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Management of metabolic syndrome by some herbs ethnic to western Himalayan region of Himachal Pradesh

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Abstract
Metabolic syndrome along with diabetes and obesity increase individual susceptibility for myocardial infarction. Postprandial hyperglycemia and triglyceridemia have been contemplated as risk factor for cardiovascular disorders in diabetic, obese and metabolic syndrome individuals. Inhibition of gastrointestinal carbohydrate and lipid digesting enzymes has been popular strategies for their management and has increased the interest identification of novel, effective and safer agents. In the present study we have evaluated *in-vitro* α-amylase, α-glucosidase, and pancreatic lipase inhibition potential some novel medicinal herbs of Western Himalayan region ethnic to Hilly region of Himachal Pradesh. Present study has identified some potential herbs for the further study.

Keywords: α-glucosidase, α-amylase, postprandial hyperglycemia, medicinal plants

1. Introduction
Control over postprandial hyperglycemia has been strategy for management of Diabetes mellitus, and similarly the control over postprandial triglyceride level in countering the obesity [1, 2]. Hyperglycemia occupy center stage of several of diabetes related micro-vascular disorders which include renal failure, blindness, diabetes-accelerated atherosclerosis, nerve damage and risk of myocardial infarction [3]. Moreover, recent studies also suggests that postprandial hyperglycemia, in addition to direct risk factors for heart diseases, also intervene in insulin resistance and glucose tolerance [4-6]. These factors are the components of metabolic syndrome, (the term used to constellate hyperglycemia, elevated triglycerides, decreased high-density cholesterol and increased blood pressure) [7]. Controlling postprandial hyperglycemia and triglyceridemia is therefore primary target to prevent cardiovascular disorders, obesity and diabetes related complications. Inhibition of intestinal digestive enzyme such as α-amylase, α-glucosidase and pancreatic lipase are suitable targets for controlling the postprandial glucose and triglyceride levels.
Since last few years, there have been a continuous interests in experimental exploration of natural inhibitors for the α-glucosidase and α-amylase inhibitors, the two enzymes involved in carbohydrate digestion, and in pancreatic lipase for regulating lipid digestion; and several reviews have compiled several of such agents [8-10]. Currently available drugs for the management of postprandial hyperglycemia or triglyceridemia are associated with side effects [11] so there is research for newer, safer and effective agents.
In the present study, medicinal herbs ethnic to hilly regions of Himachal Pradesh were taken for preliminary investigation of their antidiabetic and antiobesity effects in terms of their α-amylase, α-glucosidase and pancreatic lipase inhibition activities.

2. Materials and Methods
2.1 Chemicals
α-glucosidase (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), 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 were of HPLC grade or better.

2.2 Plant Material

Plant were collected from the Matiana region (Latitude 31°7" and longitude 77° 21"), Distt. Shimla, Himachal Pradesh in the month of April-July, 2015. The collected herbs were dried in shade for 25 days at room temperature. The dried herbs were pulverized and extracted with methanol (1:4 w/v) by simple maceration process for 4 hours at 60 °C. The extracts were concentrated using vacuum evaporator and stored in deep freezer (-20 °C) till further use. For the assay, stock solutions of the herbs (1:1, w/v) in 20% DMSO were prepared and for testing 100 µg/ml concentration of plant extract was prepared in respected buffers in triplicates.

2.3 Enzyme inhibition studies

2.3.1 α-amylase inhibition assay

The α-amylase inhibitory activity was determined according to the methods followed in earlier literatures [12, 13]. Methanolic extract (100 µg/ml) of collected parts of the herbs or acarbose (100 µg/ml) and 100 µL of α-amylase (0.5 mg/mL) prepared in 0.02M sodium phosphate buffer (pH 6.9 containing 0.006 mol/L of NaCl) were preincubated at 25 °C for 15 minutes. After preincubation, 100 µL of 1% starch solution in sodium phosphate buffer (in 0.02 M) was added to the reacting mixture. Thereafter, the reaction mixture was incubated at 25 °C for 30 min and finally the reaction was stopped by adding 200 µL of dinitrosalicylic acid (DNSA) 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. 200 µL of each extract, standard and control and negative control were taken for absorbance in 96 holes microplates which was then measured at 595 nm using iMark Microplate 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= A_c- [A_s– A_0]/ A_c x 100

A_c=Absorbance of control; A_s= Absorbance of sample with enzyme; A_0= Absorbance of sample without enzyme

2.3.2 α-glucosidase inhibition assay

For the assay, a procedure reported by Zawawi *et al*. was followed with some modifications [14]. All the solutions were made in 100 mM of phosphate buffer saline (pH 6.9) which acted as a solvent. 95 µL of phosphate buffer saline was added to 96 wells microplate followed by the addition of 25 µL of α-glucosidase (0.5 U/ml). Then 30 µL of methanolic extracts (100 µg/ml) or acarbose (100 µg/ml) was added to the mixture and incubated for 20 minutes in an incubator at 37 °C temperature. After incubation, 50 µL of *p*-nitrophenyl-α-D-glucopyranoside (5mM) was added and further incubated for 45 minutes at 37 °C. After incubation absorbance was taken at 415 nm in iMark Microplate Reader. Enzyme with substrate was positive control. The control represented 100% enzyme activity and did not contain any plant extract. In order to eliminate the absorbance produced by plant extract, appropriate extract controls were also incubated which contain all the component of reaction mixture except the enzyme. The reading were taken in triplicate and results were expressed as mean ± SEM. The percentage inhibition was calculated as

Percentage inhibition= A_c- [A_s– A_0]/ A_c x 100

A_c=Absorbance of control; A_s= Absorbance of sample with enzyme; A_0= Absorbance of sample without enzyme

2.4 Pancreatic lipase inhibition

The method used for measuring the pancreatic lipase activity has been adopted from Roh *et al* [15] with some modifications. Porcine pancreatic lipase inhibitory activity determined by using p-nitrophenyl butyrate as a substrate and using 0.1 M potassium phosphate buffer (pH 7.2, 0.1% tween 80). To 95 µl of phosphate buffer in 96 holes microplate was added 25 µl of porcine pancreatic lipase extracts (1 mg/mL) and then 30 µL of extracts (100 µg/mL) or 30 µL orlistat (100 µg/mL) and pre-incubated for 30 minutes at 37 °C. After the pre-incubation the reaction was started by adding 50 µL *p*-nitrophenyl butyrate (10 mM). After incubation at 37 °C for 40 minutes, amount of *p*-nitrophenol released in the reaction was measured at 415 nm using iMark Microplate Reader. The control represented 100% enzyme activity and did not contain any plant extract. In order to eliminate the absorbance produced by plant extract, appropriate extract controls were also incubated which contain all the component of reaction mixture except the enzyme. The readings were taken in triplicate and results were expressed as mean ± SEM. The percentage inhibition was calculated as

Percentage inhibition= A_c- [A_s– A_0]/ A_c x 100

A_c=Absorbance of control; A_s= Absorbance of sample with enzyme; A_0= Absorbance of sample without enzyme

3. Results and Discussions

Gastrointestinal enzyme inhibition have been, specifically those involved in carbohydrate and fat digestion delay the rate of digestion and therefore the absorption of digested food in the form of glucose and triglycerides. In the study, medicinal herbs ethnic to hilly regions of Himachal Pradesh were taken for preliminary investigation as shown in Table 1.

Table 1: Different herbs and their parts collected for the study

S. No	*Plant Name*	Family	Parts collected
1	*Rubus ellipticus*	Rosaceae	Leaves
2	*Fragaria nubicola*	Rosaceae	Whole plant
3	*Euphorbia royleana*	Euphorbiaceae	stem
4	*Podophyllum hexandrum*	Berberidaceae	Leaves and stem
5	*Daphne Papyraceae*	Thymelaeaceae	Leaves and stem
6	*Geranium wallichianum*	Geraniaceae	Aerial parts
7	*Valeriana wallichii*	Valerianaceae	Aerial parts
8	*Woodfordia fruticosa*	Lythraceae	Leaves
9	*Erigeron bellidioides*	Asteraceae	Aerial parts (leaves, stem and flowers)
10	*Taxus wallichiana*	Taxaceae	Leaves

For preliminary study, methanolic extract of the collected parts was prepared for each herb. The study was designed for comparative study of different methanolic extracts to the standard inhibitors of respective enzymes. A uniform concentration of 100µg/ml was selected for each extract as well as for the standard compound and evaluated. The percentage inhibition potential of methanolic extract of different herbs is shown in Table 2. As evident in the table the

methanolic extract prepared from leaves of *Rubus ellipticus* and whole plant of *Fragaria nubicola* were most effective in α-amylase inhibition activity which was even better than the standard compound acarbose. Moreover, methanolic extract prepared from whole herb *Fragaria nubicola* along with methanolic extract of aerial parts of *Geranium wallichianum* has shown very good α-glucosidase inhibition activity. The observations suggest for the presence of potent compounds that inhibit these carbohydrate digesting enzymes. As far as pancreatic lipase inhibition activity is concerned methanolic extract prepared from the aerial parts of *Geranium wallichianum* was most potent. In addition to *Geranium wallichianum* other herbs such as *Rubus ellipticus, Fragaria nubicola*, and *Taxus wallichiana* are effective in inhibition of pancreatic lipase.

α-amylase, α-glucosidase and pancreatic lipase are popular targets for the management of postprandial hyperglycemia and triglyceridemia which are components of metabolic syndrome, and specifically, in the management of the diabetes and obesity; therefore the potent herbs mentioned in the present study can be used for further studies.

Table 2: Percentage Enzyme inhibition activity of some plants collected from hilly areas of Shimla District of Himachal Pradesh

S. No	Plant	*α- glucosidase inhibition (%)± SEM at the conc. of 100 µg/ml.	*α-amylase inhibition (%) ± SEM at the conc. of 100 µg/ml.	*Pancreatic Lipase inhibition (%)± SEM at the conc. of 100 µg/ml.
1	*Rubus ellipticus*	83.16± 0.12	56.29 ± 0.15	46.30 ± 0.32
2	*Fragaria nubicola*	89.14± 0.24	72.40 ± 0.59	44.30 ± 0.65
3	*Euphorbia royleana*	31.12± 1.23	11.14 ± 1.43	29.46 ±0.33
4	*Podophyllum hexandrum*	59.69± 0.69	22.40 ± 0.46	12.30 ± 1.23
5	*Daphne papyraceae*	59.69± 1.29	36.20 ± 0.43	9.59 ± 0.45
6	*Geranium wallichianum*	65.81± 0.54	72.89 ± 0.46	52.80 ± 1.23
7	*Indian Valeraiana*	60.20± 0.22	42.20 ± 1.32	30.40 ± 0.09
8	*Woodfordia fruticosa*	58.20± 0.69	34.89 ± 1.67	26.30 ± 0.86
9	*Erigeron bellidioides*	36.22± 0.22	19.30 ± 2.34	29.60 ± 0.34
10	*Taxus wallichiana*	66.32± 0.54	62.79 ±.0.23	42.27 ± 0.54
	Standard (Acarbose)	79.08 ± 0.67	68.50 ±0.74	-
	Orlistat	-	-	62.45 ± 0.04

* All samples were taken in triplicate and each and for each triplicate three readings were taken.

4. Conclusion

Himalayan region is a rich of medicinal herbs. In the present study several herbs had shown their antidiabetic and antiobesity effects and specifically the *Fragaria nubicola, Geranium wallichianum* and *Rubus ellipticus* were most effective which can be selected for further studies for treatment and management of metabolic syndrome.

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