Alpha-Glucosidase Inhibitory And Hypoglycemic Activities Of \textit{Physalis Minima} Extract.


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\section*{ABSTRACT}
Alpha glucosidase in-vitro inhibitory activity and hypoglycemic effect by oral administration in rats of \textit{Physalis minima} ethanol extracts have been investigated. \textit{Physalis minima} extract showed in-vitro inhibitory activity of intestinal alpha glucosidase enzyme maltase. Analysis of data confirms that alpha glucosidase inhibition activity was maximum at 1000mcg/ml of \textit{Physalis minima}. The purpose of study was to know whether \textit{Physalis minima} extract could reduce intestinal absorption of monosaccharides by inhibiting disaccharide hydrolysis. The post prandial elevation in blood glucose level at 60 and 120 min after administration of maltose with \textit{Physalis minima} extracts (200 mg/kg and 400 mg/kg doses) showed significant suppression compared to control group. These results suggest that the \textit{Physalis minima} extract has potent alpha glucosidase inhibitory activity and would be effective in suppression of elevation in blood glucose after oral administration of maltose to rats.

\section*{INTRODUCTION:}
Intestinal glucosidase enzymes play an important role in carbohydrate digestion and absorption. Therefore an inhibitor of intestinal glucosidase could be expected to retard carbohydrate digestion and absorption. A reasonable way to control these carbohydrate dependent diseases would be to limit intestinal carbohydrate digestion. It has been recognized that alpha glucosidase inhibitors can be used to prevent some disorders such as diabetes, obesity, hyperlipidaemia and hyperlipoproteinaemia (1) and also show anti-HIV activity (2). It is essential for hyperglycemic conditions that the intestinal absorption of dietary carbohydrates be suppressed by inhibiting intestinal glucosidase, which delay the digestion of oligosaccharides and disaccharides to monosaccharide and reduce the rate of glucose absorption, rise in blood glucose levels and insulin response. Research has recently been conducted on glucosidase inhibitors obtained from plant sources which show reduction in postprandial blood glucose concentrations like onion (3), clove (4), tea (5). A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes, and is more strongly associated with the risk for cardiovascular disease than are fasting glucose levels (6). Potent glucosidase inhibitors such as acarbose and voglibose have already been clinically used for diabetic and obese patients.

\textit{Physalis minima} belong to Solanaceae family, distributed in South Asian countries. \textit{Physalis minima} is commonly found on the bunds of the fields, waster lands, around the houses, on roadides, etc., where the soil is porous and rich in organic matter. It is an annual herbaceous plant having a very delicate stem and leaves. The whole plant are bitter, appetizing, tonic, diuretic, laxative, and useful in inflammations, enlargement of the spleen and abdominal troubles (7). Hence, we have conducted this present study to know in vitro inhibitory activity of \textit{Physalis minima} extract on alpha glucosidase enzyme maltase and subsequent in vivo reduction of glucose absorption by inhibiting disaccharide digestion.

\section*{MATERIAL AND METHODS}
Preparation of plant material \textit{Physalis minima} was collected from local areas of Nalgonda, Andhra Pradesh
and authentified by Mr. Madhavachetty, Botanist, S.V.University, Tirupati, Andhra Pradesh. Plant was dried in the shade and ground into uniform powder using milling machine.

*Physalis minima* (500g) was extracted with 70% ethanol in Soxhlet apparatus for 24 hrs. After filtration and evaporation of ethanol, the residue obtained was 5.8%. The phytochemical screening proved that the plant consists of tannin, polyphenols, alkaloids and polysaccharides.

Male Wistar rats (150-200 g) were fed with a standard diet and water *ad libitum*. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27°C and 12 hours light / dark cycle) throughout the experimental period. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute.

**In vitro alpha glucosidase inhibitory activity:**

*Physalis minima* 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 μl of enzyme & 100 μl of extract of different concentration and Acarbose (1000mcg/ml) were taken in to different test tubes and pre incubated for 15min, at 37°C. Then 500 μl 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 (Accucheck). In case of maltase inhibitory test, maltose was used as a substrate. Table 1: represents the values of invitro studies.

**Evaluation of Hypoglycemic activity in Physalis minima fed normal wistar rat.**

Normal Wister rats were randomly divided in to 3 groups (6 rats/group) and were fasted overnight (18hrs). Animals in-group I were treated with tragacanth(1%) as control, group II animals were administered with *Physalis minima* extract 200 mg/kg and group III animals were treated with *Physalis minima* 400 mg/kg orally. Blood samples were taken from the lateral tail vein at 0, 60, 120, 180minutes. The blood glucose concentration was measured by using glucometer and noted. The noted values presents in table 2.

**Table 1: In vitro effect of alpha glucosidase inhibitory activity**

<table>
<thead>
<tr>
<th>Concentration (mcg/ml)</th>
<th>Physalis minima</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>34.31±1.259**</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>50.99±1.984**</td>
<td>51.78±2.615**</td>
</tr>
<tr>
<td>1500</td>
<td>48.08±0.525**</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>48.06±1.959**</td>
<td></td>
</tr>
</tbody>
</table>

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).

**Table 2: Evaluation of hypoglycemic activity in Physalis minima fed normal wistar rat**

<table>
<thead>
<tr>
<th>Group</th>
<th>I (control)</th>
<th>II (200mg/kg)</th>
<th>III (400mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>106.33 ± 0.5773</td>
<td>112.33 ± 1.5274**</td>
<td>112.33 ± 1.52**</td>
</tr>
<tr>
<td>1 hour</td>
<td>103.33 ± 2.0816 (9.792%)</td>
<td>101.33 ± 0.57** (7.718%)</td>
<td>103.66 ± 1.52</td>
</tr>
<tr>
<td>2 hours</td>
<td>107 ± 1 (26.413%)</td>
<td>82.66 ± 2.08** (24.036%)</td>
<td>85.33 ± 2.08**</td>
</tr>
<tr>
<td>3 hours</td>
<td>106.33 ± 1.157 (29.083%)</td>
<td>79.66 ± 1.52** (25.523%)</td>
<td>83.66 ± 0.57**</td>
</tr>
</tbody>
</table>

Values are Mean ± SD,N = 6

**P < 0.01, *P < 0.05 Vs. Control**

Figures in parenthesis indicates the percentage decrease in blood glucose level.
Evaluation of hypoglycemic activity in Physalis minima fed rat using maltose tolerance test:

Normal Wistar rats were randomly divided into 3 groups. (6 rats/group) and were fasted overnight (18 hrs). Animals in-group I were treated tragacanth(1%) along with maltose (2g/kg body weight) as control and the experimental rats are groups II animals were treated with Physalis minima extract 200 mg/kg along with maltose (2g/kg body weight)and group III animals treated with Physalis minima 400 mg/kg along with maltose (2g/kg body weight). Blood samples were taken from the lateral tail vein at 0, 60, 120, 180 minutes. The blood glucose concentration was measured by using glucometer and noted. The noted values presents in table 3.

STATISTICAL ANALYSIS:

All data were subjected to analysis of variance (ANOVA). The data (mean±standard deviation) shown are mean 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 GRACHPADPRISM version 4.2 software.

RESULTS

In vitro alpha glucosidase inhibitory activity:

Graph 1 represents the in vitro effect of alpha glucosidase inhibitory activity shows the inhibitory activity of Physalis minima ethanolic extract on maltase in vitro (experiment 1). Physalis minima ethanolic extract inhibited alpha glucosidase enzyme in a dose-dependent manner and 1000mcg/ml of Physalis minima extract resulted in 50.99% maltase enzymatic inhibitory activity compared with 1000mcg/ml of Acarbose standard.

The evaluation of hypoglycemic activity in Physalis minima fed normal wistar rat

Graph 2 represents the evaluation of hypoglycemic activity in Physalis minima fed normal wistar rat shows the changes in the levels of blood glucose in group I control and experimental Physalis minima fed groups group II and group III. Group II and III showed suppression of blood glucose elevation at 120 min and 180 min significantly (p < 0.01) compared to control. In this study, Physalis minima extract significantly (p < 0.01) suppressed blood glucose compared with control group during 120 min to 180 min period. The blood glucose level of the Physalis minima extract administered rats was identical to the level in control group during period from 60 and 120 min. These results showed that Physalis minima extract had a suppressive effect on blood glucose after oral administration of extract in rats. Percentage of reduction of blood glucose from the normal level is 29.08% and 25.52% for both 200mg/kg and 400mg/kg of ethanolic Physalis minima extract respectively.

Evaluation of hypoglycemic activity in Physalis minima fed rat using maltose tolerance test

Graph 3 represents evaluation of hypoglycemic activity in Physalis minima fed rat using maltose tolerance test the shows the changes in the levels of blood glucose in group I control and experimental Physalis minima fed group II and group III after oral administration of maltose (2g/ kg). Physalis minima treated rat groups showed suppression of blood glucose elevation at 120 min and 180 min significantly (p < 0.01) compared to control (maltose) group. In this study, Physalis minima extract significantly (p < 0.01) suppressed the postprandial elevation in blood glucose compared with control group during 120 min to 180 min period after

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<td>103.66 ± 1.5470*</td>
</tr>
<tr>
<td>1 hour</td>
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<td>112.33 ± 5.8594</td>
<td>108.33 ± 7.5055</td>
</tr>
<tr>
<td>2 hours</td>
<td>107 ± 1</td>
<td>101.67 ± 1.1547</td>
<td>98 ± 6.0827* (9.489%) (9.53%)</td>
</tr>
<tr>
<td>3 hours</td>
<td>106.33 ± 1.157</td>
<td>99.66 ± 6.6583</td>
<td>97.33 ± 7.2341 (11.279%) (25.523%)</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, N = 6

*P < 0.01, **P < 0.05 VS. Control

Figures in parenthesis indicates the percentage decrease in blood glucose level
maltose loading. The blood glucose level in treated rats was identical to the level in control group during period from 60 and 120 min. These results showed that *Physalis minima* extract had a suppressive effect on the post-prandial elevation in blood glucose after maltose oral administration in rats. Percentage of reduction of blood glucose from the elevated level is 11.27% and 25.52% for both 200mg/kg and 400mg/kg of ethanolic *Physalis minima* extract respectively.

**DISCUSSION:**

This present study shows that ethanol extract of *Physalis minima* had inhibitory activities against maltase that is present in small intestinal mucosa. This is in accordance with recent research conducted on glucosidase inhibitor obtained from plant source and their ability to suppress the postprandial blood glucose level. The methanolic extracts of *S. reticulata* and *S. oblonga* strongly inhibited
rat intestinal maltase in vitro with IC50 values 42 μg/ml and 32μg/ml respectively, Physalis minima were equivalent to the effect of S. reticulata and S. oblonga. It had been reported that digestive enzymes such as lipase, alpha amylase, and alpha glucosidase, were inhibited by proanthocyanidins and tannins in young chicks, which decreased the digestibility of protein, starch and lipid (9,10). The mechanism of inhibition on maltase intestinal enzymes by ethanolic Physalis minima extract could be done to the polyphenolic content. Arecanut extract showed inhibition of elastase and hyaluronidase on skin tissues, which was purified by each fraction of solvents and was identified as a phenolic substance that showed competitive inhibition with the substrate (11). In another study, tea polyphenol such as catechin have been found to inhibit glucosidase activity and glucose transport (12). Tannins (polyphenol) have specific property of precipitating some proteins. This precipitation is presumed to occur by the formation of hydrogen bonds between the hydroxy groups of tannins and the peptide linkages of proteins (13).

Tannins were present in sufficiently high concentrations in Physalis minima which might have significantly precipitated the enzyme maltase. In this study, an Physalis minima extract was examined for its in vitro inhibition of rat intestinal alpha glucosidase and its in vivo effect on suppression of elevating blood glucose level. Physalis minima extract treated group (200mg/kg and 400 mg/kg doses) showed significant suppression (p < 0.01) in blood glucose elevation at 120 min and 180 min (p < 0.01) compared to maltose loading control rat group. These results suggest that Physalis minima extract had a suppressive effect on post prandial elevation in blood glucose after oral administration of maltose to rats. This study is in accordance with earlier report stated that anthocyanins inhibited alpha glucosidase activity and reduced blood glucose levels after starch rich meals (14). The results strongly suggest that Physalis minima extract inhibited blood glucose elevation by inhibiting glucosidase activity, however, it may take part in other mechanism. It is necessary to investigate the mechanism of action of Physalis minima extract on glucose transport and insulin secretion. Alpha glucosidase inhibitors are used worldwide for the treatment of diabetes and alpha glucosidase inhibit reversibly the enzymatic cleavage of complex carbohydrates to simple absorbable sugars and hence slow the absorption of carbohydrate from the small intestine, thereby lowering postprandial hyperglycemia.

CONCLUSION:

In conclusion, our findings show that ethanolic Physalis minima extract inhibition on maltase may be due to several polyphenolic compounds present within the extract. More studies and in vivo experiments in diabetic conditions are required to ascertain the compounds and its mechanism of action, thereby providing a natural hyperglycemic control treatment, and thus decrease risk for diabetes, cardiovascular diseases. However, further studies are needed before Physalis minima polyphenol can be used safely as food additives and supplements.
ACKNOWLEDGEMENT:

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REFERENCES: