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Inhibitory effects of ethanolic extract of *Piper trioicum* on amylase, lipase and α -glucosidase

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Abstract

The aim of the present study was to assess the effects of ethanolic extract of *Piper trioicum* on the amylase, lipase and alpha glucosidase activity *In vitro*. Powder of whole plant of *Piper trioicum* was extracted in ethanol and the extract was assayed for the measurement of inhibitory effects on activities of enzymes. The extracts rich in bioactive phytochemicals showed inhibitory activity on the amylase, lipase and alpha glucosidase, thus suggesting that extract might be useful in the treatment to limit dietary fat and glucose absorption and the accumulation of fat in adipose tissue. The extracts of *Piper trioicum* may be safe, natural and cost effective for reducing fat and glucose absorption.

Keywords: Piper trioicum, amylase, lipase, alpha glucosidase.

Introduction

Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO notes that out of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in many ways that correlated directly with their traditional uses as plant medicines by native culture. Medicinal plants have the advantage of having little or no side effects. Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world. NIDDM (non insulin dependent Diabetes Melitus) has also been associated with an increased risk for premature arteriosclerosis due to vascular disease. An ideal treatment for diabetes would be a drug that not only controls the glycemic level but also prevents the development of arteriosclerosis and other complications of

diabetes.[1] Long before till the use of insulin became common, indigenous remedies were used for the treatment of diabetes mellitus and hyperlipidemia. There has been an increasing demand from patients for the use of natural products with anti diabetic and anti hyperlipidemic activity. This is largely because insulin cannot be used orally and insulin injections are associated with the risk of hepatic and other body functions. The undesirable side effects and contra indications of synthetic drugs, and the fact that they are not suitable for use during pregnancy, have made scientists look towards hypoglycemic agents of plant origin.[2] Many herbs and plant products have been shown to have antihyperglycemic and hyperlipidemic action.[3-4] It had been reported that digestive enzymes such as lipase, amylase and alpha glucosidase is responsible for the digestibility of protein starch & lipid.[5] The membrane-bound intestinal alpha-glucosidases hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine. Alpha-glucosidase inhibitors are saccharides that act as competitive inhibitors of enzymes needed to digest carbohydrates(specifically alpha-glucosidase enzymes in the brush border of the small intestines). Pancreatic alpha-amylase hydrolyze complex starches to oligosaccharides in the lumen of the small intestine. Alpha-glucosidase inhibitors also blocks pancreatic alpha-amylase in addition to inhibiting membrane-bound alpha-glucosidases. A high post prandial blood glucose response is associated with micro and macro cellular complications in diabetes, and is more strongly associated with the risk for cardiovascular disease than are fasting glucose levels potent glucosidase inhibitors such as acarbose and voglibose have already been clinically used for diabetic and obese patients.[6]

α -Amylase is an enzyme of low molecular mass (45000 to 50000). According to the site of action, it is a digestive juice enzyme. α -Amylase catalyzes hydrolysis of the α -1, 4-glycoside linkage in the polysaccharide molecule, forming maltose and glucose from amylose. Beside maltose and glucose, dextrans are also formed from amylopectins and glycogen, because the enzyme does not act on the 1, 6-glycoside linkages. Amylase is mostly synthesized in pancreatic acini, and partly in salivary glands. Lipases (triacylglycerol lipases EC 3.1.1.3) are enzymes which have been classically employed to carry out hydrolysis of triglycerides with concomitant production of free fatty acids. Lipase may also be elevated in chronic pancreatitis but, if severe destruction of the acinar tissue has occurred, serum levels may be below those normally detected. Obstruction of the pancreatic duct caused by a calculus or carcinoma may also result in increased serum lipase levels. Increased serum lipase may also be observed in chronic or acute renal disease, after endoscopic retrograde pancreatography or treatment with opiates.

Merina and etal explained that in diabetic rats, varying degrees of cellular damage was observed in the pancreatic islets. There was a decrease in population of cells with nuclear pyknosis, karyarerrhsis and karyolysis with degranulation and cytolysis. By regenerating pancreas, it can control the lipase elevation.[7] *Piper trioicum* belongs to Piparacae family, distributed in south asian countries. The whole plant is used as rubefacient, diuretic, hepatoprotective and used for diabetes, muscular pains, headache, toothache and internal remedy for cholera in folk medicine; the root is used as diuretic.[8] The present invitro study tested the hypothesis that ethanolic extract of *Piper trioicum* can inhibit the enzymetic activity of amylase, lipase and alpha glucosidase *In vitro*.

Materials and Methods

Preparation of ethanolic plant extracts:

Plant material of *Piper trioicum* was collected from local areas of Talakona, Andhra Pradesh and plant was authenticated by Mr. Madhavachetty, Botanist, S.V.University, Thirupati, Andhra Pradesh. Plant was dried in the shade and ground into uniform powder using milling machine.

The extraction procedures were carried out for about 18 hrs using soxhlet apparatus with 70% ethanol as a solvent. Initially the shade dried plant of *Piper trioicum* was taken in a grinder mixture to obtain a coarse powder and then passed through a 40 mesh[9] The powder (500gms) of plant was defatted with hexane and later extraction procedure was carried out using ethanol. Then residue was collected and used for experiment. The extract of *Piper trioicum* was concentrated to dryness under reduced pressure.

Preliminary phyto chemical screening [10]:

The ethanolic fractions of plant were subjected to qualitative chemical investigation for identification of Phytoconstituents.

In vitro amylase inhibitory activity:

Ethanolic extract of *Piper trioicum* was used in various concentrations (2000 μ l, 1500 μ l, 1000 μ l, 500 μ l). 1ml of substrate was taken in all the test tubes and were kept for incubation at 37⁰C for 5min. After incubation, 100 μ l of Amylase solution was added in to all the tubes then 100 μ l of extract of different dilution was added and it was incubated at 37⁰C for 15 minutes. To the resulting solution, 2,500 μ l of working coloring reagent was added then made up to 10ml with acetate buffer of pH 4.8. The absorbance was measured at 660 nm. The reading was noted.

Calculation:

Amylase activity =

(Absorbance per minute of sample/ Absorbance per minute of Calibrator) x Calibrator value

Absorbance /min of calibrator = 0.040

Value of calibrator = 100 unit/ltr.

Amylase inhibition activity = [100 – ((100 x Amylase activity)/Value of Calibrator)]

In vitro lipase inhibitory activity:

Lipase working reagent and Lipase calibrator (Lipase 450U/L) were obtained from reckon diagnostics private limited. To each 1ml of working reagent taken in to 4 individual test tubes, 0.05 ml of *Piper trioicum* extract of 500, 1000, 1500, 2000 μ l and blank solution (buffer) was added. All test tubes were incubated at room temperature or 37⁰C for 5min., then 0.05ml of calibrator was added to all test tubes and again incubated at room temperature or 37⁰C for 5min.

The rate of decrease in turbidity measured at 340nm is proportional to the lipase activity.

Lipase activity =

(Absorbance per minute of sample/ Absorbance per minute of Calibrator) x Calibrator value

Absorbance /min of calibrator = 0.030

Value of calibrator = 450 unit/ltr.

Lipase inhibition activity = $[100 - (100 \times \text{Lipase activity})/\text{Value of Calibrator}]$

***In vitro* alpha glucosidase inhibitory activity:**

Piper trioicum ethanolic extract was used to investigate the in-vitro inhibitory effect of alpha glucosidase enzymes. After fasting, small intestine of goat between duodenum and cecum(Upper Part) was cut, rinsed with ice-cold saline and homogenated with maleate buffer (pH 6). Small intestine homogenate was used as an enzyme source. The 500 μ l of enzyme & 100 μ l of extract of different concentration and Acarbose (1000mcg/ml) were taken in to different test tubes and pre incubated for 15min, at 37⁰C. Then 500 μ l of 100 mM maltose(2%) as a substrate was added to all the test tubes and incubated for 15min at room temperature and centrifuged. 0.6ml of supernatant liquid was collected from all the test tubes separately and it was mixed with 0.8ml of alkaline CuSO₄ individually The solution was heated in water bath for 8min and cooled. After cooling, phosphomolybdic acid was added to the mixture and made to 10ml with distilled water. Glucose concentration was measured using glucose kit. In case of maltase inhibitory test, maltose was used as a substrate.

Statistical analysis:

All the data was subjected to analysis of variance (ANOVA). The data (mean \pm standard deviation) shown are mean value and the significance differences was compared by using Dennett's Multiple comparison test at the p<0.05 probability level. ANOVA was carried out by using GRACHPAD PRISM version 4.2 software.

Results

The ethanolic extract of *Piper trioicum* was concentrated on water bath to a dry residue and kept in a desiccator. The percentage yield was 14.3% w/w for ethanolic extract of *Piper trioicum*. The Phyto chemical screening and quantitative estimation of the percentage crude yields of extracts studied had shown that the whole plants of *Piper trioicum* was rich in alkaloids, carbohydrates, phenolic compounds, tannins.

The experiment was performed to analyze amylase inhibition activity of ethanolic extract of our plant from 500mcg/ml to 2000mcg/ml. The data was presented in Table No: 1. Analysis of data confirms that amylase inhibition activity was maximum at 1000mcg/ml of *Piper trioicum* compared to 100U/L of standard. In case of *Piper trioicum*, inhibition activity was initially increased up to 1000 mg/ml concentration then it was decreased. According to the experimental results, it was certainly confirmed that the ethanolic extract of plant inhibits the activity of amylase enzymes.

The experiment was performed to analyze lipase inhibition activity of ethanolic extract of our plant by reckon diagnostics from 500mcg/ml to 2000mcg/ml. The data was presented in Table No: 2. Analysis of data confirms that lipase inhibition activity was maximum at 2000mcg/ml of *Piper trioicum* compared to 450U/L of standard. In case of *Piper trioicum*, inhibition activity was gradually increasing with increased concentration. According to the experimental results, it was certainly confirmed that the ethanolic extract of plant inhibits the activity of lipase enzymes.

Table: 1 Amylase activity and inhibitory effects of *Piper trioicum*

Concentration (mcg/ml)	Amylase activity		Amylase inhibitory activity	
	<i>Piper trioicum</i>	Control (100units/L)	<i>Piper trioicum</i>	Control
500	76.22±4.259**		23.78	-
1000	59.83±2.839		40.17	-
1500	68.85±2.459**		31.15	-
2000	77.04±3.756**		22.96	-
0	-	100±3.756**	-	0

The values are mean±SD of 6 values. Means with superscripts (**) within a column are significantly different from each other at $p<0.01$ and Means with superscripts (*) within a column are significantly different from each other at $p<0.05$ as determined by Dennett's Multiple comparison test. F value for PT and PM are 78.71 and 30.2 respectively, df (5, 12).

Table : 2 Lipase activity and inhibitory effects of *Piper trioicum*

Concentration (mcg/ml)	Lipase activity		Lipase inhibitory activity	
	<i>Piper trioicum</i>	Control (450 units/L)	<i>Piper trioicum</i>	Control
500	343.54±8.380*		23.65	-
1000	324.19±8.380**		27.95	-
1500	324.19±8.380**		27.95	-
2000	270.96±8.380**		39.78	-
0	-	450±14.51**	-	0

The values are mean±SD of 6 values. Means with superscripts (**) within a column are significantly different from each other at $p<0.01$ and Means with superscripts (*) within a column are significantly different from each other at $p<0.05$ as determined by Dennett's Multiple comparison test. F value for PT and PM are 88.05 and 90.41 respectively, df (5, 12).

The experiment was performed to analyze alpha glucosidase inhibition activity of ethanolic extract of *Piper trioicum* on goat intestine homogenate from 500mcg/ml to 2000mcg/ml. The data was presented in Table No: 3. Analysis of data confirms that alpha glucosidase inhibition activity was maximum at 500mcg/ml of *Piper trioicum* ($p<0.01$) compared to 1000mcg/ml of standard. In case of *Piper trioicum*, inhibition activity was gradually decreasing with increased concentration. According to the experimental results, it was certainly confirmed that the ethanolic extract of plant inhibits the activity of alpha- glucosidase enzymes such as maltase.

Table 3: *In vitro* effect of alpha glucosidase inhibitory activity

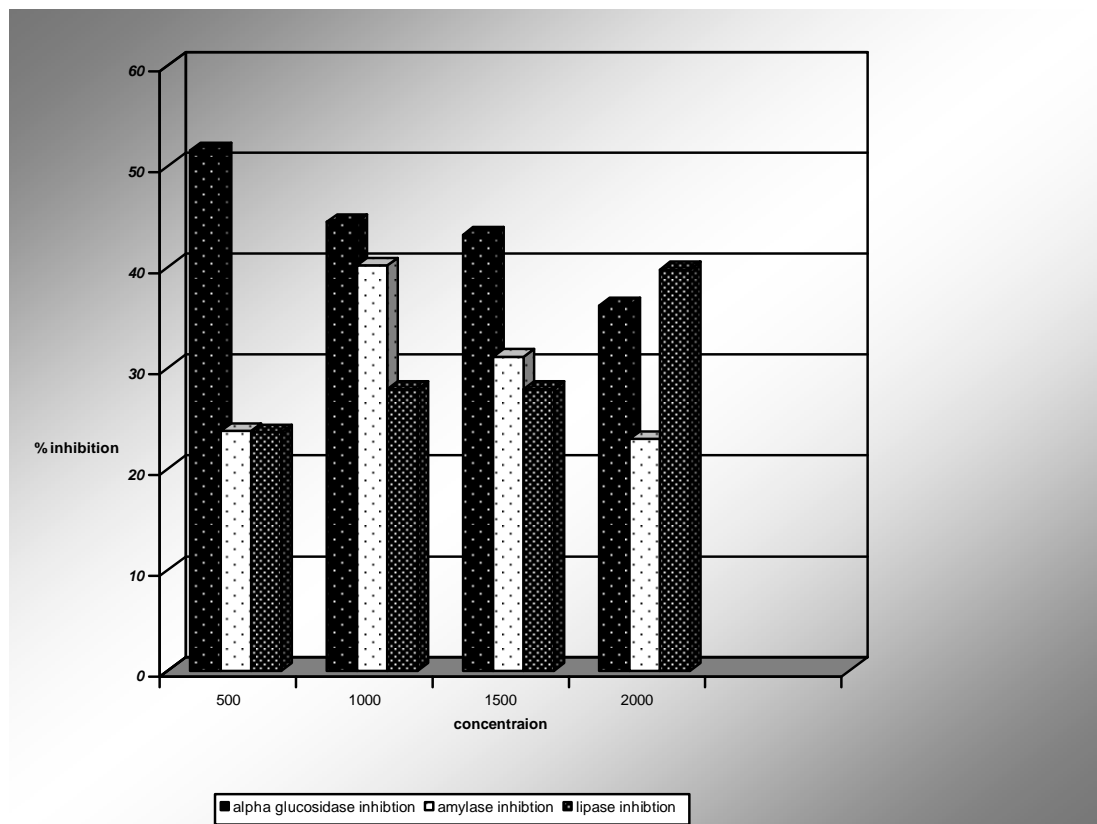
Concentration (mcg/ml)	% inhibition activity	
	<i>Piper trioicum</i>	Standard
500	51.59±0.043**	
1000	44.49±4.493**	51.78±2.615**
1500	43.24±0.370**	
2000	36.18±5.916**	

The values are mean±SD of 6 values. Means with superscripts (**) within a column are significantly different from each other at $p < 0.01$ as determined by Dennett's Multiple comparison test. F value is 90.41, df (5, 12).

Discussion

We tested our extract for inhibitory action against *In vitro* pancreatic lipase & confirmed that extract contain lipase inhibitors that act in a dose dependent manner. Graph I represents the enzyme inhibitory activity of ethanolic extract of *Piper trioicum*.

Graph 1: *In vitro* enzyme inhibitory activity of *Piper trioicum*



Starch which is the predominant ingredient of human food is rapidly degraded in the gastrointestinal tract by salivary and pancreatic α amylase to maltose which is further hydrolyzed by maltase localized in the brush border of small intestine to glucose. Glucose is immediately absorbed leading to hyperglycemia and consequently to hyperinsulinemia. Both phenomena are undesirable in diabetics and in obese patients. Inhibition of the digestion of starch leads to a decrease and a retardation of glucose absorption. In nature, α amylase inhibitors are found in wheat and other grains.[11] Several inhibitors of amylase and α glucosidase have been developed.[12] Animal experiments with high doses of absorbable alpha glucosidase inhibitors indicate that lysosomal storage of glycogen may occur.[13] The result strongly suggests that our extract inhibited the glucose level by inhibiting glucosidase activity and alpha amylase activity. NIDDM has also been associated with an increased risk for premature arteriosclerosis due to increase in triglycerides and LDL levels. An ideal treatment for diabetes would be a drug that not only controls the glycemic level but also prevents the development of arteriosclerosis & other complication of diabetes. The higher lipid levels in diabetic patient are due to increased mobilization of free fatty acids from peripheral despots & also due to lipolysis caused by hormones. According to the above results, our plant leads to inhibition of lipid peroxidation and control of lipolytic hormone like lipase. It had been reported that digestive enzymes such as lipase, amylase and alpha glucosidase were inhibited by tannins in young chicks which decrease the digestibility of protein starch & lipid.[14,15] The mechanism of inhibition on maltase intestinal enzyme by Ethanolic extracts of both plants could be due to polyphenol content. For example, tea polyphenol such as catechin have been found to inhibit glucosidase activity and glucose transport.[16] The tannin (polyphenol) has specific property such as ability to precipitate some proteins. This precipitation is presumed to occur by the formation of hydrogen bond between hydroxyl groups of tannins and the peptide linkage of the protein. As per our study, tannins present in *Piper trioicum* extracts might have significantly precipitated the enzymes such as maltase. The extracts rich in bioactive phytochemicals showed inhibitory activity on the amylase, lipase and alpha glucosidase, thus suggesting that extract might be useful as a treatment to limit dietary fat and glucose absorption and the accumulation of fat in adipose tissue.

By our study we conclude that *Piper trioicum* has significant inhibitory activity against amylase, lipase and alpha glucosidase which might be helpful in preventing of suppressing the progress of various disorders. The extracts of *Piper trioicum* may provide a safe, natural and cost effective for reducing fat and glucose absorption.

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