Research Article

In-Vitro Glucose Uptake Effect of Asparagus racemosus In Lymphocyte Culture Preparation

Godwin SE[1*] and Alvin Jose M[2]

[1] Research Scholar, PRIST University, Vallam, Thanjavur-613 403, Tamil Nadu, India/ Department of Pharmacology, Noorul Islam College of Dental Science, NIMS Medicity, Aralummoodu-695 123, Trivandrum, Kerala, India.
[2] Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode-637 205, Tamil Nadu, India.

ABSTRACT
Diabetes mellitus is a chronic metabolic disease with highest rates of prevalence and mortality affects more than 100 million people worldwide. The objective of the present investigation was to evaluate the *in-vitro* antidiabetic activity of ethanolic extract of roots of *Asparagus racemosus* (REAR). The *in-vitro* antidiabetic effect was studied by glucose uptake assay in lymphocyte culture preparation. The results of *in-vitro* study revealed that REAR increased the percentage glucose uptake when calculated in comparison with control group. The findings of this investigation concluded that REAR has antidiabetic activity in glucose uptake assay.

Keywords: Diabetes mellitus, glucose uptake, lymphocyte culture, *Asparagus racemosus*.

INTRODUCTION

The world is facing an explosive increase in the incidence of diabetes mellitus, which is a chronic metabolic disease with highest rates of prevalence and mortality affects more than 100 million people worldwide. It is caused by an absolute or relative lack of insulin and/or reduced insulin activity (insulin resistance). Hyperglycemia may be attributed to defects in pancreatic β-cells, insulin secretion, hepatic glucose output, glucose uptake of peripheral tissues and immune function[1].

The prevalence rate of diabetes is estimated to be 1-5% in India. The number of people with diabetes in India is currently around 50.8 million and expected to rise to 87 million by 2030 unless urgent preventive steps are taken[2, 3]. Hence India leads the world with largest number of diabetic patients earning the dubious distinction of being the "diabetic capital of the world"[4]. All of the pharmacological modalities show limited efficacy and certain adverse effects such as hepatotoxicity, lactic acidosis, diarrhoea, obesity or weight loss and attenuation of response after prolonged use and are expensive particularly for developing countries like India and China. Comparatively very less side effects and low cost of phytopharmaceuticals from natural resources open new avenues for the treatment of various diseases including diabetes. Therefore there is a need for phytochemical that have antidiabetic potential, which are cost effective, potent and also safe without long-term side effects[1].

The ethanolic extracts of routes of *Asparagus racemosus* contain saponins, which have reported to show the antidiabetic effect in previous studies[5,6]. Despite the availability of many antidiabetic medicines in the market, diabetes and its microvascular and macrovascular complications continues to be a major medical problem. Plant derivatives with purported antidiabetic activity are used in folk medicine and traditional healing systems around the world[7]. Herbal drugs are prescribed widely even when their biologically active ingredients are unknown[8]. Substantial efforts have been made in recent years to identify new natural and synthetic antidiabetic drugs. There is flood of scientific data about medicinal plants including those with antidiabetic potential[9].

The roots of *Asparagus racemosus* belonging to the family of Liliaceae has been recommended in Ayurvedic texts for prevention and treatment of gastric ulcers, dyspepsia, galactogogue, aphrodisiac, nervous disorders, nervine tonic, liver

diseases, inflammation, antioxytocic, anticancer, diuretic, nutritive, rejuvenating, constipating, diarrhoea, tuberculosis, cough, bronchitis, gonorrhoea, leprosy, epilepsy, fatigues, threatened abortion, diabetes mellitus and burning sensation[10,11].

Although the roots of *Asparagus racemosus* has been used in traditional medicine yet scientific validation of its use in diabetes mellitus needs to be studied. Hence this investigation was undertaken to evaluate the antidiabetic effect of ethanolic extract of roots of *Asparagus racemosus* against glucose uptake assay in lymphocyte culture preparation.

MATERIALS AND METHODS

Plant material and extract preparation

The roots of *Asparagus racemosus* was collected during May 2011, from Kaliakkavilai, Tamil Nadu. It was identified and authenticated by botanist Dr. K. Paul Raj and voucher specimen was deposited in the Herbarium, department of botany, Nesamony Memorial Christian College, Marthandam (NMCC/47/2011). The roots were washed, cut into small pieces, dried in shade and coarse powdered (2000 gm) in a mixer grinder. It was extracted with soxhlet using 95% ethanol for 72 hours, concentrated on water bath (70^0 C), kept in oven (30^0 C) for drying and stored in desiccator. The yield of ethanolic extract of REAR was 26.4 gm (1.37%).

In-vitro antidiabetic effect by glucose uptake assay

Lymphocyte culture preparation

Human peripheral lymphocytes (HPLs) were cultured in Rosewell park memorial institute (RPMI) 1640 low glucose (Himedia, Mumbai, India) media, supplemented with 10 % heat inactivated Fetal biovine serum (FBS) (Himedia, Mumbai, India), antibiotics (Penicillin and Streptomycin). Phytohaem agglutinin (PHA) (Himedia, Mumbai, India) was used as the stimulant for cell proliferation. The culture was filtered using 0.2 µm pore sized cellulose acetate filter (Sartorius, Japan) in completely aseptic conditions. Lymphocytes were separated from the blood using Hisep (Lymphocyte separation medium) (Himedia, Mumbai, India). 3 ml of lymphocytes were transferred to centrifuge tube with 3 ml diluted blood. It was then centrifuged at 2600 rpm at room temperature for 30 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band mononuclear lymphocytes above HiSep. The pellets were washed with phosphate buffered saline and diluted to 10^5 cells / ml and used for studies[12].

Individual cultures were exposed to 300 mM glucose followed by plant extracts in increasing concentrations (50, 100, 200 µg/ml) and incubated for 2 hours. Pioglitazone was used as the standard drug and a positive control with glucose alone was maintained. The glucose consumption was measured in cell free media using Glucose assay kit (Sigma, Aldrich, USA) as per manufacturer's instructions. All experiments were done in six times and mean average was used for calculations. Briefly cells were collected and spun at 7500 rpm and clear supernatant was collected and used for the assay. Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a coloured product. Oxidized o-dianisidine reacts with sulfuric acid to form a more stable coloured product. The intensity of the pink colour is measured at 540 nm and % uptake was measured using the following formula.[13]

$$\% \text{ Glucose uptake} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

RESULTS

In-Vitro antidiabetic effect of REAR by glucose uptake assay method in lymphocyte culture preparation

In-vitro antidiabetic effect of REAR by glucose uptake assay revealed that the percentage glucose uptake of REAR at 50 µg/ml was 21.83 %, 100 µg/ml was 24.05 % and 200 µg/ml was 27.21 %. But the pioglitazone 6 µg/ml had produced highest level of 74.3 % glucose uptake. (**Table No.1, Graph No.1)**

Table 1: Antidiabetic effect of REAR and their percentage glucose uptake

Groups	O.D Mean	% Glucose uptake
Control	0.316	
Standard drug P.G 6 µg/ml	0.081	74.3%
REAR 50 µg/ml	0.247	21.83%
REAR 100 µg/ml	0.240	24.05%
REAR 200 µg/ml	0.230	27.21%

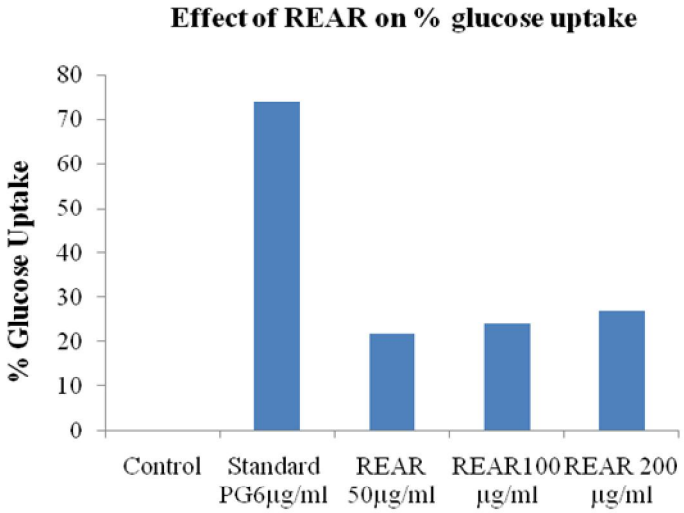

Fig.1: Effect of REAR on % glucose uptake

DISCUSSION

Hyperglycemia can be attributed to defects in pancreatic β-cells, insulin secretion, hepatic glucose output, glucose uptake of peripheral tissues and immune function. It causes premature mortality, accounting for atleast 10 % of total health care expenditure in many countries[1]. The results of *in-vitro* antidiabetic effect of REAR by glucose uptake assay revealed the increase in percentage glucose uptake in lymphocyte culture preparation. When compared to pioglitazone on increasing the dose of REAR, the % glucose uptake also increased proportionally. Pioglitazone increases the % glucose uptake by skeletal muscle via glucose transporters and the report of the REAR also showed the increase in percentage glucose uptake. So the current observations confirms the role of % glucose uptake and are indeed may be due to the activation of PPARγ by PPARγ agonist (insulin sensitisers) which are currently being used in the treatment of insulin resistance associated with type-2 diabetis mellitus and thus influenced the peripheral glucose uptake. Drugs like thiazolidinediones and Insulin cause differentiation of pre-adipocytes into adipocytes. The adipocytes then stimulate glucose uptake and this aid in reducing the blood glucose levels. Therefore, drugs which exhibit glucose uptake activity would be desirable for patients with T2DM. The drug REAR exhibited increase in % glucose uptake and thus can be explored as glucose lowering agent to treat T2DM [14, 15]. The study supports this hypothesis and given a lead to explore the role of REAR in glucose uptake.

CONCLUSION

In this investigation, REAR increased the percentage glucose uptake in lymphocyte culture preparation. Therefore, this investigation concluded that REAR may be used as an antidiabetic agent for chronic diabetes mellitus patients after confirming its efficacy and safety in well-controlled clinical trials. If it is confirmed in humans, REAR may be a potent, safe and cost effective phytomedicine to prevent premature death in diabetic patients.

REFERENCES

1. Pari L and Srinivasan S. Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in steptozotocin-nicotinamide induced diabetic rats.

Biomed Pharmacother. 2010;64(7):477- 481.
2. Srivastava B, Sinha AK, Sanjay G and Barshiliya Y. Study of hypoglycaemic and hypolipidemic activity of Eugenia jambolana pulp and seed extract in Streptozotocin induced diabetic albino rats. Asian J Pharma Life Sci. 2012;2(1):10-19.
3. Ramachandran A, Das AK, Joshi SR, Yajnik CS, Shah S and Prasanna kumar KM. Current status of Diabetes in India and need for noval therapeutic agent. J Assoc physician India. 2010;58:7-9.
4. King H, Anbert RE and Herman WH. Global burden of diabetes, 1995-2025. Prevalence, numerical estimates and projections. Diabet Care. 1998;21(9):1414-1431.
5. Marles RJ and Farnsworth NR. Antidiabetic plants and their active constituents. Phytomed. 1995;2(2): 137-189.
6. Vadivelan R, Dipanjan M and Umasankar P. Hypoglycemic, antioxidant and hypolipidemic activity of Asparagus racemosus on streptozotocin induced diabetic in rats. Adv Applied Sci Res. 2011;2(3):179-185.
7. De Sousa E, Zanatta L and Seifriz I. Hypoglycemic effect and antioxidant potential ofkaemferol-3, 7-O-(a)-dirhamnoside from Bauhinia forcata leaves. J Nat Prod. 2000;67:829-832.
8. Valiathan MS. Healing plants. Curr Sci. 1998;75:1122-1126.
9. Grover JK, Yadav S and Vats V. Medicinal plants of India with antidiabetic potential. J Ethno Pharmacol. 2002;81(1):81-100.
10. Goyal RT, Sigh J and Lal H. Asparagus racemosus an update. Indian J Medi Sci. 2003;57(9):408-414.
11. Kiritikar KR and Basu BD. Indian Medicinal plants. Vol4. ed2. International book distributers; Dehradun; 2007;2499-2550.
12. Susan J, Duthie, Aiguo Ma, Marion A and Rose. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer Res. 1996;56:1291-1295.
13. Blouin MJ, Zhao Y, Zakikhani M, Algire C, Piura E and Pollak M . Loss of function of PTEN alters the relationship between glucose concentration and cell proliferation increases glycolysis and sensitizes cells to 2-deoxyglucose. Cancer Letters. 2010;289:246–253.
14. Anandharajan R, Jaiganesh S, Shankeranarayanan NP, Viswakarma RA and Balakrishnan A. In-vitro glucose uptake activity of Aegles marmelos and Syzygium cumini by activation of Glut-4, PI3 Kinase and PPARγ in L6 myotubes. Phytomedi. 2006;13:434-441.
15. Samidha K, Ashwini K, Renuka M, Swpriya and Bhalerao. Evaluation of the adipogenic potential and glucose uptake stimulatory activity of Phyllanthus emblica and Tinospora cordifolia: an in-vitro study. Inter J Pharma Biosci. 2012;3:230-236.