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Antidiabetic activity of *Caesalpinia bonducella* F. in chronic type 2 diabetic model in Long-Evans rats and evaluation of insulin secretagogue property of its fractions on isolated islets

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Abstract

Caesalpinia bonducella F., is a shrub widely distributed throughout the coastal region of India and is ethnically used by the tribal people of Andaman and Nicober Island as a remedy of symptoms of diabetes mellitus. This ethnic report prompted the detail investigation of hypoglycemic activity of *Caesalpinia bonducella* seeds, initially on physiological hyperglycemic model and then on type 1 and type 2 subacute diabetic animal models which has already been reported. Evaluation of different extracts from *Caesalpinia bonducella* in chronic type 2 diabetic model alongwith insulin secretagogue activity of five fractions isolated from the *Caesalpinia bonducella* seed kernel are presented in this paper. Both the aqueous and ethanolic extracts showed potent hypoglycemic activity in chronic type 2 diabetic model. Two fractions BM 169 and BM 170 B could increase secretion of insulin from isolated islets.

Keywords: *Caesalpinia bonducella*; Aqueous and ethanolic extracts; Fractions; Type 2 diabetes; Pharmacological model; Insulin secretagogue property

1. Introduction

The use of ethnobotanicals has a long folkloric history for the treatment of blood sugar abnormalities. The World Health Organisation has estimated that 80% of the world's population use botanical medicine for their primary health care needs. In this context, we have almost serendipetically come across a plant named *Caesalpinia bonducella* F., (Leguminosae) commonly known as Nata Karanja. *Caesalpinia bonducella* is a prickly shrub found throughout the hotter parts of India, Myanmar, Sri Lanka, Bangladesh, reported to have antipyretic, antidiuretic, anthelmintic and antibacterial (Neogi and Nayak, 1958), antianaphylactic and antidiarrhoeal (Iyengar and Pendse, 1965), antiviral (Dhar et al., 1968), antiasthmatic (Gayaraja et al., 1978), antiemetic and antiestrogenic (Raghunathan and Mitra, 1982) properties.

Traditionally, the tribes of Andaman and Nicober Island used the aqueous decoction of the seeds of this plant, simply by rubbing on a stone, to eliminate the symptoms of diabetes mellitus. This ethnic lead necessitated the exploration of *Caesalpinia bonducella* seeds for their antidiabetic activity. Blood sugar lowering activity of *Caesalpinia bonducella* has been primarily evaluated with significant results in rabbit (Rao et al., 1994). Hypoglycemic activity of different extracts of *Caesalpinia bonducella* seed shell has been reported previously from our laboratory in physiological hyperglycemic and type 1 and type 2 diabetic model rats (Biswas et al., 1997;

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Chakrabarti et al., 2003). The present communication elaborates the study of different extracts of *Caesalpinia bonducella* seeds in chronic type 2 diabetic model and study of fractions from seeds of *Caesalpinia bonducella* on isolated islet cells of rat pancreas.

2. Materials and methods

2.1. Plant material

2.1.1. Collection, identification and extraction of plant materials

Seeds of *Caesalpinia bonducella* were procured from local market and identified by Dr. S. R. Das, Plant Survey Officer, CCRAS, Calcutta and the voucher specimen (UCM/BM/005) was preserved in our laboratory. The outer shells of the seeds were removed, air-dried and finely powdered. The powder (100 g) of the seed shell was subjected to aqueous (800 ml) and ethanolic (80%) (800 ml) extraction, separately, by soaking for 24 h followed by filtration and centrifugation at 4500 rpm for 20 min at 10 °C (650 ml). Finally, the extracts were concentrated under reduced pressure (100 ml) and lyophilized to get dry powder (approximately 6 g from aqueous and 10 g from ethanolic extract). Extracts obtained were preserved in sterile glass container at 4 °C. Different fractions from the seed kernel (1.5 kg) of *Caesalpinia bonducella* were obtained by successive extraction with petroleum-ether (40–60 °C) for 15 days, alcohol (80%) for 15 days and distilled water for 24 h.

Petroleum-ether extract was concentrated, triturated with solvent ether (5 × 100 ml). The ether extract was basified with 5% aqueous sodium hydroxide (NaOH) solution, washed with water (6 × 25 ml), dried over anhydrous sodium sulfate (Na_2SO_4), concentrated and a yellowish tri-terpenoid separated, designated as BM 169 (30 mg).

On similar processing the alcoholic extract yielded fractions BM 170A (30 mg) and BM 170 B (26 mg) which was steroid in nature and water extract produced two triterpenoids BM 171 A (600 mg) and BM 171 B (15 mg).

2.2. Biological assays

2.2.1. Experimental animal

Long-Evans rats, inbred in the Research Division, BIRDEM, Dhaka, Bangladesh were used for the present research programme. These were housed under ambient room temperature, fed with pellet diet and water ad libitum and 12 h light–dark cycle was maintained.

2.2.2. Induction of type 2 diabetes

Long-Evans rats of either sex of 48 ± 2 h old were injected with streptozotocin in citrate buffer (pH 4.5) at a dose of 90 mg/kg body weight/i.p. After 12–14 weeks, rats weighing between 150 g were selected for screening in type 2 diabetic model, by oral glucose tolerance test (OGTT). For this purpose rats were kept fasted overnight (12 h) and in the next morning blood was collected from the tail vein at 0 h. Immediately after collection of blood, glucose was fed at the dose of 2.5 g/kg body weight and blood was taken at 30, 60 and 120 min. The rats having blood glucose level of 7–12 mmol/L at 0 h and showing a highest rise to 13–20 mmol/L at 60 min, which returned to their 0 h value at 120 min were included in the study (Bonner-Weir et al., 1981).

2.2.3. Treatment groups

In the chronic model, animals were divided into five groups such as vehicle control, treated with standard drug metformin (Sigma, USA), Glibenclamide [Fison (Bangladesh) Ltd., Dhaka] and aqueous and ethanolic (80%) extracts of *Caesalpinia bonducella*. Glibenclamide was dissolved in double distilled water at 0.5% tween 20 and was administered at the dose of 1.25 mg/kg body weight. *Caesalpinia bonducella* extracts and metformin were given at a dose of 250 mg/kg body weight. The dose of the test drug has been selected on the basis of dose calibration curve (Chakrabarti et al., 2003). Each group consisted of 6–9 animals. Drugs were fed to the different groups of animals twice a day with 8 h interval for 56 doses of 28 days schedule.

2.3. Criteria of observation

2.3.1. Changes of body weight

Changes in body weight of each animal in different groups were noted on day 0, 14 and 28.

2.3.2. Collection of blood samples and estimation of biochemical parameters

For the purpose of biochemical estimation, blood samples were collected by snipping the tail vein on day 1, 14 and 28 after 3 h of the feeding of morning dose of the drugs. Blood samples were allowed to clot for 30 min and serum was separated by centrifugation.

Serum glucose levels was estimated by glucose–oxidase–peroxidase method in microwell plate (Kunst et al., 1984) on ELISA reader at 515 nm and calculated with respect to standard calibration curve.

Serum cholesterol and triglycerides were estimated on initial and final days of experiment of each model by one-step enzymatic method in ELISA reader at 500 nm (Wybenga et al., 1970; McGowan et al., 1981).

Serum insulin was measured by rat insulin ELISA kit on day 1 and day 28 in ELISA reader at 492 nm subtracting that at 630 nm (Kratzsch et al., 1990).

Serum fructosamine was estimated by fixed time colorimetric NTB method on initial and final days (Johnson et al., 1983).

Estimation of serum creatinine was done by alkaline picrate method on day 1 and day 28 (Toro and Ackermann, 1975).

On day 29 i.e., after completion of 28 days treatment, rats were sacrificed. Liver (200 mg) was taken from each animal