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

Sudhanshu Kumar Bharti, Supriya Krishnan, Amit Kumar, Kaushal Kishore Rajak, Krishna Murari, Binod Kumar Bharti, Ashok Kumar Gupta. 

Introduction

Type 2 diabetes mellitus (T2DM) is possibly the world's fastest growing metabolic syndrome of multiple aetiologies causing hyperglycaemia (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 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-1) and glucose-dependent insulinoitropic 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-1 and GIP (Vilsboll et al. 2001). GLP-1 under normal metabolic conditions improve glucose homeostasis 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).

**Keywords:** Type 2 diabetes Insulin resistance Hyperglycemia Glucose tolerance DPP-IV inhibitor

**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, HbA1c 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, HbA1c and insulin with significant (p ≤ 0.001) improvement in OGTT. The CAS extract treatment also increased (p ≤ 0.001) the KITT and prevented increase in HOMA-R level in T2DM rats. The DPP-IV inhibitory potential of CAS extract showed IC50 value of 13.96 μg/ml whilst the standard Diprotin A displayed the IC50 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.

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Abbreviations: CAS, *Castanospermum australe* seed; PX-407, poloxamer-407; T2DM, Type 2 diabetes mellitus; HbA1c, glycosylated haemoglobin; OGTT, oral glucose tolerance test; KITT, insulin sensitivity index; HOMA-R, homeostasis model assessment of insulin resistance; DPP-IV, dipeptidyl peptidase-IV; WHO, World Health Organization; GLP-1, glucagon like peptide-1; GIP, glucose-dependent insulinoitropic polypeptide; GPPN, Gly-pro-p-nitroanilide; FBG, fasting blood glucose; FI, fasting insulin; pNA, paranitroaniline; IC50, the half maximal inhibitory concentration; RT, retention time.

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Dipeptidyl peptidase-IV (DPP-IV) also named ‘glitpins’ (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 (Kiellher 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-1 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-1. This is one of the recent therapies used in the treatment of Type 2 diabetes (Lambeir et al. 2008). There is little 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 (Hennes and Keam 2006; Croxall and Keam 2008) and saxagliptin (Gallwitz 2008; Cole et al. 2008), and of other glitpins, 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), castanopermin (Hohenhutzi 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). Castanosperminesin a natural alkaloid derived from C. australae has 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 glucosidasases (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 vacuum 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 S NH, column (250 mm × 22.5 mm i.d.), and a step gradient mobile phase consisting of MeCN–H2O (90:10) as mobile phase A and MeCN–H2O (50:50) as mobile phase B. Two ml of crude sample solution (containing about 300 mg of solids) diluted to 10 ml with MeCN–H2O (80:20) was used for each injection. The column was first eluted with mobile phase A at a flow rate of 40 ml/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).
Group II: PX-407 induced T2DM rats received PX-407 in multiple dose (10 mg/kg, intraperitoneally).
Group III: PX-407 induced T2DM rats received CAS extract (100 mg/kg body weight, per orally).
Group IV: PX-407 induced T2DM rats received CAS extract (150 mg/kg body weight, per orally).
Group V: CAS extract treated rats received CAS extract (150 mg/kg body weight, per orally).

The CAS extract (100 and 150 mg/kg body weight) was dissolved in distilled water and given until the end of the study (5 weeks) to groups III, IV and V animals. During experiment, blood samples were collected by nicking the tip of tail for biochemical estimations.

**Determination of blood glucose, HbA1c, insulin level and glucose tolerance test**

Blood glucose level was estimated by glucose oxidase method (Brahman and Trinder 1972) using a commercial diagnostic kit from Span diagnosticic Limited, Surat, India. Glycosylated haemoglobin (HbA1c) level was estimated according to the method of Bannon (1982) using a commercial diagnostic kit from Monozyme India Limited, Secunderabad, India. Plasma insulin level was estimated quantitatively by using insulin ELISA kit. Oral glucose tolerance test (OGTT) was measured according to the method of Pari and Saravanan (2002) in which glucose solution (2 g/kg) was given to overnight fasted rats. Blood samples were taken at 0, 15, 30, 60 and 120 for glucose estimation.

**Determination of insulin sensitivity**

Insulin sensitivity index (KITT) is used to assess peripheral insulin resistance (Murali et al. 2002). This test measures insulin sensitivity using KITT as an index of insulin-mediated glucose metabolism. Rats were fasted overnight before giving insulin challenge. Insulin (0.2 U/100 g body weight human regular insulin; Eli Lilly, Indianapolis, IN, USA) was administered by slow intravenous injection through tail vein. Blood samples were collected at 0 min and then at 15, 30, 60 and 120 min after administration of insulin injection. Glucose was estimated by glucose oxidase–peroxidase method of Brahman and Trinder (1972). KITT was determined from the slope of a linear portion of the regression line of natural logarithm of glucose versus time and using a formula:

\[
K_{ITT} = \frac{0.693}{t_{1/2}} \times 100
\]

where \(t_{1/2}\) represents the half-life of plasma glucose decay. The half-life of plasma glucose was obtained by plotting plasma glucose concentrations versus time on semi logarithmic graph paper.

Homeostasis model assessment of insulin resistance (HOMA-R) was calculated using fasting blood glucose (FBG) and fasting insulin (FI) level and was used for the determination of hepatic insulin resistance (Uno et al., 2005). The insulin sensitivity level was calculated using the following formula:

\[
\text{HOMA-R} = \frac{F(I) (\mu U/ml) \times F(BG) (mg/dl)}{405}
\]

**DPP-IV assay of Diprotin**

DPP-IV assay was performed in triplicate following the modified method of Al masri et al. (2009). In a 96-well titer plate reader the chromogenic substrate is cleaved by the serine protease DPP-IV resulting in release of paranitroaniline (pNA), a yellow coloured product (measured at 405 nm). Diprotin A was diluted to various concentrations (0.2, 0.4, 0.8, 1.6, 3.2, 6.4 μg/ml) using Tris–HCl buffer (50 mM, pH 7.5) and the final volume was made to 35 μl. Absorbance was taken at 405 nm in a 96-well plate reader (Bio–TEK, USA). 15 μl of DPP-IV enzyme (0.05 U/ml) was added to the above mixture. One unit enzyme activity was defined as the amount of enzyme that catalyzes the release of 1 μmol pNA from the substrate/min under assay conditions.

After adding the enzyme, the mixture was pre-incubated for 10 min at 37°C to enhance binding capacity of the inhibitor. This was followed by addition of 50 μl of Gly–pro–p–nitroanilide (GPPN 0.2 mM in Tris–HCl) as a substrate. Final incubation was done at 37°C for 30 min. The reaction was terminated by addition of 25 μl of 25% glacial acetic acid. The absorbance was measured at 405 nm using a microtiter plate reader. Experiments were done in triplicates. The results obtained were compared with the negative control (no inhibitor).

**DPP-IV assay of C. austral seed extract**

The CAS extract (10 mg) was dissolved in 20 ml of distilled water to make a stock concentration 500 μg/ml. From the stock, the following working concentrations (12.5, 50, 200 and 400 μg/ml) were prepared. 20 μl of each of above stock concentrations was made to 35 μl using Tris–HCl buffer (50 mM, pH 7.5) to obtain final inhibitory concentrations of 2.5, 10, 40 and 80 μg/ml, respectively, in a total well volume of 100 μl. The assay was performed in triplicates according to standardized procedure of Diprotin A.

**Statistical analysis**

Data were expressed as the mean ± S.E.M. For statistical analysis of the data, group means were compared by one-way ANOVA with post hoc analysis. The Tukey–Kramer post hoc test was applied to identify significance among groups. The p-value < 0.05 was considered to be statistically significant. The Statistical data were evaluated by using MATLAB version 7.8.0, Natick, Massachusetts:
The Mathworks Inc., 2009. The percentage of inhibition was calculated using the following formula:

\[ \% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of inhibitor}}{\text{absorbance of control}} \times 100 \]

The half maximal inhibitory concentration (IC50) value represents the amount of inhibitor required to achieve 50% enzyme inhibition. In case of significant inhibition, the IC50 values were determined by non-linear regression by fitting to a sigmoidal dose–response equation with variable slope.

**Molecular modelling calculations**

All computational works were performed by GOLDv4.0 (2005) docking software (Jones et al. 1997) and binding affinity was calculated using X-Score (Wang et al. 2002).

**Results**

**HPLC analysis of CAS extract**

Fig. 1 shows the HPLC analysis of ethanolic extract from immature seeds of *C. australis*. The dry CAS extract powder mainly contains alkaloids castanospermine (RT = 10.91), 7-Deoxy-6-epi-castanospermine (RT = 7.04) and Australine (RT = 21.55).

**Effect of CAS extract on hyperglycemia, HbA1C levels and insulin level in addition to OGTT in experimental rat groups**

Intraperitoneal administration of PX-407 to overnight fasted normal rats (Groups II, III and IV) caused marked elevations (p ≤ 0.001) in serum glucose, glycosylated haemoglobin (HbA1C) (Table 1) and insulin levels (Fig. 3) after 72 h when compared to citrate control rats (Group I). Fig. 2a showed the blood glucose levels of experimental rats after oral administration of glucose (2 g/kg). In T2DM control rats (Group II), the peak increase in blood glucose level was observed after 1 h and remained high over next 1 h. Regular oral administration of CAS extract to rats of Group III and IV at two doses (100 and 150 mg/kg body weight) antagonized blood glucose level significantly (p ≤ 0.001) after 1 and 2 h and reduces the elevated levels of HbA1C and insulin in a dose-dependent manner when compared to T2DM control rats (Group II). On its own CAS extract treatment (Group V) did not register any significant change in the blood glucose level, HbA1C level, insulin level as well as OGTT (at 1 and 2 h) when compared to citrate control rats (Group I).

**Effect of CAS extract on insulin sensitivity in experimental rats**

The level of KITT (an index of peripheral insulin resistance) and the level of HOMA-R (an index of hepatic insulin resistance) have been shown in Fig. 2b and c, respectively. T2DM control rats (Group II) showed significant decrease in KITT with significant increase in HOMA-R level when compared to citrate control rats (Group I). Treatment with CAS extract significantly (p ≤ 0.001) increased the level of KITT and prevented increase in HOMA-R level in T2DM rats (Groups III and IV) when compared to T2DM control rats (Group II). No significant change was observed in the levels of KITT and HOMA-R in only CAS extract-treated rats (Group V) and citrate control rats (Group I).

**DPP-IV inhibition assay**

On the establishment of antidiabetic potential we had selected this plant as a candidate for DPP-IV inhibition and measured against Diprotin A (positive control). The CAS extract showed IC50 value of 13.96 μg/ml whilst the standard Diprotin A displayed the IC50 value of 1.62 μg/ml (Table 2).

**Molecular modelling calculations**

As the CAS extract showed DPP-IV inhibition, the three major reported alkaloids of seeds of *C. australis* namely Castanospermine, 7-Deoxy-6-epi-castanospermine and Australine have been docked with GOLD (2005) software (Figs. 4 and 5) and compared with berberin. We also calculated binding affinity using XSCORE (Wang et al. 2002). X-Score calculates the average of three scoring function (HS Score, HM Score and HP Score) and predicts the binding energy of the ligand. Docking results showed that all the three isoforms of castanospermine has been showing hydrogen bonding with catalytic residues of DPP-4. As compared to berberin they have slightly low Goldscore and also there is a difference of –1.5 kcal/mol of binding energy (Table 3) which is not very significant. Out of three isoforms of castanospermine, 7-Deoxy-6-epi-castanospermine showed highest ranking followed by Australine and Castanospermine.

**Discussion**

*Diabetes mellitus* is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality (Nyewee et al. 2011). Treatment that is inadequate or instituted too late predisposes the affected individual not only to the basic metabolic disturbances but also to a number of serious complications. Poloxamer-407 inductions to neonatal rats manifest hyperglycemia, an impaired response to the glucose tolerance test (Portha et al. 1979) and loss of β-cell sensitivity to glucose (Giroix et al. 1983). They are considered as a useful experimental model to study the activity of antihyperglycemic agents (Szkudelski 2001; Yamagishi et al. 2001). Hyperinsulinemia has generally been considered a marker of insulin resistance, i.e., a decrease in the effect of insulin to stimulate glucose uptake at a given serum insulin concentration (Tenenbaum et al. 2003). Hence, in addition to glycaemic control, management of hyperinsulinemia is also essential for controlling insulin resistance and limiting the complications of T2DM.

In our conduct experiment T2DM control rats (Group II) exhibited persistent hyperglycemia. Treatment with CAS extract (100 and 150 mg/kg body weight) to T2DM rats reduced the elevated blood glucose level thereby showing its antihyperglycemic activity in a dose-dependent manner. Rats treated with 100 mg of CAS extract per kg body weight showed a maximum fall of 56% in blood glucose level, whereas fall of 64% was observed with the dose of 150 mg/kg body weight.

In diabetes, there is an increased glycosylation of a number of proteins, including haemoglobin and β-crystalline of lens (Alberti and Press 1982). Hence measurement of HbA1C is useful in monitoring the effectiveness of therapy in diabetes (Goldstein 1995). The HbA1C level was found to increase in T2DM control rats (Group II) when compared to citrate control rats (Group I). The two doses of oral administration of CAS extract (100 and 150 mg/kg body weight) decreased the HbA1C level by 28% and 46% respectively in a dose-dependent manner. Administration of CAS extract to T2DM rats reduced the glycosylation of haemoglobin possibly by virtue of its free radical scavenging property and thus decreased the level of HbA1C.

Hyperinsulinemia appears to be a compensatory mechanism that responds to increased level of circulating glucose resulting in progression to insulin resistance (Goldstein 2002). The β-cells normally compensate insulin resistance by secreting more amounts
Table 1
Effect of CAS extract on blood glucose and glycosylated haemoglobin levels in experimental rat groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose (mg/dl)</th>
<th>%Glycosylated haemoglobin (HbA1c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Citrate control</td>
<td>97.17 ± 3.01</td>
<td>5.690 ± 0.067</td>
</tr>
<tr>
<td>II</td>
<td>PX-407 (10 mg/kg, intraperitoneally)</td>
<td>327.16 ± 10.77(^1)</td>
<td>12.96 ± 0.124(^1)</td>
</tr>
<tr>
<td>III</td>
<td>PX-407 + CAS extract (100 mg/kg, per orally)</td>
<td>152.92 ± 4.10(^2)</td>
<td>9.69 ± 0.117(^2)</td>
</tr>
<tr>
<td>IV</td>
<td>PX-407 + CAS extract (150 mg/kg, per orally)</td>
<td>122.18 ± 1.98(^2)</td>
<td>6.97 ± 0.068(^2)</td>
</tr>
<tr>
<td>V</td>
<td>Only CAS extract (150 mg/kg, per orally)</td>
<td>99.18 ± 1.17</td>
<td>5.69 ± 0.156</td>
</tr>
</tbody>
</table>

Note: The data are expressed in mean ± S.E.; n = 6 in each group.

\(^1\) p ≤ 0.001, compared to the corresponding value for citrate control animals (Group I).

\(^2\) p ≤ 0.001, compared to the corresponding value for T2DM control animals (Group II).

Fig. 2. Effect of CAS extract on (A) insulin level, (B) KITT level and (C) HOMA-R level in experimental rat groups. The data are expressed as mean ± S.E. (n = 6). \(*\)p ≤ 0.001 as compared to citrate control rats (Group I). \(**\)p ≤ 0.001 as compared to T2DM-treated rats (Group II).

Fig. 3. Effect of CAS extract on oral glucose tolerance test in T2DM rats. The data are expressed in mean ± S.E.; n = 6 in each group. p ≤ 0.001, compared to the corresponding value for control animals.
Table 2
DPP-IV inhibitory activity of CAS extract and Diprotin A.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>Inhibition (%)</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diprotin A (Ile-Pro-Ile)</td>
<td>0.2</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>33.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>55.1</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>74.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.4</td>
<td>86.7</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>2.5</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>59.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>68.0</td>
<td></td>
</tr>
<tr>
<td>C. australe</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Binding of 7-Deoxy-6-epi-castanospermine with active site residues of DPP-IV.

Fig. 5. Binding of Australine with active site residues of DPP-IV.

of insulin to maintain glucose homeostasis. Bonora et al. (1983) has reported that hyperinsulinemia is associated with decreased hepatic insulin clearance and hypersecretion of β-cells in mild glucose intolerance obese subjects. Our results clearly showed the condition of hyperinsulinemia in T2DM control rats (Group I). The hyperinsulinemia in T2DM rats is either due to decreased hepatic clearance of insulin or by down-regulation of insulin receptors and desensitizing post receptor pathways resulting in decreased insulin binding and degradation (Lautamaki et al. 2006). Despite hyperinsulinemia, the glucose level was greater in T2DM control rats than T2DM-treated rats. Treatment with CAS extract significantly (p ≤ 0.001) reduces the elevated levels of insulin (Groups III and IV) when compared to T2DM control rats. Treatment with 150 mg of CAS extract per kg of body weight cut down insulin level by 25% (Group IV) whilst fall of 21% was observed with the dose of 100 mg/kg. On its own CAS extract treatment (Group V) did not induce any significant change in the level of insulin. Hence, CAS extract treatment was found to be effective in reducing insulin level of T2DM rats by preventing hyperinsulinemia.

An insulin resistance state is a key phase of metabolic syndrome, constituting the major risk factor for the development of glucose intolerance and diabetes mellitus (Group 2000). Thus, interventions to decrease insulin resistance may postpone the development of T2DM and its complications. When animals were subjected to OGTT, glucose disposal was found to be significantly decreased and increased blood glucose was maintained up to 2 h in T2DM control rats (Group II). Treatment with CAS extract at two different doses, rats (Groups III and IV) showed significant (p ≤ 0.001) decrease in blood glucose level at 1 and 2 h when compared to T2DM control rats. With 100 mg of CAS extract treatment (Group III) blood glucose was found to be diminished by 14% and 23% at 1 and 2 h respectively whilst 16% and 28% reduction was observed after 1 and 2 h respectively with 150 mg/kg CAS extract treatment (Group IV). Only CAS extract treatment (Group V) did not register any significant change in the blood glucose level at 1 and 2 h during OGTT when compared to citrate control rats (Group I). Treatment with CAS extract significantly improved glucose tolerance, as indicated by reduction in peak blood glucose level at 1 and 2 h in T2DM treated rats during OGTT. The CAS extract might enhance glucose utilization by peripheral tissues and increasing the glycogen stores in the liver due to restoration of delayed insulin response, because it significantly decreased the blood glucose level in glucose loaded rats.

Table 3
GOLD score and predicted binding affinity of Castanospermine isoforms compared to Berberin.

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>GOLD score</th>
<th>Binding affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Deoxy-6-epi-castanospermine</td>
<td>41.01</td>
<td>–6.79</td>
</tr>
<tr>
<td>Australine</td>
<td>40.07</td>
<td>–6.52</td>
</tr>
<tr>
<td>Castanospermine</td>
<td>36.09</td>
<td>–6.79</td>
</tr>
<tr>
<td>Berberin</td>
<td>47.38</td>
<td>–8.21</td>
</tr>
</tbody>
</table>
Our results showed that CAS extract decreased blood glucose level, prevented hyperinsulinemia and improved glucose tolerance in T2DM rat model. Thus, KITT and HOMA-R levels were determined to check insulin sensitivity, KITT is used to assess peripheral insulin resistance (Bolzan and Bianchi 2002) whereas HOMA-R is a useful clinical index of hepatic insulin resistance (Bonora et al. 2000).

Treatment with CAS extract significantly (p < 0.001) increased the level of KITT and prevented increase in HOMA-R level in T2DM rats (Group III and IV) when compared to T2DM control rats. With 100 mg/kg CAS extract treatment (Group III) KITT and HOMA-R level was found to be increase by 22% and diminished by 23% respectively whilst 63% increment and 76% reduction was observed respectively with 150 mg/kg CAS extract treatment (Group IV). The results obtained clearly showed that KITT was significantly improved by CAS extract treatment to T2DM rats. Additionally, CAS extract treatment significantly prevented the rise in HOMA-R in T2DM-treated rats. These findings suggest that CAS extract is pharmacologically effective in improving insulin sensitivity.

The seeds of C. australie have three major alkaloids (Orwa et al. 2009). The DPP-IV inhibitory potential of CAS extract showed IC50 value of 13.96 µg/ml whilst the standard (positive control) Diprotin A displayed the IC50 value of 1.543 µg/ml (Table 2). It might be due to the presence of these three major alkaloids. The high inhibitory activity of Diprotin A might be due to its tripeptide specificity and purity. Diprotin A as an inhibitor affects the catalytic activity of DPP IV in the human central nervous system, endocrine system and on the CD-26 of immune system (Maes et al. 2007). Earlier natural DPP-IV inhibitors like berberine (an alkaloid) isolated from plants like Berberis aristata, Berberis aquifolium and Hydrastis canadensis showed effective inhibition against the DPP-IV enzyme (Al masri et al. 2009; Lubbers et al. 2007; Demuth et al. 2005). Apart from the DPP-IV inhibition, it also shows other antidiabetic properties like insulin mimetic, reduction of insulin resistance, glycolysis promotion and enhancing the GLP-1 release (Zhang et al. 2010) and preferred over metformin for hypoglycemic patients with liver diseases. Thus the three alkaloids from C. australie might have the same action mechanism as berberine. The three alkaloids from C. australie were analyzed for their DPP-IV inhibition potential by Gold (2005) docking software (Jones et al. 1997).

Conclusion

The results obtained from antihyperglycemic activity and DPP-IV enzyme inhibition assay (in vitro) explained the effectiveness of CAS in regulating diabetes. The docking results showed that 7-deoxy-6-epi-castanospermine and Australin is also acting as potent DPP-IV inhibitor as compared to berberine. Current DPP-IV drugs in market (Vildagliptin, Sitagliptin and Saxagliptin) have several side effects like tremors, headache, dizziness, low blood sugar levels especially when taken in excess of amount, nausea, feeling weak, weight gain and swelling of the legs and ankles due to excess fluid retention. If these results are extrapolated then CAS extract might prove useful in the treatment and/or prevention of hyperinsulinemia, impaired glucose tolerance and insulin resistance with DPP-IV inhibitory activity.

Conflict of interest

The authors have declared no conflict of interest.

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