Original Article

Evaluating the use of *Desmodium gangeticum* as Alpha Glucosidase and DPP-IV Inhibitor for Type-II Diabetes

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ABSTRACT

The objective of the study was to investigate the alpha glucosidase and Dipeptidyl peptidase IV (DPP-IV) inhibitory activity of aqueous extract of *Desmodium gangeticum (D. gangeticum).* α-d- Glucosidase from *Saccharomyces cerevisiae* was used as *in vitro* model to assess α-d-Glucosidase inhibitory activity of aqueous extract of *D. gangeticum*. In-vitro inhibitory effect of aqueous extract of *D. gangeticum* on DPP-IV enzyme was assessed by measuring the absorbance of reaction mixture at 405 nm using a microtiter plate reader. Results of the study demonstrated that aqueous extract of *D. gangeticum* exhibited good alpha glucosidase and DPP-IV inhibitory activity with an IC_{50} value of 950µg/ml and 255.5µg/ml respectively. The results of the study provide scientific support for the use of *D. gangeticum* in the traditional system of medicine for the treatment of Type-II diabetes. This study should help to explain the pharmacological mechanism of action and also help in the development of medicinal preparation for the treatment of Type-II diabetes.

Keywords: Type-II diabetes, Alpha glucosidase, Dipeptidyl peptidase-IV, *Desmodium gangeticum*.

INTRODUCTION

Type-II diabetes mellitus (T2DM) is a progressive, chronic metabolic disorder notable for the underlying defects in carbohydrate and lipid metabolism. It is typically characterized by several sequential steps involving impaired beta cell function, resulting in a relative insulin deficiency, followed by insulin resistance with decreased glucose transport into muscle and fat cells, accompanied by unrestrained hepatic glucose output, all of which contribute to an overwhelming glycaemic status[1]. Type-II diabetes is the commonest form of diabetes constituting 90% of the diabetic population. It is an endocrine

disease, which accounts for 9% of deaths worldwide. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. The World Health Organization has predicted that the major burden will occur in the developing countries. There will be a 42% increase from 51 to 72 million in the developed countries and 170% increase from 84 to 228 million, in the developing countries. The countries with the largest number of diabetic people are, and will be in the year 2025, India, China and United States. Previous studies on diabetes in India have highlighted not only is the prevalence of Type-II diabetes high, but also that it is increasing rapidly in the urban population[2,3].

World over, one of the major public health challenges of the 21st century is undisputedly T2DM. Oral hypoglycemic agents especially the sulphonylureas and biguanides have been commonly used for the management of diabetes, especially the Type-II, in spite of the associated adverse effects. The major attention is now focused on the use of plants and herbal remedies that would be devoid of serious side effects encountered with sulphonylureas and biguanides as alternatives in the treatment of diabetes. Therefore herbal medicines are also recommended for treatment of diabetes. One of the therapeutic approach to treat the diabetes is to control the blood glucose level, and this can be done by inhibiting the enzymes such as alpha amylase, alpha glucosidase, DPP-IV etc[1]. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Inhibitors of these enzymes have been recently developed from natural sources[4]. Thus, natural products of great structural diversity are still a good source for searching for such inhibitors, thereby motivating to explore biologically active compounds from the highly diverse plants[1].

One of the strategies adopted to treat diabetes mellitus involves inhibition of carbohydrate-digesting enzymes. α-glucosidase inhibitors (AGIs) are among the available glucose-lowering medications [3]. Mammalian α-glucosidase (α-*D*-glucoside glucohydrolase, EC 3.2.1.20), located in the brush border surface membrane of intestinal cells, is the key enzyme which catalyzes the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can retard the liberation of *D*-glucose from complex dietary carbohydrates and delay glucose absorption, reducing plasma glucose levels and suppressing postprandial hyperglycemia[5,6]. The α-glucosidase is essential to carbohydrate digestion because only monosaccharides are readily taken up from the intestine and all other carbohydrates have to be broken down enzymatically in the intestine before they can be absorbed. The other cellular glucosidases are known to be vital for the processing of Asn-linked glycoproteins and glycolipids which are involved in various biological reactions such as immune responses, metastasis of cancer and viral infections[7].

The AGIs delay, but do not prevent, the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks[3]. Inhibitors of α-glucosidase display useful anti-hyperglycaemic effects[8].

To date, some α-glucosidase inhibitors have been identified from the metabolism of micro-organisms, plants and chemical synthesis. Consequently, α-glucosidase inhibitors, such as acarbose and miglitol, have been approved for clinical use in the management of Type-II diabetes, as well as the treatment of diabetic complications. A main drawback of the currently used α-glucosidase inhibitors, such as acarbose, involves side effects such as abdominal distention, flatulence, meteorism and possible diarrhea. It has been suggested

that such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon. With this in mind, there has been a search for other effective and safe α-glucosidase inhibitors from natural materials in order to develop a physiological functional food or lead compounds for diabetes treatment[5].

The another therapeutic approach for treating Type-II diabetes is inhibition of Dipeptidyl peptidase-IV (DPP-IV), a serine protease which degrades GLP-1 into its inactive form[9]. GLP-1 is characterized as the most potent insulinotropic hormone, and is considered a therapeutic agent for the treatment of Type-II diabetes due to its combined action of stimulating insulin secretion, increasing beta-cell mass, inhibiting glucagon secretion, reducing the rate of gastric emptying and inducing satiety. The continuous infusion of this peptide decreases plasma glucose and improves β-cells function. The peptide is rapidly inactivated by dipeptidyl peptidase-IV (DPP-IV), resulting in a half-life of active GLP-1 of only approximately 1-2 minutes. Inhibition of DPP-IV increases the levels of endogenous active GLP-1 and prolongs its half-life[4,10]. This in turn leads to the suppression of blood glucose elevation. Therefore, development of DPP-IV inhibitors is being actively conducted worldwide, and control of blood glucose levels by enhancement of GLP-1 action is a new option for the treatment of diabetes.

Desmodium gangeticum (L) DC (Leguminosae) is a perennial non-climbing herb or shrub widely distributed in tropical and sub-tropical habitats and particularly abundant in India and used as medicinal herb in indigenous system of medicine (Ayurveda) as bitter tonic, febrifuge, digestive, anti-catarrhal, anti-emetic, in inflammatory conditions of chest and in various other inflammatory conditions which are due to vatadisorders. It is known as Sarivan in Hindi and Shalaparni in Sanskrit[11-13,17] (Figure: 1). This plant has been used in Ayurveda for the treatment of various diseases like typhoid fever, urinary discharges, piles, inflammations, asthma, bronchitis, vomiting, dysentery and hemicrania[14]. It is used in 'Ayurvedic' preparations like 'Dashmoolarishta' and 'Dashmoolakwaath' for the post-natal care to avoid secondary complications[15].

The aqueous extract of this plant has been reported to show severe antiwrithing activity, moderate central nervous system (CNS) depressant activity[16] and anti-leishmanial activity[17]. Gangetin, a ptero-carpnoid from *D. gangeticum* has been shown to possess anti-inflammatory and analgesic activity[18]. Total alkaloids of this species showed anticholinesterase, smooth muscle stimulant, CNS stimulant and depressant responses[19]. Chemical studies on the *D. gangeticum* revealed the presence of alkaloids, pterocarpnoids, flavonoids and isoflavonoid glycosides[12,20].

Our previous study reported the effect of aqueous extract of *D. gangeticum* in STZ-NA induced Type-II diabetic animals[21]. Further attempts were made to investigate the alpha-glucosidase and DPP-IV inhibitory effect of *D. gangeticum* to contribute to the understanding of their mechanisms of action in Type-II diabetes.

MATERIAL AND METHODS

Preparation of plant extract

Aerial parts of *D. gangeticum* were collected during the month of July-August from local herbal garden of Dehradun (Uttarakhand). The plant was taxonomically identified and authenticated at the Forest Research Institute (FRI), Dehradun. A voucher specimen (No.157028) was deposited in the Botany division of FRI,

Dehradun. Fresh aerial parts of *D. gangeticum* were washed and shade dried than coarsely powdered in a grinder. Aqueous extract was prepared by the process of maceration. Each extract were concentrated, dried *in vacuo* and the residue stored in a desiccators for further use.

Chemicals

4-Nitrophenyl-α-d-glucopyranoside, α-d-glucosidase from *Saccharomyces cerevisiae* (Sigma–Aldrich, Milwaukee, USA), acarbose (as a free sample from Biocon, Ltd., Bangalore, India); DPP-IV from porcine kidney, Gly-pro-p-nitroanilide (GPPN), Diprotin-A (Ile-Pro-Ile) and Tris-HCl Buffer were purchased from Sigma, Mumbai, India. All the other chemicals used for the experiments were of analytical grade.

Methods

α-Glucosidase (from *S. cerevisiae*) inhibitory activity

The α-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenyl-α-d-glucopyranoside (4-NPGP) according to Bisht S, *et al.*,[22] with slight modification. The assay media contained 0.1 M phosphate buffer of pH 6.9, 1 mM 4-NPGP (3.8 ml) as a substrate, 1 U/ml α-glucosidase from *S. cerevisiae* (4 µl) and plant extract (4 µl) in the range of 500-2000 µg/ml and standard (acarbose, 4 µl) drug in range of 1-100 mM. The reaction was initiated by addition of 4-NPGP at 37 ◦C for 20 min and terminated by addition of 1 M sodium carbonate (400 µl). Enzyme activity was quantified by measuring absorbance at 405 nm in triplicate. One unit of α-glucosidase activity was defined as amount of enzyme liberating 4-nitrophenyl (1.0 µM) per min. The α-glucosidase inhibitory activity (%) was calculated as follows:

% inhibition = $Abs_{control} - Abs_{extract}$ X 100 / $Abs_{control}$.

The IC_{50} value was defined as the concentration of α-glucosidase inhibitor that inhibits 50% of α-glucosidase activity and were calculated by graphical method.

DPP-IV inhibitory activity

DPP-IV inhibitory activity of Diprotin

DPP-IV inhibitory assay was performed according to the method of Al-masri, *et al.*,[23] with slight modifications. Diprotin A was diluted to various concentrations (0.5, 1.0, 2.0, 4.0, 8.0 µg/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. 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 inhibitory assay of *D. gangeticum*

Various concentration (100, 200, 400, 800, 1000 µg/ml) of aqueous extract of *D. gangeticum* were prepared and the assay was performed in triplicates according to standardized procedure of Diprotin A. The DPP-IV inhibitory activity was calculated as follow:

% inhibition = $Abs_{control} - Abs_{extract}$ X 100 / $Abs_{control}$

The IC_{50} value was defined as the concentration of DPP-IV inhibitor that

inhibits 50% of DPP-IV activity and were calculated by graphical method.

RESULTS

The present study demonstrated the inhibitory effect of aqueous extract of *D. gangeticum* on alpha glucosidase and DPP-IV enzymes.

α-glucosidase inhibitory activity of acarbose and aqueous extract of *D. gangeticum*

α-glucosidase inhibitory activity (IC_{50}) of acarbose was found to be 4 mM and the maximum percentage inhibition exhibited by acarbose was found to be at 25 mM (75.63%) (Figure: 2). The *in-vitro* alpha glucosidase inhibitory studies demonstrated that aqueous extract of *D. gangeticum* had dose dependent activity. The extract showed maximum percentage (70.247%) inhibition at dose of 1500 µg/ml, with an IC_{50} value of 950 µg/ml (Figure: 3).

DPP-IV inhibitory activity of Diprotin and aqueous extract of *D. gangeticum*

The results of the study showed that aqueous extract of *D. gangeticum* exhibited good inhibitory activity on DPP-IV enzyme. The percentage inhibition at various concentrations (100, 200, 400, 800 and 1000 µg/ml) of aqueous extract of *D. gangeticum* showed a concentration dependent increase in percentage inhibition (21.78%, 42.59%, 59.93%, 69.39% and 73.21%). The maximum percentage inhibition exhibited by aqueous extract of *D. gangeticum* was found to be 73.21% at 1000 µg/ml with an IC_{50} value of 255.5 µg/ml (Figure: 4). Similarly dose dependent percentage inhibition exhibited by various concentrations (0.5, 1.0, 2.0, 4.0, 8.0 and 10 µg/ml) of standard drug (Diprotin) were found to be 20.2%, 32.6%, 55.63%, 69.9% and 78.3% with an IC_{50} value of 1.52 µg/ml. Maximum inhibition was found to be at 10 µg/ml (78.3%) (Figure: 5).

DISCUSSION

In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels, which is called postprandial hyperglycemia or "hyper-glycemic spikes". Recently, a growing body of evidence suggests that postprandial hyperglycemia is an important and independent risk factor for atherosclerosis and some other diabetic complications with great effects than that of fasting hyperglycemia alone. Effective management of postprandial hyperglycemia therefore involves not only the maintenance of normal blood glucose levels after meal but also the prevention of many other diabetic complications. In this realm, the α-glucosidase and DPP-IV inhibitors attract most interest. α-Glucosidase plays a major role in the management of Type II diabetes by delaying carbohydrate metabolism[24] and DPP-IV inhibitors prolongs the half life of GLP-1, an insulinotropic hormone that control blood sugar levels by enhancing insulin secretion in a glucose-dependent manner, inhibiting postprandial glucagon secretion, delaying gastric emptying and stimulating growth of β-cells[25].

The results indicated that aqueous extract of *D. gangeticum* had α-glucosidase inhibitory activity with an IC_{50} value of 950 µg/ml. α-glucosidase inhibitors delay the action of alpha-glucosidases to break complex carbohydrates in to simple sugars, thereby lowering the absorption of glucose. These inhibitors play a vital role in reducing the post-prandial hyperglycemia. As a consequence of their pharmacological action, α-glucosidase inhibitors also cause a concomitant decrease in post-prandial plasma insulin and gastric inhibitory polypeptide (GIP) and a rise in late post-prandial plasma glucagon-like peptide-1 (GLP-1) levels. In individuals with normal or impaired glucose tolerance with hyper-insulinemia, α-

glucosidase inhibitors decrease hyper-insulinemia and improve insulin sensitivity[26]. The results of the present investigation demonstrated that *D. gangeticum* exhibited good inhibitory effect on DPP-IV enzyme with an IC_{50} value of 255.5 µg/ml. The another new approach in management of Type-II diabetes mellitus is based upon the effects of incretin hormones; Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), gastrointestinal hormones released from β-cells of the islets of Langerhans into the bloodstream primarily in response to meal ingestion. They enhance meal-induced insulin secretion and play an important role in maintenance of normal glucose homeostasis by a process termed as incretin effect. GLP-1 has also shown to suppress glucagon secretion, slow gastric emptying, reduce food intake and body weight. In Type-II diabetic patients, reduced incretin effect combined with constant decline in pancreatic β and α-cell function leads to progressive loss of glycemic control. This decline in β and α-cell function is evident as progressive loss of glucose-dependent insulin release and as a progression to unregulated glucagon production, respectively[27]. As described earlier the role of GLP-1 and GIP in glucose regulation is limited because of their short half life, since they are rapidly degraded and inactivated by the enzyme dipeptidyl peptidase 4 (DPP-4), resulting in loss of their insulinotropic activity. Inhibition of DPP-IV enzyme decrease the inactivation of GLP-1, thereby increasing its concentration as well as its duration of action on target tissue, thus making DPP-IV inhibitors a promising target for treatment of Type-II diabetes[28]. Thus on the basis of observed results, it can be concluded that possible mechanisms for antidiabetic activity of *D. gangeticum* in Type-II diabetic animal is their inhibitory effect on alpha glucosidase and DPP-IV enzyme.

CONCLUSION

In conclusion, the results of the study provide scientific support for the use of *D. gangeticum* in the traditional system of medicine for the treatment of Type-II diabetes. This study should help to explain the pharmacological mechanism of action and also help in the development of medicinal preparation for the treatment of Type-II diabetes.

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Figure 1. Plant of *Desmodium gangeticum*

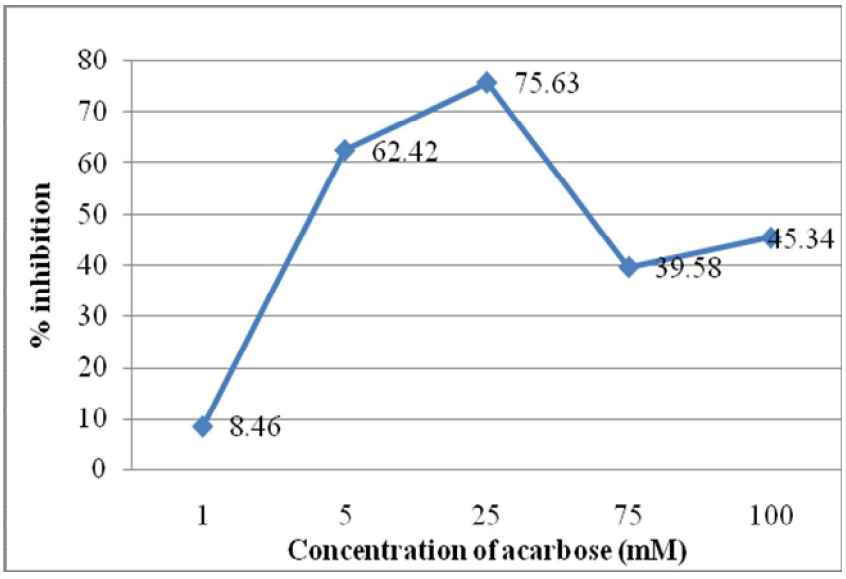

Figure 2. α-D glucosidase inhibitory activity of acarbose

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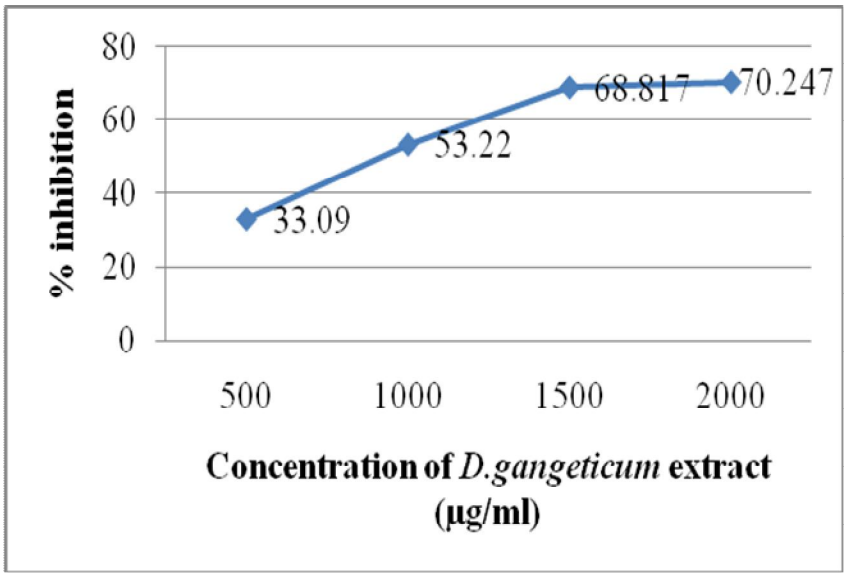

Figure 3. α-D glucosidase inhibitory activity of aqueous extract of *D. gangeticum*

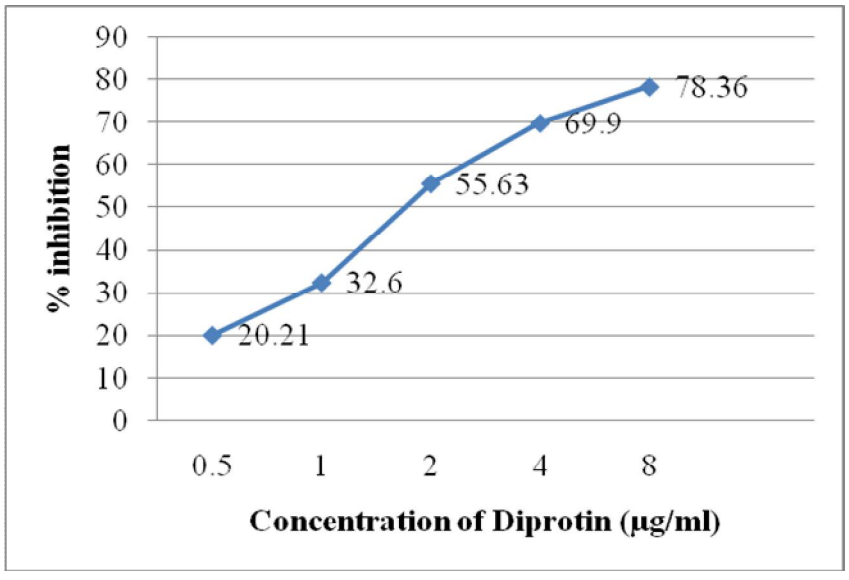

Figure 4. 4DPP-IV inhibitory activity of Diprotin

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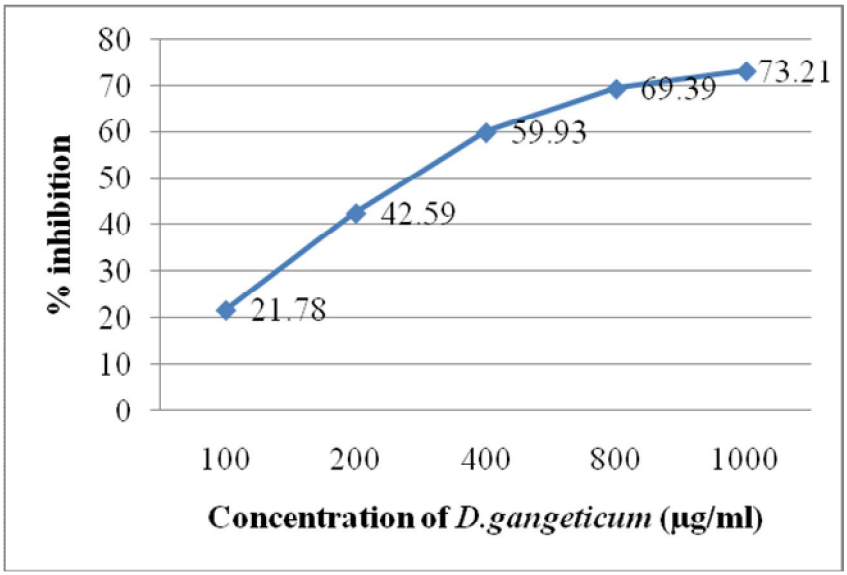

Figure 5. DPP-IV inhibitory activity of aqueous extract of *D. gangeticum*