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RESEARCH ARTICLE

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Screening Ethanolic Extract of *Aerva lanata* for α-Amylase Inhibition and *in vitro* Uptake of Glucose in Adipose Tissue and Psoas Muscle of Male Sprague Dawley Rats

S. Saisree, B. Sasibhusana Rao, G. Sudhakara, P. Mallaiah, D. Saralakumari*

Sri Krishnadevaraya University, Anantapuramu-515003, Andhra Pradesh, India

ABSTRACT

The study was intended to investigate anti-diabetic efficacy of *Aerva lanata* by determining its α-amylase inhibition activity and *in vitro* uptake of glucose in adipose tissue and psoas muscle isolated from male Sprague Dawley (SD) rats. *Aerva lanata* is reported to have many traditional and Ayurvedic uses. Male SD rats (n=3) of 150 g were sacrificed and 250 mg of respective tissues were isolated for the study. *Aerva lanata* ethanolic extract (ALE) (5-20 mg/mL) showed 13.30 to 54.08% α-amylase inhibition activity. Glucose uptake studies in *in vitro* conditions were carried out in both adipose tissue and psoas muscle in different sets - tissue alone, tissue along with (*Aerva lanata* extract: 50µg, 100µg, 150µg, insulin: 25 mU/L, insulin: 50 mU/L and *Aerva lanata* extract: 50µg + insulin: 25 mU/L, *Aerva lanata* extract: 100µg + insulin: 25 mU/L, *Aerva lanata* extract: 150µg + insulin: 25 mU/L, *Aerva lanata* extract: 50µg + insulin: 50 mU/L, *Aerva lanata* extract: 100µg + insulin: 50 mU/L, *Aerva lanata* extract: 150µg + insulin: 50 mU/L). The rate of glucose uptake by insulin action in these tissues was stabilized by ethanolic extract of *Aerva lanata* and this shows synergetic activity of insulin and *Aerva lanata*.

Keywords: *Aerva lanata*, α-amylase, Glucose uptake, Insulin.

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***Corresponding author**: Mrs. D. Saralakumari
Address: Sri Krishnadevaraya University, Anantapuramu-515003, Andhra Pradesh, India
E-mail ✉: desireddysaralakumari8@gmail.com
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INTRODUCTION

Aerva lanata (Amaranthaceae) is distributed in waste lands throughout India. [1] In different geographical locations, *Aerva lanata* has traditional and folklore uses. [2] The traditional uses of plant includes the following activities i.e., diuretic, anthelmintic, anti-diabetic. [3] The whole plant, leaf decoction, roots and leaves are used for healing wounds, cholera, inflammation due to kidney stones, diabetes, leucorrhea, spermatorrhoea, emmolient and wounds. It is also used to cure piles, hypertension, liver congestion, in various fevers-malaria, typhoid, jaundice, hemorrhages and as an antidote to snake poison. Thus, *A. lanata* is one of the important medicinal plants used for many diseases and disorders. There are reports on different pharmacological activities of *A. lanata* which includes-anti-urolithiatic and nephroprotective, anti-diabetic, diuretic, anti-microbial, anti-cancer and anti-tumor, hepatoprotective, immunomodulatory, anti-diarrhoeal and anthelmintic, anti-inflammatory, analgesic and

anti-nociceptive, anti-fertility, anti-ulcer, anti-asthmatic, anti-HIV activities [3] and also reno protective effect. [4] From our previous *in vitro* studies ALE showed anti-hemolytic and anti-urolithic activities. [5] Alpha-amylase is involved in the breakdown of long chain carbohydrates. In the treatment of diabetes, α-amylase inhibition is the potential targets in the development of lead compounds. [6] Diabetes mellitus is associated with insulin deficiency and decreased glucose uptake in skeletal muscles. [7] On observation of these scientific literature reports, the anti-diabetic potential of ALE was evaluated by determining *in vitro* α-amylase inhibition and glucose uptake activities.

MATERIALS AND METHODS

Chemicals

Analytical grade - Sodium potassium tartrate, Glucose, Starch, 3, 5–Dinitrosalicylic acid, NaOH, NaCl were obtained from Sisco Laboratories, Hyderabad.

ALE extract

Ethanolic extract of *Aerva lanata* with (Batch Number ALE/15001) was a gift sample from Green-Chem Herbal Extracts and Formulations, Bangalore, Karnataka, India.

Flow sheet of ALE preparation

Aerva lanata dried leaves and stems (Aerial parts)

▼

Ethyl Alcohol and Water Extraction

▼

Concentration

▼

Vacuum concentration and Purification

▼

Spray Drying

▼

Powdering

▼

Sieving

Process explanation

Aerva lanata leaves and stems (aerial parts) are charged to extractor along with Ethyl alcohol and water. It is extracted by heating the mass for 5-6 hours, in a closed system by re-pumping the extract to the herb bed. This process is repeated. The extracts are combined and filtered, then concentrated under vacuum. This is charged to Spray Drier unit to dry and separate the product in a powder form. This is further powdered in a Multimill to a fine mesh size. It is sieved using a Sifter to make uniform particle size. The extract was dissolved in distilled water prior to use.

Ethical clearance for animal experimentations

Sri Krishnadevaraya University got ethical clearance from CPCSEA with (Regd. No: 1889/GO/Re/S/16/CPCSEA, dt. 30th May 2016), and present work was approved by the IAEC protocol No: SKU/Biochem/04/2016. In the present study, three male Sprague Dawley rats of (6 to 8 weeks age with weight 140 ± 5 g) were acclimatized before experimentation at our animal house and these animals were used further for other experimentations.

Preparation of enzyme

α-amylase enzyme source was prepared by diluting 10 mL of the saliva to 100 mL with pH 7.0 phosphate buffer. It was centrifuged for 20 min at 8000 rpm and the supernatant was used for the assay.

Assay of α-amylase inhibition activity

To determine the α-amylase inhibitory activity, Jayaraman (1981) [8] outlined method was followed. Different concentrations of plant extract (5–20 mg/ml) were pre incubated with [α-amylase (1 Unit/ml) and 2 ml of (pH 6.9) phosphate buffer with 2 N NaCl] and to this 1 ml starch solution was added and kept for 20 min incubation. 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-dinitrosalicyclic acid) the reaction was stopped and heated in a boiling water bath for 5 min. Blank was prepared with buffer excluding plant extract and another without enzyme and at 540 nm the absorbance was measured. Replacing plant extract with distilled water a control was also prepared. A range of standard maltose (1.0-5.0 mg) was treated in similar manner. The estimation of Maltose released from Starch was done by plotting a standard graph. The α-amylase inhibition activity was calculated by

Inhibition % = Control absorbance - Sample absorbance / Control absorbance × 100

IC_{50} value is the concentration of plant extract required to inhibit 50% of α-amylase. The α-amylase inhibition activity of ALE was calculated to determine the IC_{50} value.

In vitro glucose uptake activity studies

By following the method described by (Rajesh Kumar *et al.*, 2005), [9] the study of uptake of glucose in skeletal muscle and adipose tissues were carried out. Triplicates of different sets were done twice, including tissue (250 mg) alone, tissue along with insulin (25, 50 mu/L), tissue along with ALE (50, 100, 150µg), and tissue along with both insulin and ALE (50, 100, 150µg). To measure the changes in concentration of glucose, 10 ml of aliquots were removed for every 30 min from the incubation mixture at 0 min to 150 min.

Table 1: α- amylase inhibitory activity of *Aerva lanata* ethanolic extract (ALE)

Concentration of ALE (mg/ml)	Inhibition (%)	IC_{50} Value (mg/ml)
5	13.30 ± 1.20	18.49
10	26.20 ± 1.30	
15	40.02 ± 0.72	
20	54.08 ± 0.47	

Data presented is expressed as mean ± standard error of the mean (n = 3).

RESULTS AND DISCUSSION

α-amylase inhibitory effect of *Aerva lanata* ethanolic extract

Inhibition of alpha glucosidase for controlling postprandial glucose is one of the therapeutic approaches in treating diabetes. Treatment with

disaccharide inhibitors in Type 2 diabetes patients improved both postprandial hyperglycemia and hyperinsulinemia. [10] In management of diabetes-Acarbose, Miglitol and Voglibose finds application in clinical practice. [11] But these drugs are associated with various side effects. [12] Hence, need arose to investigate amylase inhibitors from natural sources with less side effects. ALE showed 13.30%, 26.20%, 40.20% and 54.08% of α-amylase inhibition at respective concentrations, with IC_{50} of 18.49 mg/mL, and this study was supported by earlier studies in our laboratory [13-15] Table 1 represents the results of this study. Thus, the effective inhibition of α-amylase by ALE may contribute to its anti-diabetic activity.

ALE effect on sensitizing insulin

For the utilization of post prandial glucose, skeletal muscle is the crucial site and it is also the most abundant tissue in the body. In non-insulin dependent diabetes mellitus the general pathological condition is defects in skeletal muscle glucose uptake stimulated by insulin. [16] In obese/overweight persons decreased glucose uptake was observed. [17] GLUT4 drives insulin stimulated cellular glucose transport in muscle and adipocytes at plasma membrane. [18] The assay of glucose uptake effect in adipose tissue and psoas muscle of SD rats by ALE with and without insulin was done by measuring the decrease in concentrations of glucose in the incubation medium with time. Studies on different medicinal plant showed hypoglycemic activity by increasing absorption of glucose by muscle and fat tissues. [19] Present results are correlated with above results. *In vitro* glucose uptake studies of psoas muscle (Table 2) showed glucose concentrations of 33.46, 39.13, 44.43, 48.78 & 55.30 at 30, 60, 90, 120 & 150 minutes respectively and adipose tissue (Table 3) showed 11.05, 14.89, 19.03, 23.08 and 26.89 at 30, 60, 90, 120 & 150 minutes respectively. In the incubation medium with 25 & 50 units of insulin, the uptake of glucose was enhanced by 10.97 & 19.49% and 17.19 & 35.57% in both the tissues respectively at 30 minutes. With the increase in concentration of insulin in the medium, there was increase in glucose uptake by tissues.

At different concentrations (50, 100 & 150µg) in psoas muscle ALE showed glucose concentrations of 33.68, 39.30 & 25.76% respectively at 30 minutes and 23.09, 32.88 & 5.69% respectively at 90 minutes. Whereas glucose uptake concentrations of adipose tissue showed 134.93, 99.825 & 70.213% respectively at 30 minutes and 81.98, 52.55 & 36.05 at 90 minutes. This indicates the cellular concentration of glucose uptake enhancement by plant extract. The uptake of glucose with insulin in psoas muscle was 87.89 & 77.54% up to 90 minutes but decreased to 66.18 & 51.25% at 150 minutes respectively.

In adipose tissue it was 92.49 & 78.03 % at 90 minutes and 52.21 & 39.01% at 150 minutes. In the presence of plant extract enhanced insulin sensitivity by adipose tissue and psoas muscle seems useful in bringing post absorptive blood glucose clearance or correcting Insulin Resistance.

Table 2: ALE effect on glucose uptake in SD rat psoas muscle.

	Uptake of Glucose (mg/dl)				
Set Type	**30 minutes**	**60 minutes**	**90 minutes**	**120 minutes**	**150 minutes**
Muscle tissue (MT)	33.46	39.13	44.43	48.78	55.30
MT + insulin (I) (25mU/I)	37.13 (10.97)#	42.81 (9.4)	46.01 (3.56)	54.93 (12.61)	58.85 (6.42)
MT + I (50 mU/I)	39.98 (19.49)	45.89 (17.28)	51.45 (15.8)	58.79 (20.52)	61.07 (10.43)
MT + ALE (50 µg)	44.73 (33.68)	49.52 (26.96)	54.69 (23.09)	59.93 (22.86)	64.47 (16.58)
MT + ALE (100µg)	46.61 (39.30)	53.83 (37.57)	59.04 (32.88)	65.27 (33.8)	69.36 (25.42)
MT + ALE (150µg)	42.08 (25.76)	44.51 (13.75)	46.96 (5.69)	51.78 (6.15)	56.69 (2.51)
MT + ALE (50 µg) + insulin (25mU/I)	69.53 (107.8)	72.96 (86.46)	83.48 (87.89)	87.99 (80.38)	91.90 (66.18)
MT + ALE (100µg) + insulin (25mU/I)	66.57 (98.95)	69.76 (78.2)	78.88 (77.54)	81.86 (67.81)	83.64 (51.25)
MT + ALE ((150µg)+ insulin (25mU/I)	64.68 (93.31)	70.13 (79.22)	77.96 (75.47)	80.91 (65.87)	81.82 (47.96)
MT + ALE (50 µg)+ insulin (50mU/I)	67.08 (100.48)	67.81 (73.29)	69.68 (57.51)	73.32 (50.31)	78.14 (41.3)
MT + ALE (100µg)+ insulin (50mU/I)	68.89 (105.89)	69.08 (76.54)	70.48 (58.63)	71.04 (45.63)	75.15 (35.9)
MT + ALE (150µg)+ insulin (50mU/I)	64.87 (93.81)	65.93 (68.49)	68.86 (54.99)	69.83 (43.15)	72.31 (30.76)

Uptake of glucose by psoas muscle tissue

Table 3: ALE effect on glucose uptake in SD rat adipose tissue.

	Uptake of Glucose (mg/dl)				
Set Type	**30 minutes**	**60 minutes**	**90 minutes**	**120 minutes**	**150 minutes**
Adipose tissue (AT)	11.05	14.89	19.03	23.08	26.89
AT + insulin (I) (25mU/I)	12.95 (17.19)#	16.93 (13.70)	21.03 (10.51)	24.95 (8.10)	28.98 (7.77)
AT + I (50 mU/I)	14.98 (35.57)	18.99 (27.54)	24.38 (28.11)	27.33 (18.41)	30.99 (15.25)
AT + ALE (50 µg)	25.96 (134.93)	29.88 (100.67)	34.63 (81.98)	36.03 (56.11)	43.88 (63.18)
AT + ALE (100µg)	22.08 (99.82)	27.04 (81.60)	29.03 (52.55)	31.82 (37.87)	34.91 (29.83)
AT + ALE (150µg)	18.81 (70.23)	24.31 (63.26)	25.89 (36.05)	28.88 (25.13)	32.38 (20.42)
AT + ALE 50 µg) + insulin (25mU/I)	29.81 (169.77)	32.35 (117.26)	36.63 (92.49)	38.82 (68.20)	40.93 (52.21)
AT + ALE (100µg) + insulin (25mU/I)	26.67 (141.36)	29.83 (100.34)	33.88 (78.03)	35.86 (55.37)	37.38 (39.01)
AT + ALE (150µg)+ insulin (25mU/I)	22.31 (101.90)	26.62 (78.78)	29.68 (55.96)	31.56 (36.74)	33.98 (26.37)
AT + ALE (50 µg)+ insulin (50mU/I)	25.80 (133.48)	34.03 (128.54)	38.88 (104.31)	40.92 (77.30)	43.84 (63.03)
AT + ALE (100µg)+ insulin (50mU/I)	21.98 (98.91)	29.18 (95.97)	35.84 (88.33)	37.88 (64.12)	40.03 (48.87)
AT + ALE (150µg)+ insulin (50mU/I)	18.03 (63.17)	22.87 (53.59)	26.95 (41.62)	33.09 (43.37)	38.85 (44.48)

Uptake of glucose by adipose tissue

Present study showed that the ethanolic extract of *Aerva lanata* has glucose uptake stabilizing capacity and it can be an adjuvant in managing/treating diabetes.

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