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INVITRO PANCREATIC LIPASE, ALPHA AMYLASE, ALPHA GLUCOSIDASE INHIBITORY ACTIVITY OF BERGAPTEN

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ABSTRACT

In the present study the furanocoumarin, Bergapten was studied for in-vitro alpha (α)-amylase, alpha (α)-glucosidase and pancreatic lipase inhibitory activities. The aim of this work is to evaluate the inhibitory activities of the phytochemical Bergapten at different concentrations. Diabetes mellitus is a clinical condition characterized by hyperglycemia in which an elevated amount of glucose circulates in the blood plasma. Alpha amylase and alpha glucosidase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus. The present study intends to screen novel pancreatic lipase, alpha amylase and alpha glucosidase inhibitors from natural sources like plants in order to minimize the toxicity and side effects of the inhibitors currently used to control obesity and hyperglycemia. The phytochemical Bergapten exhibited significant α-amylase, α-glucosidase and pancreatic lipase inhibitory activities with an IC50 value 8.54µg/ml, 9.11µg/ml and 7.22µg/ml respectively and well compared with standard acarbose for alpha (α)-amylase and alpha (α)-glucosidase and orlistat for pancreatic lipase inhibitory activities respectively.

KEYWORDS: Bergapten, alpha amylase, alpha glucosidase, pancreatic lipase.

INTRODUCTION

Obesity is a major visible global problem and yet most neglected public health issue. It is a condition where a person has accumulated abnormal or excess body fat that causes risk to health. It is an imbalance between energy intake and expenditure.

World health Organization defines over weight and obesity as abnormal or excessive fay accumulation. It possesses a major risk for serious diet related non-communicable diseases such as coronary heart disease, hypertension, stroke, non-insulin dependent diabetes mellitus, gall bladder disease, dyslipidemia, osteoarthritis and gout and pulmonary disease.

The treatment involves dietary management, Physical activity, exercise, anti-obesity drugs and gastric intestinal surgery. The anti-obesity drugs interfere with the normal body fundamental process and lead to side effects. The anti-obesity drugs have serious adverse reactions. Hence due to high cost and potential serious side effects the natural products are an alternative method to treat obesity. The antihyperlipidemic activity is shown by the plants mainly belonging to the families Leguminosae, Laminaceae, Liliaceae, Rosaceae, Moraceae, Asteraceae, Cucurbitaceae and Araliaceae.[1,2,3]

Diabetes is a chronic disease and a serious metabolic disorder characterised by hyperglycaemia or raised blood sugar. The person is incapable to either produce or utilize insulin. According to statistics 2.8% of the world's population suffer from this disease and it is expected to increase to 5.4% by 2025.[4]

The treatments for control of diabetes include insulin therapy, pharmacotherapy, and diet therapy. are available to control diabetes. The mechanisms include stimulation of insulin secretion, increase of peripheral absorption of glucose, delay in the absorption of carbohydrates from the intestine and reduction of hepatic gluconeogenesis etc.[5,6,7]

The side effects associated with synthetic anti-diabetic drugs include skin problems, weight gain, risk of liver disease, anaemia, hypoglycaemia, abdominal gas, fluid retention, ankle swelling etc.[8] Hence anti diabetic drugs from medicinal plants have been developed which are found to be having lesser side effects.

Human pancreatic lipase is the main enzyme that breaks down dietary fats in the human digestive system. Pancreatic lipase hydrolyzes from triglycerides into glycerol esters, 2-monoacylglycerols, glycerol, and free fatty acids mainly in the intestine. Pancreatic α-amylase

is an endoglucosidase present in pancreatic juice secreted into the intestinal lumen and its main function is to hydrolyze starch into maltose, maltotriose, maltotetraose, maltodextrins, and glucose. The enzyme, α-glucosidase is responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. The inhibitory action on these enzymes lead to a decrease of blood glucose level, because the monosaccharides are the form of carbohydrates which is absorbed through the mucosal border in the small intestine. The inhibition of lipase and α-amylase, α-glucosidase inhibits absorption of dietary fat and glucose from intestines respectively and is used to treat obesity and diabetes.

Coumarins are phenolic compounds widely distributed in the plant kingdom. According to their structure, coumarins are classified as (I) simple coumarins, (II) furanocoumarins, (III) pyranocoumarins, or (IV) pyron-ring substituted coumarins and its hydroxylated, alkoxylated, and alkylated derivatives, along with their glycoside.[9] The core structure of furanocoumarins consists of a furan ring fused with a coumarin molecule. Furanocoumarins are subdivided into linear type, generically known as psoralens, where the furan ring is attached at carbons 6 and 7, such as psoralen and angular type, generically known as angelicins, where the ring is attached to carbons 7 and 8 of the coumarin structure, for example, angelicin.

The published literature has shown that furanocoumarin bioactive are often found in the following plant families: Amaranthaceae, Apiaceae (Umbelliferae), Compositae (Asteraceae), Cyperaceae, Dipsacaceae, Goodeniaceae, Guttiferae (Clusiaceae), Leguminosae (Fabaceae or Papilionaceae), Moraceae, Pittosporaceae, Rosaceae, Rutaceae, Samydaceae, Solanaceae, and Thymelaeaceae.[10,11] Furanocoumarins are found to possess several health-promoting properties that include anti-inflammatory, anti-cancer, anti-obesity, and bone-building effects (Madrigal-Bujaidar et al. 2013).

Bergapten is a natural furocoumarin, also known as 5-methoxypsoralen, and its medicinal value has been paid more and more attention. By sorting out the pharmacological literature of bergapten, we found that bergapten has a wide range of pharmacological effects, including neuroprotection, organ protection, anticancer, antiinflammatory, antimicrobial, and antidiabetes effects.[13] The present study was carried out to investigate the in- *vitro* pancreatic lipase, α-glucosidase and α-amylase inhibitory activities of the phytochemical Bergapten.

MATERIALS AND METHODS

Chemicals and reagents

Porcine pancreatic lipase (PPL), Porcine pancreatic α-amylase (EC 3.2.1.1) (PPA) and α –glucosidase, 3,5-Dinitrosalicylic acid (DNSA color reagent), Soluble starch, p-nitrophenyl- α -D-glucopyranoside (p-NPG), were obtained from SRL Laboratories (Hyderabad, India). Acarbose from Glucobay (Hyderabad, India), sodium potassium tartrate, dimethyl sulfoxide, sodium carbonate (Na_2 CO_3), sodium dihydrogen phosphate, di-sodium hydrogen phosphate and other chemicals are of analytical grade.

Determination of in-*vitro* pancreatic lipase enzyme inhibitory activity of bergapten

The pancreatic lipase in-vitro enzyme inhibition assay is based on the hydrolysis kinetics of an oleate ester of 4-methylumbelliferone (14,15) Porcine pancreas powder (0.5 mg/mL in Tris HCl buffer, pH 8.0) was used as the enzyme source. The various concentrations of the standard and test phytochemical, bergapten were prepared using dimethylsulfoxide (0.1% DMSO). A volume of 250 µL of the various concentrations of bergapten were mixed in 250 µL of pancreatic lipase solution and incubated for 10 min. Then 500 µL of 4-methylumbelliferyl oleate (0.5 mM) was added to each test tube to initiate the enzyme reaction. The amount of 4-methylumbelliferone was measured at 37 °C over 30 min using UV visible spectrophotometer at 360 nm against blank. Orlistat was used as positive control.

Determination of in-*vitro* alpha-amylase enzyme inhibitory activity of Bergapten

The inhibition of α -amylase activity was determined according to the method[16,17] descried in the literature with minor modifications. Stock solution of extract were prepared by dissolving upto 100mg of each extract in 10ml of dimethyl sulfoxide. A total of 250µl of extracts of varying concentrations (1,2,4,8,10µg/ml) was placed in a tube and 250µl of 0.02M sodium phosphate buffer(pH-6.9) containing α-amylase solution (0.5mg/ml) was added. This solution was pre-incubated at 25degree Centigrade for 10min.After which 250µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at particular time intervals and then further incubated at 25 degree Centigrade for 10min.This reaction was terminated by adding 500µldinitro salicylic acid (DNS) reagent. The tubes were incubated in boiling water (5ml) and then cooled to room temperature. The reaction mixture was diluted with 5ml of distilled water and the absorbance was measured at 540nm using UV spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The percentage inhibition was calculated by the formula:

% Inhibition = Absorbance (control) – Absorbance (extract) / Absorbance (control) × 100

Determination of alpha-glucosidase enzyme inhibitory activity of Bergapten

The inhibition of α -glucosidase activity was determined according to the method[21] described in the literature with minor modifications. One mg of α -glucosidase was dissolved in 100 ml of phosphate buffer (pH 6.8). To 100 µl of plant extracts of varying concentrations (1,2, 4, 8, 10, µg/ml), 200 µl α -glucosidase were added and the mixture was incubated at 37ºC for 20 min. To the reaction mixture 100 µl 3mM p –nitrophenyl α -D-

glucopyranoside (*p*-NPG) was added and incubated at 37 ºC for 10 min. The reaction was terminated by the addition of 2ml Na2CO3 0.1M and the α -glucosidase activity was determined spectrophotometrically at 405 nm on spectrophotometer UV-VIS (Shimadzu UV-1800) by measuring the quantity of p-nitrophenol released from *p*-NPG. Acarbose was used as positive control of α -amylase and α -glucosidase inhibitor. The concentration of the extract required to inhibit 50% of α -amylase and α -glucosidase activity under the assay conditions was defined as the IC50 value.

% Inhibition = Absorbance (control) – Absorbance (extract) / Absorbance (control) × 100

3. RESULTS AND DISCUSSION

In-*vitro* alpha-amylase enzyme inhibitory activity of bergapten: The results of the study are presented in Table 1 and Fig. 1. Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides. Among all the test doses, Bergapten has shown remarkable alpha- amylase enzyme inhibition i.e 59.4% at 10 µg/ml concentration and it was comparable with the standard drug acarbose (91.2% percentage inhibition at 10 µg/ml concentration). The IC50 value of the phytochemical, Bergapten and standard (Acarbose) was found to be 8.22 µg/ml 3.683µg/ml respectively.

Table 1: In-*vitro* alpha- amylase enzyme inhibitory activity of Bergapten.

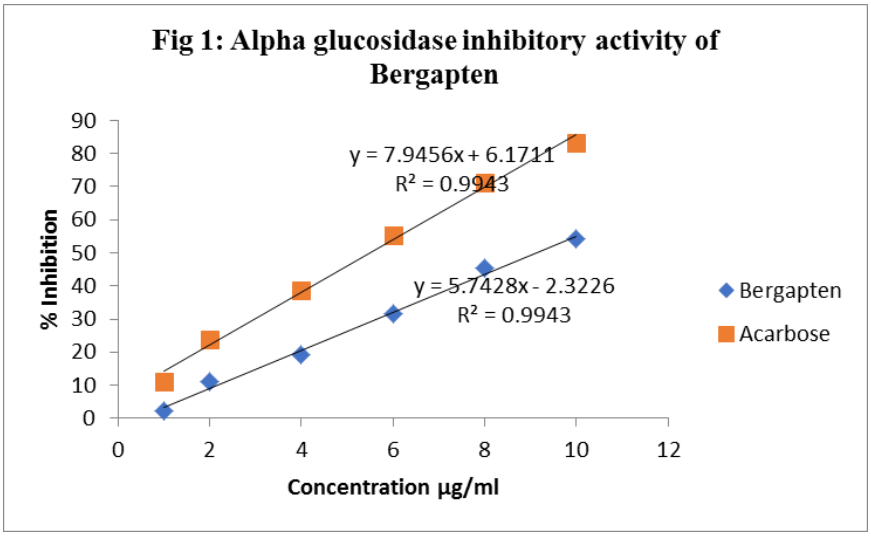
S.No	Name of sample	Concentration (µg/ml)	% Inhibition	IC 50 value (µg/ml)
1.	Bergapten	1	3.4	8.54
		2	12.86	
		4	21.78	
		6	35.7	
		8	49.56	
		10	58.63	
2.	Acarbose	1	14.31	5.21
		2	25.3	
		4	40.16	
		6	57.21	
		8	73.21	
		10	85.64	

In-*vitro* alpha- glucosidase enzyme inhibitory activity of Bergapten

The results of the study are presented in Table 2 and Fig. 2. The glucosidase is a mucosal brush border enzyme of the small intestine and catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet. α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia. The Bergapten was assessed for alpha-glucosidase enzyme inhibitory activity at different concentrations ranging from (1-10 µg/ml) and it exhibited potent α- glucosidase inhibitory activity in a dose dependent manner comparable with that of the standard drug, Acarbose. The IC50 value of the phytochemical (Bergapten) and standard (Acarbose) was found to be 9.11 µg/ml and 5.52 µg/ml respectively.

Table 2: In-*vitro* alpha- glucosidase enzyme inhibitory activity of Bergapten.

S.No	Name of sample	Concentration (µg/ml)	% Inhibition	IC 50 value (µg/ml)
1.	Bergapten	1	2.31	9.11
		2	11.26	
		4	19.34	
		6	31.52	
		8	45.38	
		10	54.28	
2.	Acarbose	1	11.24	5.52
		2	23.65	
		4	38.62	
		6	55.38	
		8	71.26	
		10	83.19	

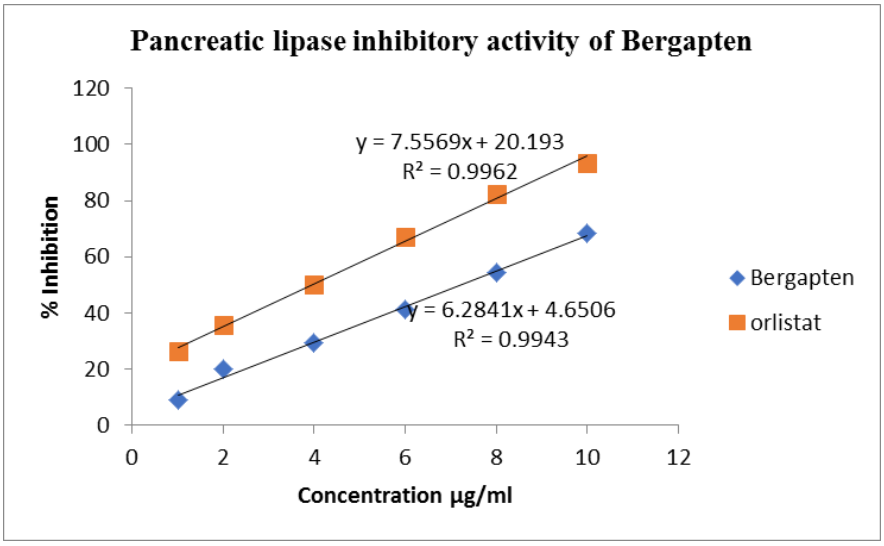


In-*vitro* pancreatic lipase enzyme inhibitory activity of Bergapten

Lipases are secreted by the pancreas when fat is present. The primary role of lipase inhibitors is to decrease the gastrointestinal absorption of fats which in turn decreases the fat intake by the body thus acting as antihyperlipdemic drugs. Bergapten was assessed for pancreatic lipase enzyme inhibitory activity at different concentrations ranging from (1-10 µg/ml) and it exhibited potent pancreatic lipase inhibitory activity in a dose dependent manner comparable with that of the standard drug, Orlistat. The IC50 value of the phytochemical (Bergapten) and standard (Acarbose) was found to be 7.22 µg/ml and 3.94 µg/ml respectively.

Table No 3: In-*vitro* pancreatic lipase enzyme inhibitory activity of Bergapten.

S.No	Name of sample	Concentration (mg/ml)	% Inhibition	IC 50 value (µg/ml)
1.	Bergapten	1	9.23	7.22
		2	20.15	
		4	29.34	
		6	41.27	
		8	54.38	
		10	68.34	
2.	Orlisat	1	26.37	3.94
		2	35.69	
		4	50.32	
		6	67.11	
		8	82.47	
		10	93.46	



5. CONCLUSION

In the present study, the phytochemical, Bergapten showed inhibition of pancreatic lipase which may be used for the treatment of obesity after prior in vivo studies. The drug, also inhibited the action of the enzymes, alpha-amylase and alpha-glucosidase which in turn decreases blood glucose levels, making the drug effective in the management of diabetic complications. Hence, the phytochemical, Bergapten can be used as an adjuvant for the management of obesity and complications associated with diabetes mellitus.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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