Vol. 11/1 (2011) 31-38

JOURNAL OF NATURAL REMEDIES

Alpha-glucosidase inhibitory and hypoglycemic activities of *Piper trioicum* extract.

D.Sathish Kumar*, S.N.Raju, A.Harani, David Banji, K.N.V Rao, Otilia Banji

Nalanda College of Pharmacy, Nalgonda-508 001, Andhra Pradesh, India.

Abstract

Alpha glucosidase *in vitro* inhibitory activity and hypoglycemic effect by oral administration in rats of *Piper trioicum* ethanol extracts have been investigated. *Piper trioicum* extract showed *in vitro* inhibitory activity of intestinal alpha glucosiadse enzyme maltase. Analysis of data confirms that alpha glucosidase inhibition activity was maximum at 500 mcg/ml of *Piper trioicum*. The purpose of study was to know whether *Piper trioicum* 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 Piper trioicum extracts (200 mg/kg and 400 mg/kg doses) showed significant suppression compared to control group. These results suggest that the *Piper trioicum* 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.

Keywords: *Piper trioicum*, hypoglycemic activity, alpha-glucosidase inhibition.

1. 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, onion [3] clove [4], tea [5]. A high postprandial blood glucose response is associated with micro-and macro-vascular complications in diabetes, and

* Corresponding author
Email: satmpdina@yahoo.co.in

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.

Piper trioicum belongs to Piparacae family, distributed in South Asian countries. The whole plant is used for diabetic, as rubefacient, diuretic, hepatoprotective, muscular pains, headache, toothache and internal remedy for cholera in folk medicine, the root is used as diuretic[7] , we have conducted this present study to know *Piper trioicum* extract in vitroinhibitory activity on alpha glucosidase enzyme such as maltase and subsequent *in vivo* of glucose absorption by inhibiting disaccharide digestion.

2. Material And Methods

2.1 Plant Material

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

Piper trioicum (500 g) was extracted with 70% ethanol in Soxhlet apparatus for 24 hrs. After filtration and evaporation of ethanol, the residue obtained was 14.3%. Phyto chemical screening of the ethanolic extract of *Piper trioicum* for the presence of favonoids, gycosides, saponins, tannins, alkaloids and triterpenes were carried out in accordance with procedures previously described [8].

2.2. Experimental animals

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 27^0C 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.

2.3. In vitro alpha glucosidase inhibitory activity

Piper trioicum ethanolic extract was used to investigate the *in vitro* effect of alpha glucosidase enzymes. After fasting, goat small intestine between duodenum and above cecum 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 (1000 mcg/ml) were taken in to different test tubes and pre incubated for 15 min, at 37^0 C then 500 µl of 100 mM maltose (2%) as a substrate was added to all the test tubes and incubated for 15 min at room temperature and centrifuged. 0.6 ml of supernatant obtained following was collected from all the test tubes separately and it mixed with 0.8 ml of alkaline $CuSO_4$ individually The solution was heated in water bath for 8min and cooled. After cooling, phosphomolybdic acid was added to the mixture and made to 10 ml with distilled water. Glucose concentration was measured using glucose kit. In case of maltase inhibitory test, maltose was used as a substrate.

2.4. Acute toxicity test

The ethanolic extract of *Piper trioicum* was screened for acute toxicity, following the standard method (OECD/OCDE No: 420, ANNEX 2C). Albino mice of either sex weighing 25 gms were divided in to 2 groups and each consisting of 3 mice. Animals were maintained on normal diet and water prior to and during the course of experiment. The suspension of ethanolic extract was prepared in 1%W/V of

tragacanth and was administered by gastric intubations. The acute toxicity studies were tested at the doses of 300 and 2000 mg/ kg.

2.5. Evaluation of Hypoglycemic activity in Piper trioicum 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, groups II animals were treated with *Piper trioicum* extract 200 mg/kg and group III animals treated with *Piper trioicum* 400 mg/kg orally. 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.

2.6. Evaluation of hypoglycemic activity in Piper trioicum fed rat using maltose tolerance test

Normal Wistar rats were randomly divided in to 3 groups. (6 rats/group) and were fasted overnight (18hrs). Animals in-group I weretreated tragacanth (1%) along with maltose (2 g/kg body weight) as control and the experimental rats are groups II animals were treated with *Piper trioicum* 200 mg/kg along with maltose (2 g/kg body weight) and group III animals treated with *Piper trioicum* 400 mg/kg along with maltose (2 g/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.[9]

2.7. Statistical analysis

All data were subjected to analysis of variance (ANOVA). The data (mean ± standard deviation) shown are mean value and the significance differences was compared by using Dennett's Multiple comparison test at the $p<0.01$ probability level. ANOVA was carried out by using GRACHPADPRISM version 4.2 software.

3. Results

3.1. Phytochemical analysis

The phytochemical screening was proved that the plant consisted of tannin, polyphenol, alkaloids and polysaccharide.

3.2. In vitro alpha glucosidase inhibitory activity

Figure 1 and table 1 represent the *in vitro* effect of alpha glucosidase inhibitory activity shows the effect of *Piper trioicum* ethanolic extract activities on maltase in vitro. *Piper trioicum* ethanolic extract inhibited glucosidase inhibitory activity in a dose - dependent manner and 500 mcg/ml of *Piper trioicum* extract resulted in 51.59% maltase inhibitory activity compared with 1000 mcg/ml of Acarbose standard.

3.3. Acute toxicity

The mice treated with oral and i.p. administration of *Piper trioicum* ethanolic extract up to 2000 mg/kg did not produce any toxic effects in mice. No mortality was observed and *Piper trioicum* ethanolic extract was found to be safe at given doses.

3.4. Evaluation of Hypoglycemic activity in Piper trioicum fed normal wistar rat.

Figure 2 and table 2 represent the evaluation of hypoglycemic activity in *Piper trioicum* fed normal wistar rat the changes in the levels of blood glucose in group I control and experimental *Piper trioicum* 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, *Piper trioicum* 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 *Piper trioicum* extract administered rats was identical to the level in control group during period from 60 and 120 min. These results showed that *Piper trioicum* 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 39.58% for both 200 mg/kg and 400 mg/kg of ethanalic *Piper trioicum* extract.

3.5. Evaluation of hypoglycemic activity in Piper trioicum fed rat using maltose tolerance test

Figure 3 and table 3 represent evaluation of hypoglycemic activity in *Piper trioicum* fed rat using maltose tolerance testthe shows the changes in the levels of blood glucose in group I control and experimental *Piper trioicum* fed group II and group III after oral administration of maltose (2 g/kg). *Piper trioicum* treated rat groups showed suppression of blood glucose

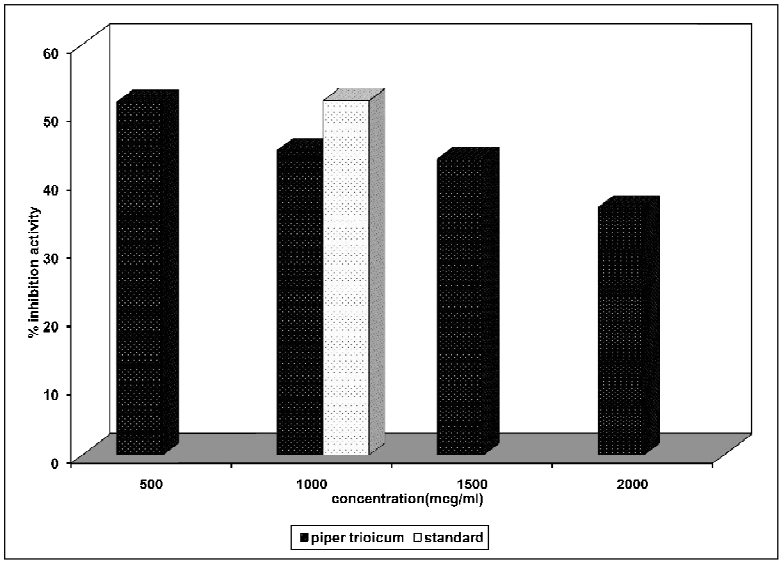

Figure 1: *In vitro* effect of alpha glucosidase inhibitory activity

Table1: *In vitro* effect of alpha glucosidase inhibitory activity

Concentration	% inhibition activity	
(mcg/ml)	Piper trioicum	Standard
Control	00.19±1.924	-
500	51.59±0.025**	-
1000	44.49±2.594**	51.78±1.51**
1500	43.24±0.213**	-
2000	36.18±3.416**	-

Values are Mean + SE, N= 6. **P<0.01, *P<0.05 Vs. Control

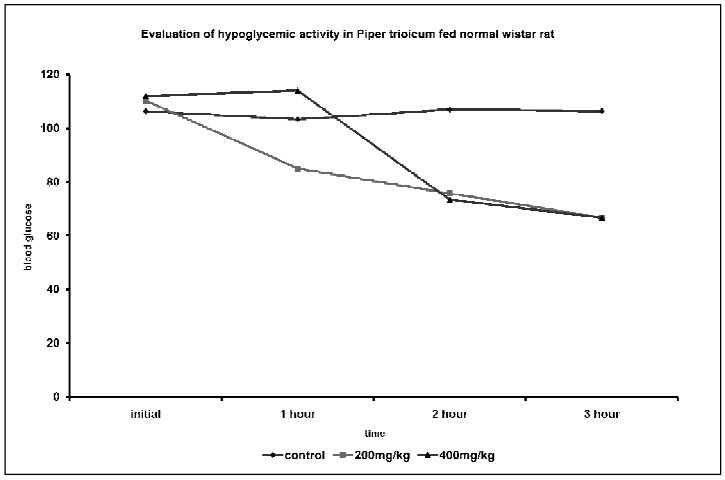

Figure 2: Evaluation of hypoglycemic activity in *Piper trioicum* fed normal wistar rat

Table2: Evaluation of hypoglycemic activity in *Piper trioicum* fed normal wistar rat

Group	I (control)	II (200mg/kg)	III (400mg/kg)
Initial	106.33 ± 0.333	110.33 ± 0.333*	112 ± 1.528**
1 hour	103.33 ± 0.333	85 ± 5.132*	114 ± 3.512
		(22.95%)	(10.71%)
2 hours	107 ± 0.881	75.66 + 1.453**	73.33 ± 0.88**
		(31.423%)	(34.52%)
3 hours	106.33 ± 0.333	66.66 + 0.666**	66.66 ± 1.856**
		(39.58%)	(39.58%)

Values are Mean + SE, N= 6. **P<0.01, *P<0.05 Vs. Control. Figures in parenthesis indicates the percentage decrease in blood glucose level

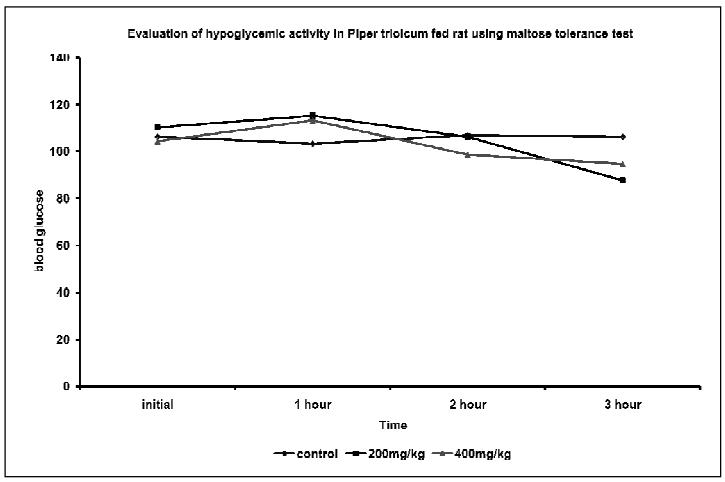

Figure 3: Evaluation of hypoglycemic activity in *Piper trioicum* fed rat using maltose tolerance test

Table 3: Evaluation of hypoglycemic activity in *Piper trioicum* fed rat using maltose tolerance test

	I (control)	II (200mg/kg)	III (400mg/kg)
Initial	106.33 ± 0.8819	110.33 ± 0.66*	104.33 ± 0.8819
1 hour	103.33 ± 0.333	115.33 ± 0.881**	113.3 ± 1.856
2 hours	107 ± 0.881	106.33 ± 0.881**	98.66 ± 0.881**
		(8.089%)	(11.38%)
3 hours	106.33 ± 0.8819	87.66 + 0.881**	94.66 ± 1.453**
		(23.99%)	(14.973%)

Values are Mean ± SE, N= 6. **P<0.01, *P<0.05 VS. Control. Figures in parenthesis indicates the percentage decrease in blood glucose level

elevation at 120 min and 180 min significantly ($p< 0.01$) compared to control (maltose) group. In this study, *Piper trioicum* extract significantly ($p< 0.01$) suppressed the postprandial elevation in blood glucose compared with control group during 120 min to 180 min period after 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 *Piper trioicum* 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 23.99% and 14.97% for both 200 mg/kg and 400 mg/kg of ethanalic *Piper trioicum* extract respectively.

4. Discussion

This present study shows that ethanol *Piper trioicum* extract 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 S. oblonga* inhibited rat intestinal maltase *in vitro* with an values were 42 μg/ml and 32 μg/ml respectively, *Piper trioicum* 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 [10,11]. The mechanism of inhibition on maltase intestinal enzymes by ethanolic *Piper trioicum* 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 [12]. In another study, tea polyphenol such as catechin have been found to inhibit glucosidase activity and glucose transport[13]. Tannins (polyphenol) have specific property of precipitating some proteins. This is presumed to occur by the formation of bonds between the hydroxy groups of and the peptide linkages of proteins[14].

Tannins were present in high concentrations in *Piper trioicum* which might have precipitated the enzyme maltase. In this study, a *Piper trioicum* extract was examined for its *in vitro* of rat intestinal alpha glucosidase and its in vivo on suppression of elevating blood glucose level. *Piper trioicum* extract treated

group's (200 mg/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 *Piper trioicum* extract had a suppressive effect on post prandial elevation in blood glucose after oral administration of maltlose 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 [15]. The results strongly suggest that *Piper trioicum* 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 *Piper trioicum* 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.

In conclusion, our findings show that ethanolic *Piper trioicum* 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 *Piper trioicum* polyphenol can be used safely as food additives and supplements.

5. Acknowledgement

The authors are grateful to the Managament, Nalanda college of pharmacy for providing support to carry out this work.

References

1. Puls, W., U. Keup, H. P. Krause et al., (1977). *Naturwissen-schaften* 64: 536 537..
2. Ratner, L. (1992),. *AIDS Res. Hum. Retroviruses* 8(2): 165-173.
3. Nisioka,T., Watanabe.J., Kawabata, J., Niki,R., (1997), *Biosci. Biotechnol. Biochem*, 61 ,1138 -1141.
4. Toda,H., Kawabata,J. Kasuari.T., (2000),. *Biosci. Biotechnol. Biochem* .64, 294-298
5. Honda, M, Hara,V., (1993), *Biosci. Biotechnol. Biochem* 57,123-124.
6. Del Prato, S. (2000), *Int J Clin Pract Suppl.* 112, 3S - 8S.
7. Madhava Chetty K, Sivaji K, Tulasi Rao K, (2008) Flowering plants of chithor district, Andhra Pradesh, India, Students offset printers, Tirupati, first edition, pg no 301.
8. Kokate CK, (2004), Practical Pharmacognosy, Vallabh Prakashan: New Delhi; 123-124.
9. Senthil Amudhan M, Hazeena Begum V. (2008). Phcog Mag. 4 (15), 223-227.
10. Yuste, P.; Longstaff, M.; Mc Corquodale, C. (1992). *Br. J. Nutr.* 67, 57- 65.
11. Longstaff, M.; McNab, J.M. (1991). *Br. J. Nutr.* 65, 199-216.

112. Kuk.-Kook Lee, J.-J.Cho, J. Park, J. D. Choi (2001), *International Journal of Cosmetic Science*. 23(6) : 341- 346.

13. Kobayashi, Y.; Suzuki, M.; Hideo, S.; Arai, S.; Yukihiko, H.; Suzuki, K.; Miyamoto, Y.; Shimizu, M. (2000).. *J. Agric. Food Chem 48 :* 5618 - 5623.

14. Loomis, W.D., Battaile J. (1966). *Phytochemistry* 5:423.

15. McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. (2005), *J. Agric. Food Chem.*, 53, 2760 - 2766.