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Antidiabetic activity of cycloart-23-ene-3β, 25-diol (B2) isolated from *Pongamia pinnata* (L. Pierre) in streptozotocin–nicotinamide induced diabetic mice

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ABSTRACT

The aim of the present investigation was to evaluate the antidiabetic activity of cycloart-23-ene-3β, 25-diol (called as B2) isolated from stem bark of *Pongamia pinnata* in streptozotocin–nicotinamide induced diabetic mice. Diabetes was induced in mice by injecting streptozotocin (200 mg/kg, i.p.) after 15 min nicotinamide (110 mg/kg, i.p.). The mice were divided into following groups; I — nondiabeteic, II — diabetic control, III — glybenclamide (10 mg/kg, p.o.), IV — B2 (1 mg/kg, p.o.) and V — B2 (3 mg/kg, p.o., only for acute study). Serum glucose was determined periodically. Body weight, food and water intake were recorded daily. Oral glucose tolerance test was performed on day 28. Biochemical and enzyme antioxidant parameters were determined. Histology of pancreas was performed. B2 and glybenclamide treatment reduced serum glucose in acute study. However in chronic study, increase in body weight and decrease in food and water intake was observed. Increased glucose utilization was observed in oral glucose tolerance test. Both glybenclamide and B2 increased serum and pancreatic insulin. Glycosylated haemoglobin, serum cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, globulin, bilirubin, lactate dehydrogenase, urea and uric acid were decreased significantly after B2 treatment. B2 treatment decreased liver malondialdehyde but increased superoxidase dismutase and reduced glutathione. Histologically, focal necrosis was observed in the diabetic mouse pancreata but was less obvious in treated groups. The mechanism of B2 appears to be due to increased pancreatic insulin secretion and antioxidant activity.

1. Introduction

A number of plant species are known worldwide to have hypoglycaemic (Kumar et al., 2006) hypolipidemic (Kumari et al., 2006) or both activities (Sharma et al., 2006). Despite the presence of known antidiabetic medicines in the pharmaceutical market, screening for new sources from natural plants is still attractive because they contain substances that are effective and safe in diabetes mellitus. *Pongamia pinnata* (L.) Pierre (Fabacae) is popularly known as 'Karanj' or 'Karanja' in Hindi and Indian beech in English (Krishnamurthi, 1998). Recently, we have reported the antihyperglycaemic activity of petroleum (Badole and Bodhankar, 2009a) and alcoholic extract (Badole and Bodhankar, 2008) of stem bark of *P. pinnata* (L.) in alloxan induced diabetic mice. We have also reported concomitant administration of petroleum ether extract of stem bark of *P. pinnata* (L.) Pierre with synthetic oral hypoglycaemic drugs in alloxan induced diabetic mice (Badole and Bodhankar, 2009b). Then we have reported the antihyperglycaemic activity of cycloart-23-ene-3β, 25-diol (B2) in alloxan induced diabetic mice (Badole and Bodhankar, 2009c).

The aim of the present investigation was to evaluate the antidiabetic activity of cycloart-23-ene-3β, 25-diol (called as B2) isolated from stem bark of *P. pinnata* in streptozotocin–nicotinamide induced diabetic mice.

2. Materials and methods

2.1. Cycloart-23-ene-3β, 25-diol (B2) identification and characterization

Isolation and characterization of cycloart-23-ene-3β, 25-diol (B2) has been previously reported (Badole and Bodhankar, 2009b). Chemical structure of B2 is shown in the Fig. 1.

2.2. Drugs and chemicals

Streptozotocin (Sigma chemical Co. USA), nicotinamide (Sigma chemical Co. USA), GOD/POD kit (Acuurex, India), glybenclamide (Ranbaxy, India), tween-80 (Research-Lab, India) were purchased from respective vendors. All chemicals used were of analytical grade.

2.3. Animals and research protocol approval

Swiss albino male mice (25–30 g) were purchased from National Toxicology Centre, Pune, India. Animals were housed in an air-

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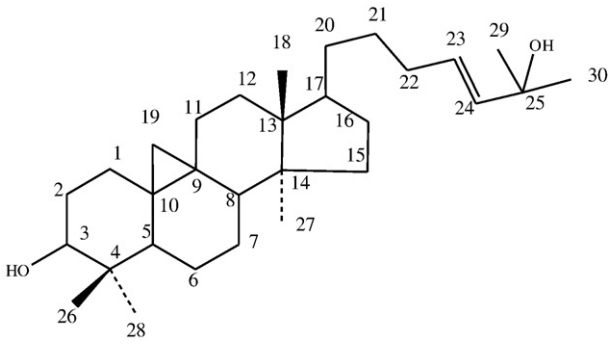

Fig. 1. Structure of cycloart-23-ene-3β, 25-diol (B2).

conditioned room at a temperature of 22 ± 2 °C and relative humidity of 45 to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India) except when starvation was required. Water was provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

2.4. Drugs solution

B2 was emulsified with 2% tween-80 and glybenclamide was dissolved in distilled water. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline.

2.5. Induction of diabetes and determination of serum glucose

Overnight fasted mice were treated with nicotinamide (110 mg/kg, i.p.). Streptozotocin (200 mg/kg, i.p.) were injected 15 min after nicotinamide injection in all the groups except group I which was non-diabetic. Animals were fed with glucose solution (5%) for 12 h to avoid hypoglycaemia. Hyperglycaemia was confirmed after 3 days. Steady state of hyperglycaemia was reached after 10 days. Serum glucose was determined by the glucose oxidase peroxidase method (Tindar, 1969). Mice having serum glucose between 300–400 mg/dl were selected for the study.

2.6. Acute and chronic study of B2 on serum glucose and oral glucose tolerance test

The mice were divided into following groups ($n = 6$) *viz*; Group I — non-diabetic, II — diabetic control, III — glybenclamide (10 mg/kg, p.o.), IV — B2 (1 mg/kg, p.o.) and V — B2 (3 mg/kg, p.o., only for acute study). Because of limited quantity of compound only one dose of B2 (1 mg/kg, p.o.) was selected for chronic study.

Acute study involved determination of serum glucose at 0, 2, 4, 6 and 24 h after glybenclamide and B2 administration.

Chronic study involved repeated administration of glybenclamide and B2 for 28 days (once a day) at predetermined time and serum glucose was determined in samples withdrawn after 6 h of glybenclamide and B2 administration on day 7, 14, 21 and 28. Oral glucose tolerance test was carried out at the end of the 28th day. D-glucose (2.5 g/kg, p.o.) was administered in the above mentioned groups at the 4th h of pre-treatment with either glybenclamide or B2. Serum glucose was determined before and 2 h after glucose administration (Badole and Bodhankar, 2009a).

2.7. Effect on body weight, food and water intake

During the study period of 28 days, the mice were weighed daily using electronic balance. Food intake was determined by measuring the difference between the pre-weighed food and the weight of the remaining food in hopper and spilled food after every 24 h. Water intake was measured by recording the quantity of water remaining in the feeding bottle. Body weight, food and water intake of mice were recorded daily but data is presented only of day 0 (after 10 days injection of streptozotocin–nicotinamide) and subsequent weeks.

2.8. Haematological and biochemical parameters

On day 29, blood samples were collected by retro orbital puncture technique by using capillary tubes containing disodium ethylene diamine tetra acetate (anti-coagulant). The following haematological parameters were evaluated in the collected blood samples: total haemoglobin, red blood corpuscles count, white blood corpuscles count, neutrophils, lymphocytes, eosinophils, monocytes, basophils and platelet count using fully automated hematology analyzer (Sysmex KX-21, Japan). Glycosylated haemoglobin was determined by glycosylated haemoglobin kits by Nycocard reader (Axis shield, Norway). Biochemical parameters viz; aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, globuline, total protein, bilirubin (liver function test); urea, uric acid, creatinine (renal function test); cholesterol, triglycerides, high density lipoprotein, low density lipoprotein (lipid profile) and lactate dehydrogenase were determined by kits specific for the test (Microlab 300, Merck, Netherland).

2.9. Serum and pancreatic insulin assay

Serum insulin was assayed by Accubind, ELISA Micorwell Insulin kit by ELISA reader (Sunrise-basic TECAN, Austria) on day 0 (before treatment) and day 29.

The mice were sacrificed on day 29 by cervical dislocation and the pancreas of each mouse was isolated and weighed. Small piece of pancreas was cut by scissor and stored in 10% neutral formalin solution for histological analysis. Remaining portion of pancreas was homogenized in an ice cold concentrated hydrochloric acid: ethanol (1:4, v/v) and centrifuged at 4 °C at 5204 g. The supernatant obtained after centrifugation was pooled and stored in amber colour vials at −20 °C until assayed.

2.10. Histopathology of mouse pancreas

The isolated pancreas were trimmed into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3–5 μm in thickness and stained by hematoxyline-eosin stain. The specimen was mounted by disterene phthalate xylene (D.P.X). The photomicrographs of each tissue section were observed using Cell imaging software for Life Science microscopy (Olympus soft imaging solution GmbH, Munster, Germany). Pancreatic tissue was processed for Gomori staining for morphology of pancreatic β cells (Gomori, 1950).

2.11. Effect on enzymatic biomarkers of oxidative stress

Liver was isolated from all mice and were cut into small pieces, placed in chilled 0.25 M sucrose solution and blotted on a filter paper. The tissues were then homogenized in 10% chilled tris hydrochloride buffer (10 mM, pH 7.4) by tissue homogenizer (Remi Motors, Mumbai, India) and centrifuged at 5974 g for 15 min at 0 °C using Eppendorf 5810-R high speed cooling centrifuge.

The biomarkers selected were malondialdehyde, reduced glutathione, superoxidase dismutase and total protein. Malondialdehyde in supernatant of the mice liver was determined by method of Slater and Sawyer (1971). The assay of reduced glutathione was carried out by method Moron et al. (1979). The superoxidase dismutase activity was determined by the method of Misera and Fridovich (1972). Protein concentrations were determined using the method of Lowry et al. (1951).