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Antihyperglycemic activity with DPP-IV inhibition of alkaloids from seed extract of *Castanospermum australe*: Investigation by experimental validation and molecular docking

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ABSTRACT

The antidiabetic actions of *Castanospermum australe* Cunn., seed (CAS) extract were evaluated in Poloxamer-407 (PX-407) induced T2DM rats. The CAS extract (100 and 150 mg/kg body weight) was administered orally once a day for 5 weeks after the animals were confirmed diabetic. A significant increase in blood glucose, HbA_1c and serum insulin levels were observed in T2DM rats in comparison to citrate control rats. Treatment with CAS extract in T2DM rats reduced the elevated levels of blood glucose, HbA_1c and insulin with significant ($p \leq 0.001$) improvement in OGT. The CAS extract treatment also increased ($p \leq 0.001$) the K_{ITT} and prevented increase in HOMA-R level in T2DM rats. The DPP-IV inhibitory potential of CAS extract showed IC_{50} value of 13.96 μg/ml whilst the standard Diprotin A displayed the IC_{50} value of 1.543 μg/ml. Molecular docking of the three reported alkaloids from the seeds of *C. australe* showed comparable DPP-IV inhibition with berberine. Our data suggest that CAS extract (150 mg/kg body weight) normalizes hyperglycemia in T2DM rats with strong DPP-IV inhibitory potential. The molecular docking showed that among the three alkaloids of seed extract 7-Deoxy-6-epi-castanospermine is a potent DPP-IV inhibitor similar to berberine.

Introduction

Type 2 diabetes mellitus (T2DM) is possibly the world's fastest growing metabolic syndrome of multiple aetiologies causing hyperglycemia (Nyenwe et al. 2011; Al masri et al. 2009). The progression of T2DM begins with an impairment of glucose tolerance (Zimmet and Thomas 2003) and is often associated with a state of insulin resistance (Robertson and Harmon 2006). According to the World Health Organization (WHO), T2DM is the World's fifth leading cause of death and it is estimated that it will be surpass 366 million by the year 2030 (Wild et al. 2004). A popular theory on meal-induced insulin secretion, the 'incretin effect' states that glucose or any other drug is more effective on the pancreatic cells when administered orally than given through intravenous or subcutaneous injections (Vilsboll and Holst 2004). Major glucose regulating incretin hormones are glucagon like peptide-1 (GLP-I) and glucose-dependent insulinotropic polypeptide (GIP) produced from the L-cells and K-cells of the intestinal mucosa, respectively. Recently it was shown that in T2DM there is a decrease in the incretin effect and rapid degradation of short-lived GLP-I and GIP (Vilsbøll et al. 2001). GLP-I under normal metabolic conditions improve glucose homeostas by promoting α- and β-cell function, insulin secretion, glycogenesis in muscles and liver (Fehmann and Habener 1992). On the other hand it down regulates the level of gastric emptying and gastric acid secretion to reduce postprandial glucose spikes (Nauck et al. 1997). However, GLP-1 has short plasma half-life of only 1–2 min (Mentlein et al. 1993).

Abbreviations: CAS, *Castanospermum australe* seed; PX-407, poloxamer-407; T2DM, Type 2 diabetes mellitus; HbA_1c, glycosylated haemoglobin; OGTT, oral glucose tolerance test; K_{ITT}, insulin sensitivity index; HOMA-R, homeostasis model assessment of insulin resistance; DPP-IV, dipeptidyl peptidase-IV; WHO, World Health Organization; GLP-I, glucagon like peptide-1; GIP, glucose-dependent insulinotropic polypeptide; GPPN, Gly-pro-p-nitroanilide; FBG, fasting blood glucose; FI, fasting insulin; pNA, paranitroaniline; IC_{50}, the half maximal inhibitory concentration; RT, retention time.

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Dipeptidyl peptidase-IV (DPP-IV) also named 'gliptins' (EC 3.4.14.5) is a soluble plasma enzyme found in the capillary bed of the gut mucosa (Lambeir et al. 2003). Other organs like kidney, liver and intestine are reported to have DPP-IV enzyme (Kieffer and Habener 1999). This enzyme belongs to the family of serine proteases, containing 766 amino acids with Asp-His-Ser at the active site. DPP-IV cleaves the alanine and proline from the N-terminal ends of GLP-I and GIP making them biologically inactive (Deacon et al. 1995). Administration of DPP-IV inhibitors block the enzyme and thereby prolongs the half life and biological activity of GLP-I. This is one of the recent therapies used in the treatment of Type 2 diabetes (Lambier et al. 2008). There is like vildagliptin (NPS 2010), sitagliptin (Kim et al. 2005), saxagliptin (Augeri et al. 2005) used as antihyperglycemic agents. Of the five DPP-IV inhibitors three already commercialized are sitagliptin (Lyseng-Williamson 2007; Zerilli and Pyon 2007), vildagliptin (Henness and Keam 2006; Croxtall and Keam 2008) and saxagliptin (Gallwitz 2008; Cole et al. 2008), and of other gliptins, such as alogliptin (Deacon 2008; Pratley 2009) and linagliptin (Rungby 2009; Tiwari 2009) are currently in late phase of development.

There has been renewed interest in medicinal plant as source of drugs and many plants have been used to treat diabetes (Holman and Turner 1991; Larner 1985; Rao et al. 1997). It is assumed that herbal medicine can be effective alternative to oral hypoglycemic agents in the treatment of T2DM, where pancreatic islets are not totally destroyed (Koehn and Carter 2005). The *Castanospermum australe* Cunn. (Family, Papilionaceae) also known as the black bean or Moreton Bay chestnut is cultivated in India as an ornamental tree (Ali 1977). In traditional medicine, the pods of the plant are reported as astringent (Chopra et al. 1956) and in treatment of post-prandial hyperglycemia in diabetic patients (Nash et al. 1985). On literature survey it was found that the wood of *C. australe* contains castanogenin and bayogenin (Eade et al. 1963) whereas castanogenol from bark (Rao et al. 1969), castanospermine (Hohenchutz et al. 1981) and australine (Molyneux et al. 1988) from seed are reported. Saponins from the fresh leaves of *C. australe* have also been characterized (Ahmed et al. 1992, 1994). Castanospermineis a natural alkaloid derived from *C. australae* have endowed with entirely new hypoglycemic compounds. The compound is water soluble and can be readily isolated in large quantity through a rather simple purification scheme (Rao et al. 1969).

Though other properties of *C. australe* have been reported, their effect on hyperinsulinemia, glucose intolerance, insulin sensitivity and DPP-IV inhibition activity are scanty in the literature. Therefore, the present study was conceived to investigate the effect of *C. australe* seed extract (CAS) on hyperinsulinemia, glucose intolerance and insulin sensitivity as well as DPP-IV inhibition by *in vitro* assay in poloxamer-407 induced T2DM model of rats. Before proceeding with the plant extracts we have standardized and modified the method of Al masri et al. (2009) by using the Diprotin A as the standard inhibitor of DPP-IV. Diprotin is a tripeptide (Ile-Pro-Ile) and effective inhibitor of DPP-IV in Type 2 diabetes (Alponti et al., 2011). It has been reported that seeds of *C. australe* contain an indolizidine alkaloid castanospermine, a trihydroxyindolizidine alkaloid 7-Deoxy-6-epi-castanospermine and a pyrrolizidine alkaloid Australine which have been shown to inhibit several glucosidases (Orwa et al. 2009). Further after establishment of antidiabetic property of CAS we have performed molecular docking of the three aforesaid alkaloids.

Materials and methods

Experimental animals

Healthy albino Wistar rats were housed under good hygienic conditions and allowed to acclimatize for 15 days under controlled condition of illumination (a 12-h light:dark cycle) and temperature 20–25 °C. They were maintained on standard pellet diet (Lipton rat feed Ltd., Pune, India) and water *ad libitum* throughout the experimental period. The experimental study was approved by the Institutional Animal Ethics Committee of JamiaHamdard, New Delhi, India.

Drugs and chemicals

Poloxamer-407 was procured from Sigma Chemicals Co., St. Louis, MO, USA. The enzyme-linked immunosorbent assay (ELISA) kit for insulin assay was purchased from Mercodia (Uppsala, Sweden). Hyperlipidemic diet supplement like olive oil, cholic acid, and cholesterol were purchased from Zeel Pharmaceuticals (Mumbai, Maharashtra, India). DPP-IV from porcine kidney, Gly-pro-p-nitroanilide (GPPN), Diprotein-A (Ile-Pro-Ile) and Tris–HCl buffer were purchased from Sigma, Bangalore, India. All the other chemicals used for the experiment were of analytical grade.

Sample preparations and HPLC analysis

The plant *C. australe* was collected from ruderals of Hajipur and Patna locality, Bihar, Patna. The plant was identified and authenticated at source and a voucher specimen (CAS-103/2011) has been deposited in the post graduate department of biochemistry, Patna University, Patna for future reference. The immature seeds (250 g) of *C. australe* were milled and extracted using 70% ethanol in Soxhlet apparatus for 8 h. Extract was evaporated till dryness using a vaccum evaporator and the final crude product obtained was stored at 4 °C for future use. For high performance liquid chromatography (HPLC) analysis the CAS extract was fractionated by preparative IC that included a Sep Tech STBOOC preparative liquid chromatograph, a Phenomenex IB-SIL 5 NH, column (250 mm × 22.5 mm i.d.), and a step gradient mobile phase consisting of MeCN–H_2O (90:10) as mobile phase A and MeCN–H_2O (50:50) as mobile phase B. Two ml of crude sample solution (containing about 300 mg of solids) diluted to 10 ml with MeCN–H_2O (80:20) was used for each injection. The column was first eluted with mobile phase A at a flow rate of 40 mY min for 21.5 min, then with mobile phase B using a step gradient for an additional 6.5 min. The collected fractions were taken to dryness using a rotary evaporator operated at 25 s and 3 mm Hg.

Induction of diabetes

The animal model for the current study was based on multiple administration of freshly prepared PX-407 dissolved in injectable distilled water and administered at a dose of 10 mg/kg body weight (in 1 mM of cold citrate buffer at a pH of 4.5) to an adult rat once a day for five week. For induction of diabetes, initially the normal rats were kept 24 h without food and water. The weights of normal rats were taken. In a beaker 11 ml stock solution was taken and 50.2 mg of PX-407 was dissolved in it. Of this solution 0.5 ml was injected to each rat intraperitoneally once a day for five week by insulin syringe; afterward food and water was supplied. Rats with fasting blood glucose level of 200 mg/dl or higher were considered to be diabetic and were used in the study.

Experimental design

The rats were divided into five groups comprising of six animals in each group as follows:

Group I: Citrate control rats received citrate buffer (0.1 ml/10 g, intraperitoneally).