ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is a progressive metabolic disease characterized by hyperglycemia, insulin resistance, dysfunctional or impaired insulin secretion due to β-cell dysfunction and reduced gut hormone (incretins) secretion from gut. T2DM is associated with the risks of myocardial infarction, stroke and microvascular complications as the disease progresses further. The global prevalence of diabetes is estimated to increase, from current to 7.8% by the year 2030. WHO has predicted that the major burden will occur in developing countries. Studies conducted in the last decade in India, have shown high prevalence of diabetes and also indicated that diabetes is increasing rapidly in the urban population. It is estimated that there are approximately 33 million adults (12% of overall diabetics in world) with diabetes in India. This number is likely to increase to 57.2 million in India by the year 2025. Majority of people world wide are suffering from T2DM and India being the major contributor has become the diabetes capital of the World. Though there are many drugs currently available for the treatment of T2DM including the recently launched Exenatide and Liraglutide (Victoza) of Incretin mimetic class and januvia (Sitagliptin) of DPP-IV inhibitor or incretin enhancer class of drugs, they all suffer from having potential long term safety concerns. Herbals (often known as herbal drugs, herbal preparations or dietary supplements including Cinnamon) offer an exciting opportunity for multi-modal therapeutics due to their ability to effectively trigger multiple signaling pathways. However, studies on standardization and mechanistic aspects are sparse. Cinnamon has been considered by Ayurvedic physicians for the treatment of metabolic diseases including diabetes; however mechanisms of actions are not studied in depth. A detailed literature search was carried out for understanding herbal plants with known anti-diabetic potential, target proteins or enzymes and nuclear receptors involved in glucose and lipid homeostasis. Literature review was also done to understand role of genes and coactivators involved in energy expenditure, role of PPARs in glucose and lipid regulation and currently available drugs in clinic, β- cell dysfunction in diabetes, hormones and key β-cell GPCRs (FFAR1, GRP119) involved in glucose regulation and glucose-stimulated insulin secretion (GSIS) along with role of incretins (GLP-1, GIP) in glucose homeostasis, and use of C.zeylanicum as anti-diabetic herb.

Objective of this research was to study the effect of hydroalcoholic Cinnamomum zeylanicum extract prepared using aqueous ethanol (50:50, v/v; CZE-3) and aqueous
methanol (30:70, v/v; CZE-2) on glucoregulatory enzymes, PPARs, pancreatic GPR40 and effects on the expression levels of pancreatic β-cell genes involved in maintaining glucose homeostasis that might be responsible for anti-diabetic effects of C. zeylanicum and rationalize its usage as anti-diabetic supplement. There are studies which demonstrate the inhibitory effects of aqueous Cinnamomum extract on protein tyrosine phosphatase 1B enzyme and some that describe the role of Cinnamaldehyde (one of the constituent) as anti-diabetic agent. Organs like, liver, gut, pancreas, muscle and adipose tissue play an important role in the regulation of physiologic glucose levels in response to food intake and type 2 diabetes. There are various preclinical and clinical studies that has demonstrated beneficial role of cinnamon in glucose and lipid metabolism in diabetic patients. Many regulatory enzymes, transcription factors and pancreatic GPCRs are involved in the regulation of glucose and lipid metabolism in vivo. However, effects of Cinnamomum extract on these enzymes and pancreatic GPCRs have not been studied so far. Thus, studying the effects of C. zeylanicum extract [aqueous ethanol, (50:50 v/v; CZE-3) and aqueous methanol, (30:70 v/v; CZE-2)] on these glucoregulatory enzymes, pancreatic GPCRs and modulatory effects on expression levels of key pancreatic β-cell genes like GLUT2, FFARs, PDX-1 and insulin could unravel the mechanism(s) for anti-diabetic effect. We used HEK293/T cells transiently transfected with PPAR and luciferase reporter construct to test the transactivation potential of different C. zeylanicum extract prepared using acetone, aqueous methanol and aqueous ethanol (CZE-1, CZE-2 and CZE-3). A pancreatic β-cell line (HIT-T15) was used to test the insulin secretagogue activity and gene expression studies. Out of this, CZE-3 demonstrated maximal activity in PPAR, PTP1B inhibition and insulin secretion assays and thus chosen for further studies. Purified recombinant hDPP-IV and PTB1B enzyme was used to test the activity of extract.

We demonstrate that aqueous ethanol extract (CZE-3) of C. zeylanicum has ligands that activate the pancreatic β-cell GPCR, GPR40 (FFAR1), the peroxisome proliferator activator receptor-gamma (PPARγ), inhibits PTP1B protein that negatively regulates insulin signaling and also modestly inhibited DPP-IV enzyme that cleaves the biologically active incretin hormone, glucagon like peptide (GLP-17-36 & GLP-1 7-37). To ascertain the quality of extraction and authentication, we standardized both CZE-2 and CZE-3 for the presence of major markers like cinnamaldehyde and cinnamic acid using reverse phase HPLC. Both the markers were
present in CZE-2 and CZE-3. Further, CZE-3 was extensively studied in vitro for its insulin secretogogue potential in pancreatic HIT-T15 cells, PPARα, γ transactivation using HEK293/T cells, PTP1B inhibition and DPP-IV inhibitory action (if any) using purified enzymes and for effects on intracellular calcium increase in CHO cells stably expressing human GPR40 receptor. We demonstrate that CZE-3 activates PPARγ and this activation is further enhanced in vitro upon overexpression of key co-activators like PGC1α and SRC1 in HEK293/T cells. We also demonstrate that CZE-3 causes glucose-stimulated insulin secretion (GSIS) in pancreatic β-cell line (HIT-T15) in presence of 15 mmols/lit glucose, most likely via activation of surface GPR40 receptor present in this line. Thus, we attribute this effect as one of the responsible mechanisms for in vivo glucose homeostasis. These results correlated with real-time qPCR studies in these cells wherein we observed an up-regulation of insulin mRNA.

In order to confirm the mechanism for CZE-3 mediated insulin secretion in β-cell, we evaluated its effect in FLIPR based assays in hGPR40-CHO cells. CZE-3 dose-dependently augmented intracellular calcium responses via activation of GPR40 receptor in these cells. We also tested CZE-3 for its effect on the expression of key pancreatic β-cell genes; like insulin, pdx-1, gpr40, glucokinase (GK), glucose transporter viz glut2 in HIT-T15 cells. CZE-3 treatment caused significantly increased pdx-1 expression levels in HIT-T15 cells treated for 60 and 90 min. Oral administration of CZE-3 (120 mg/kg, p.o) in C57/BL6J male mice (n=8), caused significant improvement in % glucose lowering as compared to vehicle control group. Oral administration of CZE-3 (150 mg/kg, p.o) in male BKS Cg.Dock 7 db/db mice (n=8, age: 12 week old), also showed a significant improvement (**P <0.01) in glucose excursion (total AUC) as compared to vehicle control group.

Thus we conclude that aqueous ethanol extract (CZE-3) of C.zeylanicum exhibits in vivo anti-diabetic effect via acting on multiple pathways; (i) CZE-3 showed activation of PPARγ in vitro. It potently inhibits (ii) PTP1B enzyme which is involved in negative regulation of insulin receptor (IR), (iii) inhibits DPP-IV enzyme thereby may exhibit potential for causing sustained elevation of in vivo GLP-1 levels upon oral administration. (iv) It causes GSIS in pancreatic β- cell line likely via activation of GPR40 receptor leading to increased intracellular calcium levels as confirmed by studies in CHO cells stably overexpressing hGPR40 R. CZE-3 causes in vivo glucose homeostasis by all of the above mechanisms and its use as food supplement and medication for diabetic patients would be beneficial.