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International Journal of Diabetology & Vascular Disease Research (IJDVR)
ISSN:2328-353X

Effect of Aqueous Extracts of some Ayurvedic Medicinal Plants on Tissues Involved in Glucose Homeostasis *in vitro*

Research Article

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Abstract

Aqueous extracts of some antidiabetic Ayurvedic plant treatments such as Trigonella foenum-graecum Linn (TFG), Pterocarpus marsupium Roxb (PM), Gymnema sylvestre R.Br (GS) and Curcuma longa Linn (CL) were tested for their biological effects on glucose uptake by mouse muscle tissue and insulin secretion from mouse pancreas under both basal and hyperglycemic culture conditions. The results indicate that none of these treatments have a significant effect on either glucose uptake by muscle or insulin secretion from the pancreas under basal conditions. Under hyperglycemic conditions all treatments except GS demonstrate significant effects on glucose uptake by muscle tissue, and all treatments were effective at increasing insulin secretion from pancreas. This suggests that all these plant treatments enhance glucose homeostasis by stimulating either insulin secretion (GS) or enhancing glucose uptake (TFG) or activating both (CL and PM).

Keywords: Ayurvedic Medicinal Plants; Diabetes; Insulin Secretion; Glucose Uptake.

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Received: May 14, 2015
Accepted: June 20, 2015
Published: July 01, 2015

Citation: Suresh K. Mohankumar, James R. McFarlane (2015) Effect of Aqueous Extracts of some Ayurvedic Medicinal Plants on Tissues involved in Glucose Homeostasis *in vitro*. *Int J Diabetol Vasc Dis Res*, 3(6) 105-107. **doi:** http://dx.doi.org/10.19070/2328-353X-1500021

Introduction

Incidence of Type 2 diabetes (T2D) is increasing worldwide. A number of undesirable side effects are associated with current conventional treatments and they are not always effective in maintaining euglycemia or preventing later complications associated with the disease. Consequently the search for new antidiabetic compounds is intense. Ayurveda a traditional Indian system of medicine advocates a wide range of medicinal plants to treat T2D. Plants such as Trigonella foenum-graecum Linn, Pterocarpus marsupium Roxb, Gymnema sylvestre R.Br and Curcuma longa Linn, either individually or in combination have been traditionally used as an Ayurvedic remedy in T2D and related complications [1]. The antidiabetic effect of these plants has been studied in animals [2-6] as well as in humans [7-9]. Despite the numerous studies that have demonstrated that these plants reduce blood glucose levels in a number of species including humans, the biological mechanisms, major site of action and active ingredients for these hypoglycemic effects are not known. Hence, the present study aimed to investigate the effect of aqueous extracts of these plant treatments on glucose uptake by mouse muscle tissues and also insulin secretion from mouse pancreas under normal and hyperglycemic conditions *in vitro*.

Materials and Methods

Plant material preparation

Gymnema sylvestre R.Br. (Apocynaceae) leaves (GS) and Curcuma longa Linn. (Zingiberaceae), rhizome (CL) were purchased from Austral herbs, Uralla, NSW 2358, Australia. Pterocarpus marsupium Roxb. (Febaceae), hardwood (PM) was directly imported from India. Trigonella foenum-graecum Linn. (Febaceae), seeds (TFG) were purchased from local commercial health foods market, Armidale, NSW 2350, Australia. GS, CL and TFG extracts were prepared by soaking 100g of the material in 1 litre of water at ambient temperature overnight. Aqueous extracts of heartwood of PM was prepared by soaking one block (30-35gm) of PM in 10 litre of water at ambient temperature for 10 days. The extracts obtained were then filtered (Whatman no.1) to remove any particles and the filtrate was further centrifuged at 5000rpm for 15 min to remove fine particles and stored at –20°C. Immediately before use the extracts were sterilized through a 0.22µm syringe filter (Sarstedt Australia Pty Limited, South Australia 5095).

Animals and tissue preparation

Male Swiss mice of adult age, weighing approximately 18-22g were obtained from the physiology mouse colony at the University of New England, Armidale, NSW 2351, Australia. They were euthanased via CO_2 asphyxiation. The pancreas and the skeletal

muscle from the abdomen were removed and placed on ice in phosphate buffered saline (PBS) of a neutral pH. Muscle and pancreas tissues were dissected in to strips approximately 5mm long, 2mm wide, 2mm thick and 2mm long, 2mm wide, 2mm thick respectively and rinsed with PBS immediately before the incubation. All of the animal experimentation was approved by University of New England Animal ethics committee and are in accordance with NH & MRC guidelines for animal experimentations. All the experiments were carried out in quadruplicates and repeated once.

Tissue culture bioassay

Pancreas or muscle tissues (5 pieces/well) were separately transferred to the 24 well tissue culture plates containing 1ml of pre sterilized Dulbecco's modified Eagle's medium (Invitrogen Australia Pty Ltd, Australia) through 0.45µm syringe filters, supplemented with 0.1% bovine serum albumin (Sigma Aldrich Pty Ltd, Australia) and 0.01% antibiotic and antimycotic solution (Invitrogen Australia Pty Ltd, Australia). Glucose concentration in the media was 5 mmol/L or 12 mmol/L in order to mimic normal and hyperglycemic culture conditions respectively. Incubation were performed in a humidified atmosphere of 5% CO_2 in air at 37°C for 24 hours, with the treatments (10µl) is compared with saline (0.9% NaCl) as a control, tolbutamide-0.1 mmol/L and metformin - 2 mmol/L (Sigma Aldrich Pty Ltd, Australia) as a positive control in pancreas and muscle culture respectively. Immediately after the experiment, media and tissue samples were stored at -20°C until further examined. All the doses of treatments and positive control were derived from respective preliminary dose response curve experiments (data not shown). A dose that represents a maximal efficacy was chosen and used in this study.

Glucose analysis

The frozen culture media samples were thawed at room temperature and 100µl of muscle culture media samples was analyzed using DADE clinical analyzer (DADE-XL, Dupont, USA) for glucose content. The glucose method was an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method and this method is more specific than general reducing sugar methods [10]. Glucose uptake was calculated based on the difference in glucose concentration between control wells with and without tissues.

Insulin assay

Pancreas culture media insulin concentration was determined by radio-immuno assay (RIA). Insulin concentration of the pancreas culture media samples were calculated in µIU/ml using a linear equation derived from log/logit transformation of the RIA standard curve. Briefly, 100µl of culture media samples and standards (in DMEM) were added to RIA tubes in duplicate followed by the addition of 100µl of anti-bovine insulin (1:8000) and 100µl of radioactive insulin tracer (20,000 cpm/100µl). All these tubes vortexed and incubated at 4°C overnight. After overnight incubation 1.1 ml of 20% polyethylene glycol (PEG) and 50µl of charcoal striped horse serum were added and further incubated for 15-20 min at room temperature then centrifuged at 3000 rpm for 30 minutes followed by aspiration. The pellet containing the bound fraction was immediately counted for γ-decay using an automatic Gamma counter (Wallac, 1470 WIZARD).

Statistical analysis

Experimental data were analyzed statistically, using the general linear model procedure in SAS statistical software (SAS Institute Inc. Cary, NC, USA). The data were evaluated using 2-way ANOVA followed by Student-Newman Keuls post hoc test. Values were considered to be significantly different at $p < 0.05$ and presented as mean ± standard error.

Results

The effects of TFG, CL, GS and PM treatments on glucose uptake and on insulin secretion under hyperglycemic conditions have been converted as folds of control and presented in Table 1.

Muscle glucose uptake

There was no significant difference in muscle glucose uptake under basal culture conditions between the control and the extracts ($p > 0.05$). Under hyperglycemic conditions plant treatments, CL, TFG and PM, significantly increased the glucose uptake activity ($p > 0.05$). However, under similar experimental conditions, GS failed to produce a significant glucose uptake activity. Treatment with CL extract showed a peak glucose uptake activity among other plant treatments and this effect was significantly different from the control and also directly comparable with the effect of

Table 1. Effects of some Ayurvedic plant treatments on tissues involved in glucose homeostasis *in vitro*.

Treatment	Glucose uptake in folds of control		Insulin in folds of control	
	Basal	Hyperglycemic	Basal	Hyperglycemic
Control	1.00 ± 0.07^{A}	1.02 ± 0.02^{A}	1.00 ± 0.05^{AB}	1.00 ± 0.06^{A}
TFG	0.97 ± 0.07^{A}	1.54 ± 0.03^{C}	1.00 ± 1.31^{AB}	2.05 ± 0.14^{B}
GS	0.84 ± 0.07^{A}	0.96 ± 0.05^{A}	0.94 ± 0.03^{A}	2.44 ± 0.15^{C}
CL	0.98 ± 0.06^{A}	1.66 ± 0.11^{C}	1.00 ± 0.11^{AB}	2.54 ± 0.16^{C}
PM	0.97 ± 0.06^{A}	1.26 ± 0.07^{B}	1.04 ± 0.05^{AB}	3.76 ± 0.42^{D}
Metformin	1.08 ± 0.03^{A}	1.65 ± 0.18^{C}	-	-
Tolbutamide	-	-	1.11 ± 0.06^{B}	3.36 ± 0.15^{E}

Data are expressed in mean ± standard error

Means without a common letter are significantly different ($p < 0.05$)

metformin.

Pancreas insulin secretion

Under basal culture conditions, all the treatments showed no significant difference from control in stimulating insulin secretion from pancreas ($p > 0.05$). However, all treatments under hyperglycemic conditions showed a significant increase in the media insulin and they were significantly different from the control ($p < 0.05$). The maximal media insulin was observed with the PM treatment and it was significantly greater than the media insulin of tolbutamide treatment ($p < 0.05$).

Discussion

The present study for the first time reports the effects of these treatments on insulin secretion and action under both and basal and hyperglycemic culture conditions. Glucose uptake under basal and non-basal culture conditions has been reported to be the one of the most sensitive indicators of in vitro functional viability of the tissues [11]. The results of glucose uptake from the present experiments, compares favorably with earlier reports in the literature, and moreover significant response of the tissues when treated with metformin substantiates that the tissue culture protocol is functional as well as metabolically viable [12, 13]. Similarly, the significant response of pancreas with tolbutamide and also their significant stimulatory response from basal to hyperglycemic conditions demonstrate that the *in vitro* assay technique is metabolically functional and reproducible.

Of these plant treatments, TFG, CL and PM showed a significant glucose uptake activity, suggesting these plant treatments may have either protective effects on tissues to keep them metabolically viable under hyperglycemic conditions or mimic insulin action or act via insulin-mediated enhanced peripheral glucose uptake like metformin [14]. However the GS treatment failed to produce enhanced activity under similar conditions, which supports the earlier findings, where it has been suggested that GS extract have no effect on peripheral glucose uptake [15].

Insulin secretion by pancreatic β-cells in response to glycemic control is maintained by glucose, other nutrients, neurotransmitters and other hormones [16]. However glucose is the primary regulator of insulin synthesis and secretion. The enhanced insulin secreting activity of plant treatments in response to glucose, suggest that these treatments have either protective or regenerative activity on β-cell metabolism that leading to insulin secretion.

Conclusion

In conclusion, all these plant treatment enhance glucose homeostasis by increasing either insulin secretion (GS) or peripheral glucose uptake (TFG) or both (CL and PM). The findings of antidiabetic properties in tissue culture-based bioassay support the ethnopharmacological use of these Ayurvedic plant and also indicates the possible mechanistic ways in which they are achieving glucose lowering effects. Moreover, the *in vitro* model and methods described in this study could be used for the development of new antidiabetic drugs. Nonetheless, these promising *in vitro* results must be ascertained in various *in vitro* and *in vivo* animal models of insulin resistance and diabetes.

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