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In Vitro Antidiabetic and Hypolipidemic Activity of Selected Medicinal Plants

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Abstract

Diabetes mellitus is one among the fundamental ailments prevailing around the world. New helpful methodologies are being researched to direct postprandial glucose levels because of serious symptoms of commercially available anti-diabetic drugs. Alpha-amylase and glucosidase are responsible for postprandial glucose levels in this manner, distinctive plant extracts with alpha-amylase and glucosidase inhibitory action are being explored that may diminish postprandial blood glucose levels, hence being an intriguing and novel remedial focus for diabetes mellitus treatment. Hindrance of gastrointestinal starch and lipid digesting enzymes has been mainstream methodologies to tackle with the diabetes. In the present investigation we have assessed in-vitro α-amylase, α-glucosidase, DPP4 and pancreatic lipase inhibition potential of some plants.

Keywords

Diabetes, α-amylase, α-glucosidase, pancreatic lipase, postprandial hyperglycemia, DPP4 inhibiton.

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INTRODUCTION

Inappropriate glucose digestion influences cell balance of glucose and lipid digestion amid beginning and improvement of Type 2 diabetes [1]. Deregulation of these digestion systems prompt postprandial hyperglycemia and later cause noninsulin-dependent type 2 diabetes [2]. Diabetes mellitus (DM) is a perpetual infection caused by acquired or procured lack in insulin discharge and by diminished affectability of the organs to discharged insulin [3]. Type 2 diabetes what's more, non-alcoholic fatty liver disease (NAFLD) have insulin obstruction as one of their side effects [4] and instinctive fat has been accounted for from different investigations as a noteworthy hazard factor for insulin opposition and type 2 diabetes [5]. The impairment of insulin responsiveness had been accounted for to be started by lipids and their subsidiaries. This advances the unsteadiness of plaque in the arterial wall and furthermore contributes to inflammation in liver – a condition entirely connected to obesity and fatty liver malady [6].

The end products of α-amylase hydrolysis of starch are maltose, maltotriose, a-dextrins and some

glucose. These items are hydrolysed into their segment monosaccharides by chemicals present on the brush fringe of the little intestinal cells for the most part called α-glucosidases which are maltase, sucrase, isomaltase and lactase [7-9]. This causes a sudden ascent in blood glucose level (hyperglycemia) which is a genuine complication related with type 2 diabetes. Pancreatic, endothelial, hepatic, lipoprotein lipases are individuals from the human lipase super family and have auxiliary similitude. Pancreatic acinar cells emit pancreatic lipase (triacylglycerol acyl hydrolase EC 3.1.1.3), a vital compound of pancreatic juice in charge of assimilation of dietary triglycerides in the small intestine and produces postprandial hyperglycemia. Additionally, late examinations likewise proposes that postprandial hyperglycemia, notwithstanding direct hazard factors for heart illnesses, likewise mediate a role in insulin opposition and glucose resistance [10-12]. These variables are the segments of metabolic disorder, (the term used to constellate hyperglycemia, raised triglycerides, diminished high density cholesterol and expanded blood pressure) [13].

It is realized that eating nourishment leads to the arrival of numerous hormones that control gut motility, the release of gastric and pancreatic enzymes, the withdrawal of the gallbladder, and the assimilation of different nutrients. A few hormones encourage the procedure of glucose evacuation by stimulating the release of insulin from the pancreas. The two principle hormones engaged with this endocrine signalling from the gut are glucagon-like peptide-1 (GLP-1) and glucose-dependentinsulinotropic polypeptide (GIP) [14]. GLP-1 assumes a vital role in regulation of blood glucose level due to their biological activities, for example, stimulating the release of insulin, expanding β-cell mass, restricting the release of glucagon, decreasing the rate of gastric emptying and prompting satiety. Be that as it may, GLP-1 is quickly metabolized by the enzyme called dipeptidyl peptidase IV (DPP-IV) into dormant form. Thusly, the GLP-1 has a short half life, around for 1– 2 min. Inhibition of DPP-IV keeps up the level of GLP-1 and maintains its half life. DPP-IV inhibitor can possibly be a novel, effective and significant specialist to treat type 2 diabetes mellitus. The use of DPP-IV inhibitor has fewer side effects like hypoglycemia, increased body weight and GIT issue [15].

Controlling postprandial hyperglycemia what's more, triglyceridemia is in this manner essential focus to avoid cardiovascular disorders, obesity and diabetes related inconveniences. In this way, inhibition of these enzymes can diminish the postprandial hyperglycemia and could be a key strategy in the managements of diabetes mellitus [16]. Since most recent couple of years, there have been a nonstop interests in experimental investigation of characteristic inhibitors for the α-glucosidase and α-amylase inhibitors, the two enzyme associated with carbohydrate digestion, and in pancreatic lipase for controlling lipid absorption; and a few surveys have gathered a few of such constituents [17-19]. As of now accessible medications for the the management of postprandial hyperglycemia or triglyceridemia are related with risk factors [20-21] so there is need for noval, more secure and effective therapeutic agents.

MATERIALS AND METHODS

Chemicals

α-glucosidase, pancreatic lipase, acarbose, orlistat, p-nitrophenylbutyrate were purchased from Sigma Aldrich. α-amylase from Aspergillus oryzae (Himedia), Starch (Himedia), pancreatic lipase (Sigma aldrich), p-nitropheny-α-D-glucopyranosyl (Himedia), acarbose (Sigma aldrich), orlistat (Sigma aldrich), p-nitrophenylbutyrate (Sigma Aldrich), Gly-Pro p-nitroanilide toluene sulfonate salt (Sigma aldrich), Sitagliptin (Sigma aldrich), Dimethylsulphoxide DMSO (Himedia), Sodium dihydrogen phosphate (Himedia), Disodium hydrogen phosphate (Himedia), Potassium dihydrogen phosphate (Himedia), Dipotassium hydrogen phosphate (Himedia), Sodium potassium tartrate (Himedia), 2-4 Dinitrophenyl salicylic acid (Himedia), 96 well microplates (Himedia), microplate reader (iMark Microplate Reader S/N 17766). Other reagents and chemicals used were of HPLC grade.

Plant Material

Casuarina equisetifolia leaves, *Emblica officinalis* fruit, *Murrayakoenigii* leaves and *Tinospora cordifolia* bark were collected from Kurukshetra University, Kurukshetra, Haryana, India. The collected plant material was dried in shade and pulverised. Hydro alcoholic extract was prepared for each plant material by maceration process. The concentrated extracts were obtained by using rotary evaporator and stored in deep freeze for further use. Stock solutions were prepared in 20% DMSO.

ENZYME INHIBITION ASSAY

α-glucosidase inhibitory activity [22]

100mM of phosphate buffer saline (ph 6.9) was used to prepare solutions. 95µL of phosphate buffer saline was added to 96 wells microplate. After this 25µL of α-glucosidase (0.5U/ml) and 30 µL of hydroalcoholic extract (100µg/ml) or acarbose (100µg/ml) were added to the mixture and placed in incubater at 37°C

for 20 min. 50 µL of p-nitrophenyl-α-D-glucopyranoside (5mM) was added after incubation of 20 min and also further incubated for 45 min at same temperature. Absorbance was taken at 415 nm using iMark Micro plate Reader. The readings were taken in triplicate and expressed as mean ±SEM. The percentage inhibition was calculated using given formula

Percentage inhibition= Ac- [As– A0]/ Ac x 100

Ac=Absorbance of control; As=Absorbance of sample with enzyme; A0=Absorbance of sample without enzyme

α-amylase inhibition assay [23-24]

Hydroalcoholic extract (100 µg/ml) and acarbose (100 µg/ml) and 100 µL of α-amylase (0.5 mg/mL) was prepared in 0.02M sodium phosphate buffer (pH 6.9 containing 0.006 mol/L of NaCl) and preincubated at 25°C for 15 minutes. After preincubation, 100 µLof 1% starch solution in sodium phosphate buffer (in 0.02 M) was added to the reacting mixture and further incubated at 25°C for 30 min. 200 µL of dinitrosalicylic acid (DNSA) was added to stop the reaction and incubated in a boiling water bath for 10 min, and then cooled to room temperature. The reaction mixture was then diluted to 1 mL by adding distilled water. Absorbance was measured at 595 nm using iMark Micro plate Reader. The readings we taken in triplicate and α-amylase inhibitory activity was calculated as percentage inhibition. The inhibitory activity was calculated as Percentage inhibition= Ac- [As– A0]/ Ac x 100

Ac=Absorbance of control; As=Absorbance of sample with enzyme; A0=Absorbance of sample without enzyme

DPP4 Inhibition assay [25]

The assay was conducted according to procedure adopted by Yarizade et al with some modifications. Gly-Pro p-nitroanilide toluene sulfonate salt was used as substrate and sitagliptin as standard DPP4 inhibitor. Briefly the assay mixture contained 75 µL Tris buffer, 50 µL extract or standard drug and 25 µL DPP4 enzyme and incubated for 15 min at 37^0C. The reaction was started by adding 50 µL of substrate (Gly-Pro p-nitroanilide toluene sulfonate salt) and stopped by adding sodium acetate after 1 hour. The absorbance was taken at 415 nm using iMark Micro plate Reader and percentage inhibition was calculated. The results obtained were compared with negative control i.e no inhibitors.

Pancreatic lipase inhibition assay [26]

All solutions were prepared in 0.1 M potassium phosphate buffer (pH 7.2).p-nitrophenylbutyrate was used as a substrate.95 µlof phosphate buffer, 25 µl of porcine pancreatic lipase extracts (1 mg/mL), 30 µLof hydroalcoholicextracts (100 µg/mL) or 30 µL orlistat (100 µg/mL) were added in 96 well micro plate and preincubated for 30 minutes at 37 °C.50 µL p-nitro phenyl butyrate (10 mM) was added to reaction mixture to start the reaction and incubated at 37 °C for 40 minutes. Amount of p-nitrophenol released in the reaction was measured at 415 nm using iMark Micro plate Reader. The control represented 100% enzyme activity and did not contain any plant extract. The readings were taken in triplicate and results were expressed as mean ± SEM. The percentage inhibition was calculated as

Percentage inhibition= Ac- [As– A0]/ Ac x 100

Ac=Absorbance of control; As=Absorbance of sample with enzyme; A0=Absorbance of sample without enzyme

RESULT AND DISCUSSION

For preliminary study, hydroalcoholic extract of the collected parts was prepared for each plant. A comparative study was conducted for different hydroalcoholic extracts to the standard inhibitors of respective enzymes. A uniform concentration of 100 µg/ml was selected for each extract as well as for the standard compound and evaluated. The percentage inhibition potential of hydroalcoholic extract of collected plant materials is shown in Table 1.

As shown in Table 1 hydroalcoholic extract of *Casuarina equisetifolia* is most effective in glucosidase inhibition and effect is nearly same as produced by standard drug acarbose. In case of α-amylase inhibition hydroalcoholic extract of *Casuarina equisetifolia* is most effective among selected plants. However hydroalcoholic extract of *Murraya koenigii* also showed potent α-amylase inhibition. As far as pancreatic lipase inhibition assay is concerned hydroalcoholic extract of *Murraya koenigii* was found to be most effective. *Casuarina equisetifolia* was found to be most effective DPP4 inhibitor and effect is near to the standard drug effect. In the present study plants contain polyphenols that inhibit the enzymeα-amylase and α-glucosidase and also facilitate insulin response and enhances secretion of glucose dependent insulinotropic polypeptide and glucagon like GLP-1. α-amylase, α-glucosidase, DPP4 inhibitors and pancreatic lipase are well known targets for the management of postprandial hyperglycemia and triglyceridemia which are parts of metabolic disorder, also, explicitly, in the management of the diabetes and obesity; accordingly, the effective plants referenced in the present study can be utilized for further studies.

Table 1: Results of percentage enzyme inhibition assay

Sr. No	Plant	α-glucosidase inhibition	α-amylase inhibition	Pancreatic lipase inhibition	DPP4 Inhibition
1	*Casuarina equisetifolia*	79.27±0.34	69.85±1.51	53.82±0.42	68.75±1.23
2	*Emblica officinalis*	40.89±1.21	27.2±0.62	34.68±0.17	49.99±0.62
3	*Murrayakoenigii*	71.26±0.25	55.57±0.56	61.21±0.19	65.39±0.17
4	*Tinosporacordifolia*	42.21±0.48	29.85±1.51	43.82±0.42	39.23±0.24
5.	Standard (Acarbose)	79.23±0.22	79.23±0.22	-	-
6.	Standard (Orlistat)	-	-	60.45±0.67	-
7.	Standard (Sitagliptin)	-	-	-	72.35±0.21

All readings were taken in triplicate and represented as % ± SEM.

CONCLUSION

In the present study a comparison was made for enzyme inhibition assays for different plant extracts. In the present study *Casuarina equisetifolia* and *Murraya koenigii* were found to be most effective and can be selected for further studies related to treatment and management of diabetes.

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