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Alpha amylase inhibitory activity and *in vitro* glucose uptake in psoas muscle and adipose tissue of male wistar rats of leaf methanolic extract of *Achyranthes aspera*

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Abstract
The aim of the study was to investigate the anti diabetic activity by determination of the alpha amylase inhibitory activity and *in vitro* glucose uptake in isolated psoas muscle and adipose tissue of male wistar rats. The plant used in this study, *Achyranthes aspera,* is traditionally an important herb that possesses therapeutic properties used as medicine in India to cure many diseases. This plant was used in all the traditional system of medicine, Ayurveda, Unani, and Sidha from the ancient time in India. 180 g male wistar rat was sacrificed for isolation of psoas muscle and adipose tissue. 250 mg of tissue was used for this study. The inhibition caused by 0.5% Mercuric chloride was arbitrarily fixed as 100% inhibition. *Achyranthes aspera* leaf methanolic extract (AALMEt) (5- 25 mg/mL) was showed 29.75 – 71.97% amylase inhibitory activity. *In vitro* glucose uptake studies were carried out in both psoas muscle and adipose tissue in different sets i.e tissue alone, with insulin (25 mU/L & 50 mU/L), plant extracts (50, 100 & 150 mg/mL), plant extracts along with insulin. Psoas muscle was showed increased absorbance from 50 to 100 mg/mL and decreased absorbance from 100 to 150 mg/mL of plant extract alone and in the presence of insulin too but in adipose tissue glucose uptake was decreased with increased plant extract concentration in plant extract alone and along with insulin too.

Keywords: *Achyranthes aspera,* $HgCl_2$, α-Amylase, psoas muscle tissue, adipose tissue, glucose uptake.

Introduction
India is a very good source for medicinal plants. Maharsi Caraka has categorized *Achyranthes aspera* as sirovirecanopage- adjunct to cleansing nasal therapy, vamanopaga-adjunct to emesis, mutra virecaniya- diuretic and krmighna-anthelminitec. It is also classified in many Ayurvedic taxts as kasaghna anti-tussive, chardinasaka-anti-emetic, arsoghna-anti-haemorrhoidal, adhmana nasaka-ameliorates cervical adenitis. Diabetes is a metabolic disorder of carbohydrate, protein and lipid with hyperglycemic condition results from within saficient or without insulin effect. Diabetes is major world health problem today. Control or prevention of diabetes is needed [1]. *Achyranthes aspera* Linn belonging to family Amaranthaceae, is commonly found as a weed on way side throughout Indian. It is known as Apamerga in Sanskrit, Chirchitta in Hindi. The plant is used for treating asthmatic, cough, snakebite, hydrophobia, Urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, real dropsy and abdominal pain [1-4]. The present investigation aim is to evaluated *Achyranthes aspera* leaf methanolic extract *in vitro* alpha amylase inhibitory activity and effect on glucose uptake in psoas muscle and adipose tissue of male wistar rats.

Materials and Methods
Materials
All the chemicals used were of analytical grade. Methanol, glucose, NaCl, Sodium potassium tartrate, Starch, NaOH, 3,5 – Dinitrosalicylic acid were obtained from Sisco Research Laboratories Pvt Ltd, Hyderabad**,** India**.**

Collection of Plant material & Preparation of plant extract
A. aspera was collected during Nov –Dec 2010 of the natural population growing in and around Reddipalli village, Anantapuramu, Andhra Pradesh, India. The plant was authenticated by Prof T.Pullaiah, and ocher specimen was maintained in the Dept. of Botany, Sri Krishnadevaraya University, Anantapuramu (Dt), Andhra Pradesh, India. (S.K.U.B.H. No: 11202, F.A.P. No: 42001, 16/12/ 2010).

The leaves were separated from the plant, washed thoroughly 2 - 3 times with running water and once with sterile distilled water. Cleaned leafs were shade dried at room temperature for 25 days. The dried plant material was ground into fine powder. The obtained powder was extracted with methanol for 12 h using Soxhlet apparatus. These extracts were concentrated at 40 ^{o}C under reduced pressure with rotary evaporator and dried using lyophilizer. The dried extracts were stored in brown airtight container at 4 ^{o}C until for further use.

Preparation of enzyme

Enzyme source

10 mL of the saliva was collected and diluted to 100 mL with cold phosphate buffer pH 7.0. The solution was centrifuged at 8000 rpm for 20 min and the clean supernatant was used amylase assay. Clear supernatant resulted after completion of 10% saliva in phosphate buffer (0.1 M, pH 7.0, containing 2 N NaCl) was used as α-amylase enzyme source.

Amylase assay

α-Amylase activity was determined by the method out lined by Jayaraman (1981) [6]. AALMEt was dissolved in 0.1 M Phosphate buffer (pH 7.0) to get a concentration of 0, 5, 10, 15, 20, and 25 mg/mL. Then 1.0 mL of plant extract of each concentration was added to 0.2 mL of enzyme source and incubated for 10 min. To this 1.0 mL of soluble Starch (1% w/v), 2.3 mL of 0.1 M Phosphate buffer (pH 7.0) containing 2N NaCl were added and incubated at room temperature for 20 min. Then the reaction was stopped by adding 0.5 mL of DNS reagent (12.0 g of Sodium potassium tartrate in 8 mL of 0.25 M NaOH and 96 mM 3, 5-dinitrosalicylic acid) and the contents were heated in a boiling water bath for 5 min, cooled and the absorbance was measured at 530 nm against buffer blank. A series of standard maltose (1.0 to 5.0 mg) was treated in a similar manner. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. The standard graph was constructed using pure anhydrous maltose. The assay was run along suitable blank (without enzyme)

To measure the amylase inhibitory activity 1 mL of different concentrations of mercuric chloride (0.1% - 0.5%) were pre-incubated with α-amylase and carried out as described above. The inhibition caused by the 0.5% mercuric chloride was arbitrarily fixed as 100% inhibition. The decrease in the inhibitory activity of the crude plant extract was defined on the basis of these parameters. The extract showed about 29.75 to 71.97% the anti diabetic activity was determined through the inhibition of α-amylase which was expressed as a percentage of inhibition). The concentration of $HgCl_2$ and plant extracts required to inhibit 50% of α-amylase activity under the conditions was defined as the IC_{50} value. The α-amylase inhibitory activities of plant extracts and $HgCl_2$ were calculated and its IC_{50} values were determined.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control (no inhibitor)} - \text{Absorbance of sample}}{\text{Absorbance of control (no inhibitor)}} \times 100$$

Glucose uptake studies in rat psoas muscle and Adipose tissue

The muscle and adipose tissues after isolation were processed and incubated following the method described earlier (Rajesh kumar *et al*., 2005) [7]. Psoas muscle and adipose were isolated from two anesthetized adult rats and placed immediately in Kreb's ringer buffer (KRB) containing 11.1 mM glucose. Muscle/adipose tissues were cut into pieces of equal mass; about 250 mg were placed in KRB containing 11.1 mM glucose container, and pre-incubated for 5 min in CO_2 incubator under 95% O_2 and 5% CO_2 atmosphere. Four sets (in triplets) including tissue alone, tissue with insulin (25, 50 mU/L), tissue with both insulin and methanolic extract of AALMEt (50, 100 and 150 µg) and tissue with and methanolic extract of AALMEt (50, 100 and 150 µg) alone were incubated in CO_2 incubator under 95% O_2 and 5% CO_2 atmosphere for 2.5 h. Aliquots of 10 µL were removed from incubation mixture at 0, 30, 60, 90, 120 and 150 min, and changes in glucose concentration were measured using Excel Diagnostic kit by GOD-POD method.

Results and Discussion

The AALMEt exhibited α-amylase inhibitory activity in a concentration dependent manner with 29.75%, 40.63%, 55.16% and 71.97% inhibition at concentrations of 5, 10, 20 and 25 mg/mL of the extract respectively, with an IC_{50} value of 18.14 ± 0.31 mg/mL. The results of the study are represented in Table 1 & fig 1. $HgCl_2$ inhibitory activity was shown in the fig 2.

In *in vitro* glucose uptake studies control incubations (i.e., buffer with 11.1 mM glucose without added insulin or plant extract) showed reductions in glucose concentrations by 30.31, 37.32, 42.63, 49.16 and 52.45% at 30, 60, 90, 120 and 150 min respectively with psoas muscle and 13.69, 22.67, 27.67, 31.67, and 35.95% at 30, 60, 90, 120 and 150 min respectively with adipose tissue. With 25 and 50 units of insulin in the incubation medium the glucose uptake by both psoas muscle and adipose tissue was enhanced by 14.67 & 28.46% and 14.17 & 29.22% respectively compared to corresponding control values at 30 min. Further, as insulin concentration increased in the medium the uptake of glucose by both tissues are also increased, but this increase is not proportional to the increased concentration of insulin.

The AALMEt at different concentrations (50, 100, 150µg) caused maximum uptake of glucose by psoas muscle amounting to 58.56, 66.97 and 55.19% respectively at 90 min which are significantly greater than control (Table 2) whereas glucose uptake studies of adipose in the presence of 50, 100 and 150 µg concentration of plant extract showed maximum uptake of glucose at 30 min of incubation period amounting to 34.67, 19.68 and 17.37% respectively, which are also significantly higher than control studies (Table 3). This indicates an increased cellular concentration of glucose by enhanced uptake of glucose from media in the presence of plant extract. Direct effect in the absence of insulin indicates that the extract has either insulin like effect or direct stimulatory effect on enzymes involved in the metabolism of glucose.

However, in the presence of insulin and plant extract the per cent glucose uptake was increased more than additive values of individual studies with insulin alone and extract alone in both tissues. With 50, 100 and 150 µg concentrations of the extract maximum effect was obtained with 50 & 100 µg of the extract by itself alone as well as in combination with insulin. Thus results are discussed only with 50 & 100 µg of the AALMEt. It is clear from the results (Table 2) in the psoas muscle 50 & 100 µg of the plant extract by itself increased glucose uptake by about 37.36 & 57.09% up to 90 min, after which the effect decreased to 30.89 & 45.64% by 150 min. However, in combination with insulin, increase in glucose uptake was 98.56 & 101.17% up to 90 min, but it decreased to 77.30 & 79.62% by 150 min. Similarly, in adipose tissue 50 µg

of the plant extract by itself increased glucose uptake by 153.25% in 30 min, after which the effect decreased to 60.29% by 150 min. However, in combination with insulin, the extract increased the uptake of glucose by 178.74% in 30 min, which was further decreased to 54.79% by 150 min compared to the corresponding values of controls (Table 3). As per the data, the presence of AALMEt stabilizes the rate of glucose uptake via insulin action by these tissues, which is an indication of synergetic activity of insulin and *A.aspera* extract.

From the results, it can be concluded that use of these plant extracts will be greatly beneficial to reduce the rate of digestion and absorption of carbohydrates and thereby contribute for effective management of diabetes by decreasing the post-prandial hyperglycemia.

Some of the Indian herbal extracts like *Tamarindus indica, Catharanthus roseus, Caesalpinia bonducella* [8] and *Asystasia dalzelliania* [9] used in the ayurveda for treatment of diabetes are also reported to exhibit α-amylase inhibitory activity.

One therapeutic approach which may prove to be beneficial for treatment of diabetes is to decrease the post-prandial hyperglycemia which can be achieved by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes in the digestive tract. The glucosidase enzyme such as α-amylase is responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the postprandial plasma glucose rise. Further purification is needed for more information about these natural enzyme inhibitors in *A. aspera* leaf methanolic cured extract.

In muscle cells and adipocytes insulin stimulated cellular glucose transport is driven by the appearance of the insulin responsive glucose transporter, GLUT4 at the plasma membrane. While insulin stimulated glucose uptake by adipose tissue accounts for less than 10% of the whole body glucose uptake, surprising results have been observed in mice carrying an adipose-specific deletion of the GLUT4 gene. These animals rapidly develop marked muscular and hepatic IR, preceding hyperglycemia, despite normal fat mass, muscle and hepatic TG content, and normal serum leptin levels [10]. In contrast, mice carrying a muscle-specific deletion of GLUT4 develop hepatic and adipose IR secondary to the resulting hyperglycemia; such a resistance can be treated by correcting the glycemia with phlorizin [11]. To the same extent lipodystrophic patients develop a generalized IR [12]. Taken together these data suggest that adipose tissue plays a more important role in whole body glucose homeostasis than was previously thought. In conclusion, while adipose tissue glucose uptake accounts for a small part of whole body glucose disposal, an understanding of the mechanism of insulin actions involved in this tissue is of utmost importance.

Our studies on isolated psoas muscle and adipose indicated that the AALMEt enhanced the uptake of glucose in these tissues in a short time of 30 min in the absence of insulin, i.e. directly and the effect was more in the presence of insulin. Direct effect of AALMEt in the absence of insulin indicates that the extract has either insulin-like effect on skeletal muscle and adipose tissue or direct stimulatory effect on the enzymes involved in the metabolism of glucose. Increase of glucose uptake in the presence of plant extract along with insulin suggests that possibility of increased binding of insulin to receptors or increases in the number of insulin receptors or increased the insulin sensitivity in muscle and adipose tissue. The enhanced uptake of glucose may lead to increased utilization of glucose from the blood. Thus the enhanced insulin sensitivity in the presence of plant extract by psoas muscle and adipose tissue seems to be useful in bringing about post absorptive blood glucose clearance or correcting the IR. The principle compound which is responsible for the enhancement of the glucose uptake is not identified and some more research has to be done on this aspect.

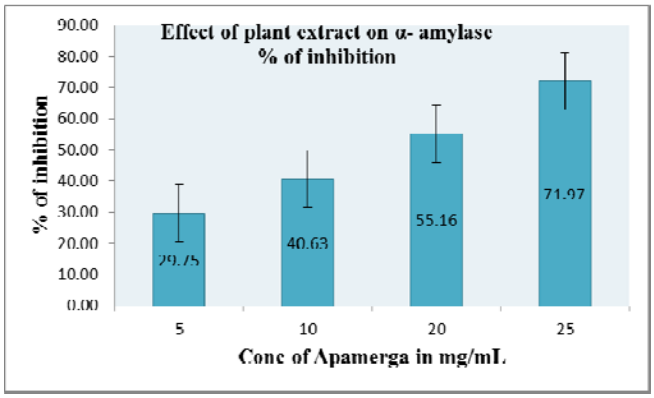

Fig 1: α- Amylase inhibitory effect of AALMEt.

Table 1: α- Amylase inhibitory effect of AALMEt and IC_{50} – 50% inhibitory concentration.

Conc. of AALMEt mg/mL	Inhibition (%)	IC_{50} VALUE mg/mL
5	29.75	18.14 ± 0.31
10	40.63	
20	55.16	
25	71.97	

IC_{50} – 50% inhibitory concentration

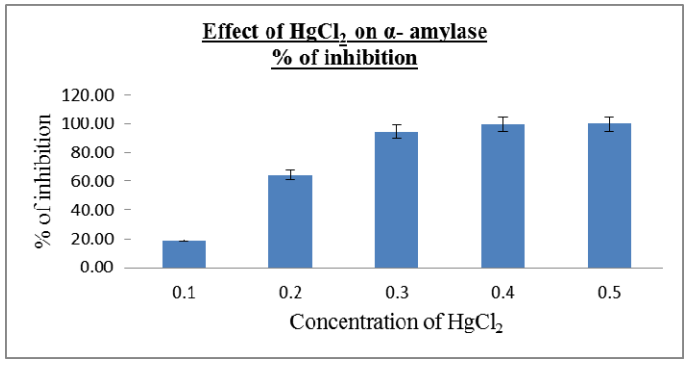

Fig 2: α- Amylase inhibitory effect of $HgCl_2$

Table 2: Effect of AALMEt on uptake of glucose by psoas muscle isolated from wistar rat

Glucose uptake (mg/mL)					
Set	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**
Muscle tissue(MT)	30.31	37.32	42.63	49.16	52.45
MT+Insulin(I) (25 mU/l)	34.75 (14.67)	42.95 (15.07)	48.34 (13.39)	56.07 (14.05)	60.86 (16.04)
MT+I (50 mU/l)	38.93 (28.46)	46.63 (24.93)	54.07 (26.82)	59.33 (20.69)	61.11 (16.52)
MT+AALMEt (50 µg)	46.63 (53.88)	49.69 (33.12)	58.56 (37.36)	65.07 (32.36)	68.65 (30.89)
MT+AALMEt (100 µg)	55.46 (83.00)	59.81 (60.24)	66.97 (57.09)	74.22 (50.98)	76.38 (45.64)
MT+AALMEt (150 µg)	45.44 (49.96)	50.64 (35.68)	55.19 (29.45)	61.83 (25.77)	66.88 (27.51)
MT+AALMEt (50 µg)+I(25 mU/l)	72.45 (139.07)	76.48 (104.89)	84.65 (98.56)	90.10 (83.28)	92.99 (77.30)
MT+AALMEt (100 µg)+ I(25 mU/l)	76.42 (152.16)	81.01 (117.05)	85.77 (101.17)	91.48 (86.07)	94.21 (79.62)
MT+AALMEt (150 µg)+ I(25 mU/l)	56.60 (86.77)	63.70 (70.66)	68.47 (60.59)	77.29 (57.23)	80.47 (53.43)
MT+AALMEt (50 µg) +I(50 mU/l)	74.58 (146.10)	80.98 (116.97)	86.68 (103.31)	91.71 (86.54)	94.89 (80.91)
MT+AALMEt (100 µg) +I(50 mU/l)	79.13 (161.12)	86.58 (131.97)	88.44 (107.43)	91.09 (85.29)	96.75 (84.47)
MT+AALMEt 1(50 µg)+ I(50 mU/l)	68.28 (125.32)	74.90 (100.68)	78.02 (83.00)	85.18 (73.27)	90.44 (72.44)

*Full in glucose concentration of the medium indicates glucose uptake by psoas muscle
Values in brackets indicate percentage increase of individual set when compared with psoas muscle alone (MT)

Table 3: Effect of AALMEt on uptake of glucose by adipose tissue isolated from wistar rat

Glucose uptake (mg/mL)					
Set	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**
Adipose tissue(AT)	13.69	22.67	27.67	31.67	35.95
AT+Insulin(I) (25 mU/l)	15.63 (14.17)	24.68 (8.84)	29.67 (7.22)	33.66 (6.29)	37.67 (4.78)
AT+I (50 mU/l)	17.69 (29.22)	27.68 (22.09)	33.66 (21.64)	37.66 (18.91)	42.66 (18.65)
AT+AALMEt (50 µg)	34.67 (153.25)	42.66 (88.17)	47.64 (72.17)	48.55 (53.31)	57.63 (60.29)
AT+AALMEt (100 µg)	19.68 (43.75)	27.65 (21.95)	32.66 (18.03)	39.73 (25.44)	40.66 (13.09)
AT+AALMEt (150 µg)	17.37 (26.88)	24.85 (9.6)	29.31 (5.93)	36.70 (15.89)	39.48 (9.80)
AT+AALMEt (50 µg)+I(25 mU/l)	38.16 (178.74)	43.65 (92.52)	48.66 (75.86)	51.62 (63.00)	55.65 (54.79)
AT+AALMEt (100 µg)+ I(25 mU/l)	34.67 (153.25)	40.66 (79.33)	45.64 (64.94)	47.64 (50.43)	50.65 (40.88)
AT+AALMEt (150 µg)+ I(25 mU/l)	23.17 (69.25)	28.67 (26.44)	33.15 (19.80)	36.67 (15.79)	39.66 (10.31)
AT+AALMEt (50 µg) +I(50 mU/l)	40.66 (197.01)	46.65 (105.74)	51.64 (86.63)	54.64 (72.55)	57.63 (60.29)
AT+AALMEt (100 µg) +I(50 mU/l)	37.66 (175.09)	42.66 (88.16)	48.66 (75.85)	50.62 (59.84)	53.64 (49.19)
AT+AALMEt 1(50 µg)+ I(50 mU/l)	26.17 (91.16)	31.66 (39.63)	37.66 (36.10)	42.66 (34.69)	46.66 (29.78)

*Full in glucose concentration of the medium indicates glucose uptake by psoas muscle
Values in brackets indicate percentage increase of individual set when compared with psoas muscle alone (MT).

Reference

1. Papadakis MA, Tierney LM, Mcphee SJ. Current medical diagnosis and treatment. International edition. Newyork: Lange medical books/Mc Graw-Hill. 2002, 1203-1215.
2. Jain SP, Puri HS. Ethnomedical plants of Janusar-Bawar hilld Uttar Pradesh, India. J Ethnopharmacol. 1984; 12:213-22.
3. Jhon D. One hundred useful raw drugs of the Kani tribes of Trivendrum forest division, Kerala, India. Int J Crude Drugs Res. 1984; 22:17-39.
4. Singh YN. Traditional medicine in Fiji, Some Herbal folk cures used by Fiji Indians. J Ethanopharmacol. 1986; 15:57-88.
5. Bhattari MK. Medical ethno botany in the Rapti Zone. Nepal. Fitoterapia 1993; 64:483-89.
6. Jayaraman J. Laboratory Manual in Biochemistry, Wiley

Eastern Ltd. New Delhi, India. 1981, 122-123.
7. Rajesh Kumar G, Achyut Narayan K, Geeta W, Murthy PS, Ramesh C, Kapil M *et al*. Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* (L.) in experimental animal. Current Sci. 2005; 88:1244-1254.
8. Bhukar MA, Bhise BS. *In viro* assay of alpha amylase inhibitory activity of some indigenous plans. Int J Chem Sci. 2012; 10:457-462.
9. Satish Kumar, Vivek Kumar, Chandrashekhar MS. *In-vitro* anti-oxidant and alpha-Amylase inhibitory activity of isolated fractions from methanolic extract of *Asystasia dalzelliana* Leaves. International Journal of Pharm Tech Research. 2011; 3:889-94.
10. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E *et al*. Adipose selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature. 2001; 409(6821):729-33.
11. Kim ZK, Zisman A, Fillmore JJ, Peroni OD, Kotani K, Perret P *et al*. Glucose toxicity and the development of diabetes in mice with muscle specific inactivation of GLUT 4. J Clin Invest. 2001; 108:153-160.
12. Vigouroux C, Bourut C, Guerci B, Ziegler O, Magre J, Capeau J *et al*. A new missense mutation in the calcium-sensing receptor in familial benign hypercalcaemia associated with partial lipoatrophy and insulin resistant diabetes. Clin Endocrinol. 2000; 53:393-398.