

Dipeptidyl Peptidase- IV Inhibitory Activity of *Berberis aristata*

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) is an insulinotropic gut hormone. It enhances meal-induced, glucose-dependent insulin secretion (incretin effect) and restores glucose competency to the beta-cells of pancreas. GLP-1 also inhibits meal-induced gastric acid secretion and gastric emptying, thereby reducing postprandial glucose excursions which is an advantage in Type 2 diabetes. However, the major limiting factor of GLP-1 is its susceptibility to degradation by dipeptidyl peptidase IV (DPP-IV) enzyme. Also GLP-1 has a short plasma half life of only 1-2 minutes. Therefore, it becomes a requisite to identify DPP-IV inhibitors that act as potential antidiabetic agents. Though there are few commercial DPP-IV inhibitors (Vildagliptin, Sitagliptin and Saxagliptin) are available in the market, they have some undesirable side effects. In this context, our study had focused to screen few medicinal plants for DPP-IV inhibiting activity. Among the eight plants screened, we have identified the methanolic extract of the bark of tree turmeric (*Berberis aristata*) has the ability to inhibit dipeptidyl peptidase IV activity (*in vitro*). The crude bark extract had shown IC₅₀ value of 14.4µg/ml and the standard diprotin-A displayed 1.5µg/ml.

Keywords: GLP-1; DPP-IV; *Berberis aristata*; Diprotin A; *In vitro* assay.

INTRODUCTION

Type 2-diabetes is a multifaceted chronic metabolic disorder causing hyperglycemia (Tony, 2007; Nyenwe, et al., 2011; Al masri, et al., 2009). Hyperglycemia is mainly observed in people with increased concentration of fats and carbohydrates in their body; assisted with low physical activities. The manifestation is mainly due to insulin resistance, pancreatic β-cells injury or infection leading to its malfunctioning and lowered insulin secretion (Barnett and Kumar, 2009). According to the World Health Organization (WHO), Type 2 Diabetes 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 Type 2 diabetes 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 promote β -cell growth, 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 minutes (Mentlein, et al., 1993).

DPP-IV (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., 2003). There are DPP-IV inhibitors like vildagliptin (Vilhauer, et al., 2003), sitagliptin (Kim, et al., 2005), saxagliptin (Augeri, et al., 2005) used as an antihyperglycemic agents.

In this study, we have screened the following eight plants for DPP-IV inhibition by *in vitro* assay: *Pongamia pinnata* (seed), *Szygium cumini* (seed), *Punica granatum* (seed), *Terminalia chebula* (fruit), *Terminalia arjuna* (bark), *Cassia auriculata* (flower), *Salvadora persica* (bark) and *Berberis aristata* (bark). 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).

MATERIALS AND METHODS

Chemicals used: DPP-IV from porcine kidney, Gly-pro-p-nitroanilide (GPPN), Diprotein-A (Ile-Pro-Ile), Tris-HCl Buffer. All chemicals were purchased from Sigma, Bangalore.

Sample preparation: *Pongamia pinnata* (seed), *Szygium cumini* (seed), *Punica granatum* (seed), *Terminalia chebula* (fruit), *Terminalia arjuna* (bark), *Cassia auriculata* (flower), *Salvadora persica* (bark) were purchased from Amman Ayurvedics Vellore. These plant parts were ground to yield a powdered form for solvent extraction. Grounded plant parts (250g) were extracted using methanol (300 ml) in Soxhlet apparatus for 8 hours. Bark of *Berberis aristata* (Family-Berberidaceae) was collected from the natural plantation of Rajasthan. It is a woody plant, native to Himalayas in India and Nepal. The bark (250g) was milled and extracted using methanol (300ml) in Soxhlet apparatus for 8 hours. Extract was evaporated till dryness using a vacuum evaporator and the final crude product obtained was of yellow-black colour, which was stored at 4°C for future use.

DPP-IV assay of Diprotin: DPP-IV assay was performed following the modified method of Al-masri, et al., (2009). In a 96-well titer plate reader, inhibition assay was conducted in triplicates. The substrate is chromogenic, cleaved by the serine protease DPP-IV resulting in release of Paranitroaniline (pNA), a yellow coloured product

which was measured at 405nm. Diprotin A was diluted to various concentrations (0.2, 0.4, 0.8, 1.6, 3.2, 6.4µg/ml) using Tris-HCl Buffer (50mM, pH 7.5) and the final volume was made to 35µl. Absorbance was taken at 405 nm in a 96-well plate reader (Bio-TEK, USA). 15µl of DPP-IV enzyme (0.05U/ml) was added to the above mixture. One unit enzyme activity was defined as the amount of enzyme that catalyzes the release of 1µ mol pNA from the substrate/min under assay conditions. After adding the enzyme, the mixture was pre-incubated for 10 minutes at 37°C to enhance binding capacity of the inhibitor. This was followed by addition of 50µl of Gly-pro-p-nitroanilide (GPPN 0.2mM in Tris-HCl) as a substrate. Final incubation was done at 37°C for 30 minutes. The reaction was terminated by addition of 25µl 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 *Berberis aristata*: Berberis crude extract (10mg) was dissolved in 20ml of distilled water to make a stock concentration 500µg/ml. From the stock, the following working concentrations (12.5, 50, 200, 400µg/ml) were prepared. 20µl of each of above stock concentrations was made to 35µl using Tris-HCl Buffer (50mM, pH 7.5) to obtain final inhibitory concentrations of 2.5, 10, 40, 80µg/ml respectively in a total well volume of 100µl. The assay was performed in triplicates according to standardized procedure of Diprotin A.

Statistical Analysis: All values are represented as Mean ± Standard Deviation. The Statistical data were evaluated by using GraphPad Prism5 software. The percentage of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of inhibitor}) \times 100}{\text{Absorbance of control}}$$

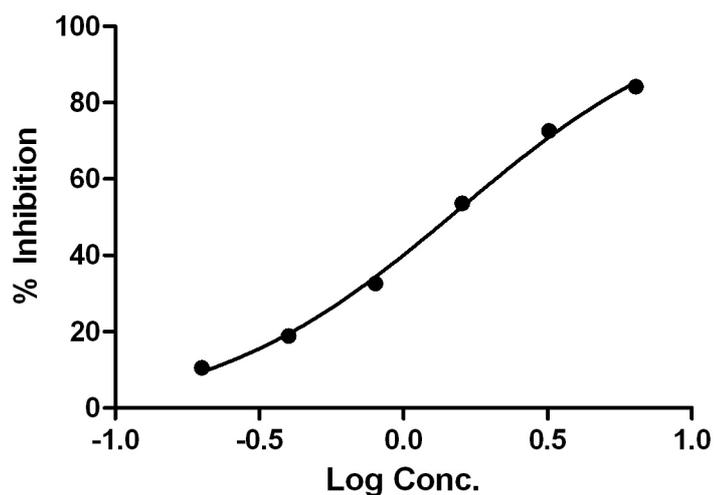
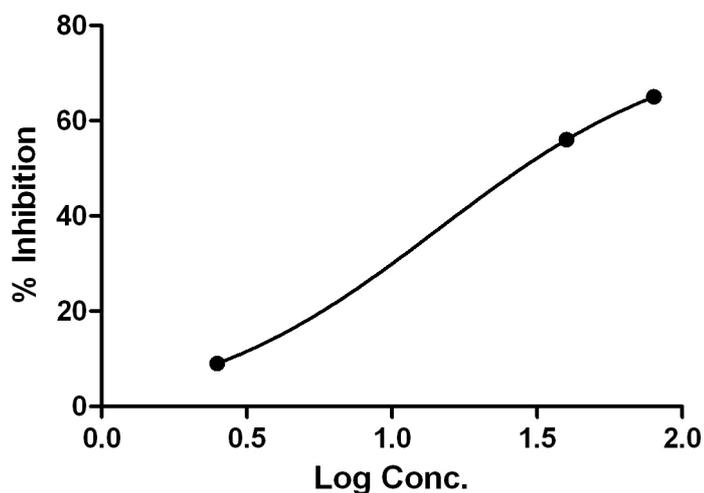
The IC₅₀ value represents the amount of inhibitor required to achieve 50% enzyme inhibition. In case of significant inhibition, IC₅₀ values were determined by non-linear regression by fitting to a sigmoidal dose-response equation with variable slope.

RESULTS AND DISCUSSION

Natural DPP-IV inhibitors like berberine, a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids, show effective inhibition against the DPP-IV enzyme. This active phytochemical can be isolated from plants like *Berberis aristata* (Tree Turmeric), *Berberis aquifolium* (Oregon grapes), *Hydrastis canadensis* (Goldenseal) (Demuth, et al., 2005). Among the eight plants screened, *Syzygium cumini* had shown high inhibition (87%). This was followed by *Berberis aristata* (73%), *Terminalia chebula* (51%), *Punica granatum* (28%) and *Pongamia pinnata* (15%) and others had shown very low inhibition. As the bark of *Berberis aristata* contains berberine, we had selected this plant as a candidate for our future experiments. The DPP-IV inhibitory potential of *Berberis aristata* bark extract and the positive control Diprotin A was measured. The plant extract showed IC₅₀ value of 14.46 µg/ml (Table-1, Figure-2) and the standard Diprotin A displayed the IC₅₀ value of 1.543µg/ml (Table-1, Figure-1). The reason to observe 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).

Table-1: DPP-IV inhibitory activity of *Berberis aristata* and Diprotin A.

Sample	Concentration ($\mu\text{g/ml}$)	Inhibition (%)	IC ₅₀ ($\mu\text{g/ml}$)
Diprotin A (Ile-Pro-Ile) Positive control	0.2	10.5	1.54
	0.4	18.9	
	0.8	32.6	
	1.6	53.6	
	3.2	72.6	
	6.4	84.2	
<i>Berberis aristata</i>	2.5	9.0	14.46
	40	56.0	
	80	65.0	

**Figure-1: DPP-IV inhibitory activity of Diprotin A****Figure-2: DPP-IV inhibitory activity of *Berberis aristata***

The bark has an alkaloid berberine that has an antioxidant, antimicrobial, antitumor, anti-inflammatory, and antidiabetic potential (Dutta and Panse, 1962). Activity of *Berberis* is similar to DPP-IV inhibitor Benzoquinolizines (Lubbers, et al., 2007) and it was demonstrated that commercially available pure form of berberine is an effective

DPP-IV inhibitor (Al-masri, et al., 2009). 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-I release. Berberine significantly reduced the fasting blood glucose (FBG), HbA_{1c}, and triglycerides in type 2 diabetic patients (Zhang, et al., 2010). It lowered blood glucose level through increasing insulin receptor expression. Berberine is preferred over metformin for hypoglycemic patients with liver diseases. Ethanol extract of the roots of *Berberis aristata* had shown effective reduction in serum glucose along with increase in HDL cholesterol level in alloxan induced diabetic rats (Bupesh, et al., 2009).

Current DPP-IV drugs in market (Vildagliptin, Sitagliptin and Saxagliptin) have several side effects like tremors, headache, dizziness, low blood sugar levels specially when taken in excess of amount, nausea, feeling weak, weight gain and swelling of the legs and ankles due to excess fluid retention (Idris, et al., 2007). These drugs have to be taken as combination therapy with other drugs to achieve desired results.

CONCLUSION

The results obtained from DPP-IV enzyme inhibition assay (*in vitro*) explained the effectiveness of *Berberis aristata* in regulating diabetes. This study has to be performed on rats to know the extent of emulation of *in vitro* results and its physiological significance to *in vivo* system.

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