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

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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. 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.
diseases, inflammation, antioxycotic, anticancer, diuretic, nutritive, rejuvenating, constipating, diarrhoea, tuberculosis, cough, bronchitis, gonorrhoea, leprosy, epilepsy, fatigues, threatened abortion, diabetes mellitus and burning sensation\textsuperscript{10,11}. Although the roots of \textit{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 \textit{Asparagus racemosus} against glucose uptake assay in lymphocyte culture preparation.

**MATERIALS AND METHODS**

**Plant material and extract preparation**

The roots of \textit{Asparagus racemosus} was collected during May 2011, from Kallakkavilai, 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\\degree C), kept in oven (30\\degree 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 \textmu m 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 polymuclear 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\textsuperscript{12}.

Individual cultures were exposed to 300 mM glucose followed by plant extracts in increasing concentrations (50, 100, 200 \textmu g/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.\textsuperscript{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**

\textit{In-vitro} antidiabetic effect of REAR by glucose uptake assay revealed that the percentage glucose uptake of REAR at 50 \textmu g/ml was 21.83 \%, 100 \textmu g/ml was 24.05 \% and 200 \textmu g/ml was 27.21 \%. But the pioglitazone 6 \textmu g/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

<table>
<thead>
<tr>
<th>Groups</th>
<th>O.D Mean</th>
<th>% Glucose uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.316</td>
<td></td>
</tr>
<tr>
<td>Standard drug P.G 6 µg/ml</td>
<td>0.081</td>
<td>74.3%</td>
</tr>
<tr>
<td>REAR 50 µg/ml</td>
<td>0.247</td>
<td>21.83%</td>
</tr>
<tr>
<td>REAR 100 µg/ml</td>
<td>0.240</td>
<td>24.05%</td>
</tr>
<tr>
<td>REAR 200 µg/ml</td>
<td>0.230</td>
<td>27.21%</td>
</tr>
</tbody>
</table>

**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 at least 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 sensitiser) which are currently being used in the treatment of insulin resistance associated with type-2 diabetes 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**