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Antidiabetic activity of alcoholic root extract of *Caesalpinia digyna* in streptozotocin-nicotinamide induced diabetic rats

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ABSTRACT

Objective: The present investigation deals with evaluation of antidiabetic (Type 2) activity of standardized alcoholic root extracts of Caesalpinia digyna in STZ-nicotinamide induced diabetic rats. **Methods:** Alcoholic root extract of Caesalpinia digyna (ACD), obtained from Soxhlet extractor was standardized by HPLC. Type 2 diabetes was induced by single intraperitoneal injection of nicotinamide (110 mg/kg) followed by streptozotocin (65 mg/kg). Diabetic rats ware administered ACD at doses of 250, 500, and 750 mg/kg (p.o.) and different parameters such as normoglycemic and oral glucose tolerance test were evaluated. The study also included estimations of blood plasma glucose, lipid profile, liver glycogen, body weight and anti-oxidant status in normal and diabetic rats. **Results:** Normoglycemic rats did not reduce the blood glucose level, whereas oral glucose tolerance test showed better tolerance of glucose in treated rats. The alcoholic extract showed a dose dependent reduction in fasting blood glucose level i.e. more pronounced at 750 mg/kg ($P<0.05$). ACD showed significant reduction in plasma lipid like triglycerides, total cholesterol and improvement in high density lipo-protein cholesterol (HDL-C) in treated groups. The decrease in lipid peroxides and increase in superoxide dismutase (SOD) and catalase (CAT) in liver clearly showed the antioxidant potential while rat hemi-diaphragm glucose uptake study revealed increases in peripheral glucose uptake of treated rats. **Conclusions:** Results showed that standardized alcoholic extract of C. digyna possessed significant antidiabetic activity which may be attributed to increase in glycogen storage, hypolipidemic and antioxidant activity thus, rationalizing its traditional use.

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 the eastern Himalayas, Assam, West Bengal, Madhya Pradesh, and the Eastern Ghats of Andhra Pradesh. The root has marked astringent properties. It is given internally in phthisis and scrofulous affections; when sores exist, it is applied externally as well. It is also used in diabetes[1]. In some parts of the Burma the root, pounded and mixed with water, is drunk as a febrifuge and also said to have an intoxicating effect[1]. The drug also exhibits antifatigue effect in rats. 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. The root extract and bergenin isolated from it have shown significant antioxidant activity[2]. Bergenin isolated from the plant has been shown to posses antiulcerogenic, hepatoprotective, antiviral, antidiabetic/antiobesity (by in vitro inhibition of PTP1B (protein tyrosine phosphatase 1B), anti-arrhythmic, antioxidant, antiarthritic, burn wound healing and trypanocidal activities[3].

It is currently estimated that about 150 million people worldwide suffer from diabetes. This number is expected to be increase to 300 million by the year 2025. However, among the two major types of diabetes i.e. Type 1 and Type 2. Type 2 diabetes mellitus is the commonest form of diabetes constituting 90-95% of the diabetic population. It was also documented that the number of people diagnosed with Type 2 diabetes mellitus globally is estimated to be at 2-3% of the

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world population and is rising at a rate of 4-5% per year[4,5]. Hence, the present study was conducted to assess Type 2 antidiabetic activity of standardized alcoholic roots extract of C. digyna.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) and bergenin were 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, whereas other biochemical 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 identified by Prof. V. Chelladurai, Research Botanist, Palayamkottai, Tamilnadu, India. The voucher specimen was deposited in the herbarium of Department of Pharmaceutics, Banaras Hindu University for future reference (Specimen number-COG/CD-08).

2.3. Preparation of extract

The roots were chopped to small pieces and shade-dried. The dried roots were powdered and passed through sieve no. 20 and extracted (2.5 kg) in a Soxhlet extractor for three days using alcohol. The extract was than concentrated under reduced pressure to dryness. The alcoholic extract yielded a dark brown solid residue weighing 375.09 g (15% w/w). Extract was preserved in a desiccator till further use. Further preliminary phytochemical screening was conducted for the presence of various phytoconstituents[6]. Based on the results obtained from phytochemical screening, total phenolic (TP) content of extract was determined by Folin-Ciocalteu method[7].

2.4. Standardization of extract

Crude alcoholic extract of C. digyna was standardized by established method using bergenin as a standard[8]. HPLC analysis was performed with a Waters HPLC system, USA with PDA detector. A Cosmosil C18 column (150 mm x 4.6 mm, 5 μm particle) was used for the analysis. The mobile phase used was a mixture of acetonitrile:water 30:70 (v\v) delivered at a flow rate of 0.8 ml min-1, and run time of 5 min. The data was collected at wavelength of 275 nm. Peak of bergenin was identified by comparison with retention time of standard bergenin (3.20 min).

2.4.1. Sample Preparation

Crude alcoholic extract of C. digyna (30 mg) was taken in a volumetric flask and dissolved using HPLC grade methanol and volume was finally adjusted to 10 ml accurately. The mixture was sonicated for 30 min at room temperature and filtered through a 0.45 μm nylon filter to obtain a clear solution. Solution was injected (25 μl) into the HPLC system directly for the analysis.

2.4.2. Preparation of Standard Solutions for Calibration Curves

For quantification, an external standard method was utilized. Peak areas from the HPLC chromatogram were plotted against the known concentrations of stock solutions at varying concentrations. Equations generated by linear regression were used to establish concentrations of bergenin in alcoholic extract of C. digyna. About 10 mg of a standard bergenin weighed accurately was dissolved into a 10 ml volumetric flask in methanol to obtain stock solutions. For calibration curves, the stock solution was diluted with methanol to obtain the concentration in the range of (0.25-2.5 μg/ml).

2.5. Animals

Healthy male albino rats of Charles foster strain (150-220 g) were obtained from the Central Animal House, (Reg. No. 542/02/ab/CPCSEA) Banaras Hindu University, Varanasi, India, and were maintained under standard environment conditions (22-28 ℃, 60-70% relative humidity, 12h dark:light cycle) and were fed with standard rat feed (Mona Laboratory Animal Feeds) 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.

2.6. Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 425. Alcoholic extract of C. digyna (5 g/kg) was suspended in 0.5% w/v carboxy methyl cellulose (CMC) and given p.o. to overnight fasted rats and animals were observed individually for 48 h and their behavioral and neurological changes such as tremors, convulsions, salivation, diarrhea, sleep and lacrimation in drug treated rats were observed for sign of acute toxicity[9].

2.7. Experimental

2.7.1. Normoglycemic study of the ACD in overnight fasted rats

Rats were divided into five groups of six rats in each group. Group I animals were orally administered equal volume carboxy methyl cellulose (CMC) solution, which served as control. Group II rats were given the oral hypoglycemic, glibenclamide at a dose level 10 mg/kg; body weight; p.o. The rats in group III-V were given CMC suspension of ACD orally at dose levels of 250, 500, and 750 mg/kg body weight, respectively. Blood samples were collected just prior to and 1, 2, 4 and 6 h after administration of the extract from the retro-orbital plexus of eye. Plasma was separated and