anti-arrhythmic, antioxidant, anti-arthritic, burn wound healing and trypanocidal activities [10]. In our recent investigation we have reported the antidiabetic activity of ethanolic root extract of *C. digyna* in STZ-nicotinamide induced diabetic rats [11]. In support to the above study present investigation was conducted to assess Type 2 antidiabetic (*in-vivo*) activity of bergenin, a major constituent of *C. digyna*.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) was obtained from Sigma-Aldrich Co., St. Louis, USA. Solvents were purchased from SD Fine Chemicals Ltd., Mumbai, India. All the chemicals used were of analytical grade. For estimation of blood glucose and other biochemical tests, kits were obtained from Span Diagnostic Ltd, India.

2.2. Plant material

The dried roots of *C. digyna* were purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India, and was identified by Prof. V. Chelladurai, Botanist (Retd.), Palayamkottai, Tamilnadu, India. The voucher specimen has been deposited in the herbarium of Department of Pharmaceutics, Banaras Hindu University for future reference (Specimen number-COG/CD-08).

2.3. Isolation of bergenin

The coarsely powdered root (mesh size 20) of *C. digyna* was extracted (1 kg) with 2500 ml of ethanol (95%) in a Soxhlet extractor for three days. The extract was concentrated to one fourth of its volume which was further kept at room temperature for 24 h. A crystalline solid was found to settle down by the bottom of the container, which was separated, washed with acetone and crystallized from methanol. Repeated recrystallation of combined crystalline solids with methanol yielded a colorless crystalline compound.

2.4. Characterization of bergenin

The percentage yield of isolated compound was 0.065% w/w. The homogeneity and purity of the isolated compound was confirmed by TLC using an ethyl acetate: methanol (7:3 v/v) as the mobile phase with respect to standard bergenin (Sigma-Aldrich Co., St. Louis, USA). R_f value was found to be 0.16 and a spot was detected under 10% sulphuric acid in methanol. The isolated colorless crystalline compound was confirmed as bergenin (Fig. 1). Its melting point was found to be 237 °C. Further, it was characterized by IR, NMR and mass spectroscopy. Melting point was recorded by using Sonar melting point apparatus, ISO 9001-2000 in open capillary tubes. The IR spectra study was performed in SHIMADZU FT/IR 8400 infrared spectrophotometer using KBr disk method for sample preparation. ^{1}H NMR and ^{13}C NMR spectroscopical analysis was performed on DDR X — 500 m/z Bruker Deltonics, Germany NMR spectrophotometer. The ESI-MS was recorded on a MICROMASS QUATTRO II triple quadrupole MASS spectrometer having a JASCO PU-980HPLC pump connected to it and acetonitrile was used as a solvent at 80°C source temperature. IR bands (KBr, υ cm^{-1}): 3425 (Ar–OH), 2891, 2727 (C–H, CH_2, CH_3), 1707(C═O), 1612, 1525, 1460, (C═C, Ar), 1413, 1375(C–H_{bend}, CH_2, CH_3); TOF-MS ES+: m/z 329 (M+1) with base peak at m/z 209 which confirms the tricyclic nature of bergenin. The ^{1}H NMR showed the presence of total 16 protons. The ^{1}H NMR exhibits a singlet signal for three protons at δ 3.80 for the methoxy group and one proton singlet at δ 7.00 in the aromatic region suggesting that all the five other carbon atoms of the aromatic rings are substituted. Two phenolic groups are seen as broad singlets at δ 8.30 and 9.36. The signals assigned to the hydrogen atoms attached to glucopyranosyl moiety are at δ 4.77, 4.81, 3.59, 3.72 and 3.88 for each proton and δ 3.31 and 3.22 for protons attached to CH_2OH of the glucopyranosyl moiety. The three hydroxyl groups of glucopyranosyl appeared at δ 3.99 (^{1}H, t, J=1.01), δ 5.25 (^{1}H, d, J=1.01) and at δ 5.59 (^{1}H, d, J=1.08). In ^{13}C NMR shows the appearance of 14 carbons peaks. The nature of each carbon was confirmed by Distortedness Enhancement by Polarization Transfer (DEPT-90 and DEPT-135) technique. In DEPT-90 only CH carbons appeared in the positive mode. DEPT-90 showed the presence of 6 CH carbons. ^{13}C NMR (400 MHz, DMSO-d_6); δ ppm: 117.74, 115.26, 147.84, 140.43, 150.78 (ArC), 109.64 (ArCH), 163.12 (C═O), 72.57, 73.77, 79.60, 70.47, 61.13 (Alkyl CH), 81.69 (CH_2OH), 59.72 (OCH_3). The structure of the isolated compound was further confirmed by comparing its melting point, IR, NMR and mass spectrum of bergenin reported in literature [12].

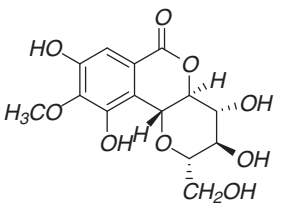

Fig. 1. Structure of bergenin.

2.5. Pharmacological evaluation

2.5.1. Animals

Male albino rats of Charles foster strain (135–175 g) were obtained from the Central Animal House, (Reg. No. 542/02/ab/CPCSEA), Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. They were maintained under standard environmental conditions (22–28 °C, 60–70% relative humidity, 12 h dark:light cycle). The animals were fed with standard rat feed (Mona Laboratory Animal Feeds, Raman Dairy Vikas Udyog, India) and water ad libitum. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. All the experimental procedures conducted after the approval of ethical committee (No. Dean/2009-10/579) and were in strict accordance with institutional animal ethical committee guidelines for the care and use of laboratory animals.

2.5.2. Oral glucose tolerance test

Overnight fasted animals were divided into five groups (n=6) and were administered with glucose (2 g/kg) orally by means of gastric intubation. Animals in second, third and