



MODULATION OF THE PLASMA GLUCOSE, INSULIN AND LIPID PROFILE LEVELS OF NEONATAL STREPTOZOTOCIN (NSTZ) INDUCED PRE-DIABETIC RATS BY BITTER GOURD (*MOMORDICA CHARANTICA*)

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Abstract

The main aim of this study is to investigate the effect of bitter gourd on long term pre-diabetes induced blood glucose, insulin and plasma lipid profile alterations in neonatal streptozotocin (nSTZ) induced rat model. Male Sprague Dawley (SD) rat pups (n=35) of two-day old were taken and injected with STZ (90 mg/kg body weight) dissolved in 0.1M citrate buffer, pH 4.5. Control pups (n=10) received only vehicle. Oral glucose tolerance test (OGTT) was conducted at 2nd and 10th month. All rats were maintained on AIN93G/M diet in individual cages and a sub set of pre-diabetic animals received 5% bitter gourd in the diet. Majority of nSTZ rats exhibited impaired glucose tolerance (2h glucose >140mg/dl) or pre-diabetes by 2 months and maintained up to 11th month. Feeding of bitter gourd to these pre-diabetic rats partially prevented these changes. Pre-diabetes induced blood glucose, insulin and lipid alterations were partially prevented by bitter gourd through its hypoglycemic and antioxidant potential. This study will give an additional insight into molecular action of bitter gourd in preventing the pre-diabetic abnormalities thereby leading to the development of therapeutic strategies in controlling pre-diabetic complications.

Key words: Pre-diabetes, HOMA-IR, bitter gourd, lipid profile and STZ

Introduction

T2DM is preceded by a latent and asymptomatic phase called pre-diabetes (PD). This condition includes fasting and postprandial glucose disorders known as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), respectively. The prevalence of PD in India varies from 8.1 to 29.8% (Ramachandran *et al.* 2001; Ramachandran *et al.* 2003; Mohan *et al.* 2007; Radha *et al.*, 2007), further increasing the burden of T2DM and subsequent short- and long-term complications. The prevalence of PD is increasing worldwide; it is projected that more than 471 million people will have PD by 2035 (Guariguata *et al.* 2014). PD can be readily identified through screening and is potentially modifiable through simple and cost-effective measures,

providing us with effective ways to combat the health care burden of diabetes (Gillies *et al.* 2008).

Alteration of lipid metabolism is one of the factors that have been implicated in the development of diabetic complications. This is because in insulin deficiency or insensitivity, hormone sensitive lipase is activated, leading to increased mobilization of free fatty acids (FFAs) from the peripheral depots with decreased peripheral utilization. The excess FFAs are catabolized to acetyl CoA. The acetyl CoA cannot be readily utilized as the availability of oxaloacetate is reduced and TCA cycle activity is sluggish. The result is that the excess acetyl CoA is channeled to the pathway of cholesterol biosynthesis leading to hypercholesterolemia and hyper-lipidaemia characterized by elevated levels of cholesterol, triacylglycerol and low density lipoprotein (LDL)-cholesterol (Eleazu *et al.* 2014).

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The glycation and oxidation of LDL will delay their catabolism and promote their uptake by macrophages which process is dysregulated. This is the starting point of atherosclerosis leading to myocardial infarction. Furthermore, alteration in HDL activity further increases the risk of coronary heart disease in people with diabetes (Vasudevan and Seekumari 2013). The mainstay of the management of diabetes mellitus is pharmacological (exogenous insulin or hypoglycemic drugs administration) or non-pharmacological (diet, exercise and surgery) treatments, all of which measures have their various shortcomings as per reversing the course of the disease (Ejike *et al.* 2014). In addition, the costs and adverse effects associated with the use of synthetic drugs in the management of this disease, coupled with the abundance of plant materials with promising antidiabetic potentials, have led to the recommendation by the World Health Organization for the management of diabetes mellitus, using herbal medicine (World Health Organization 1980). Moreover, these medicinal plants play important roles in the lives of rural people, particularly those in the remote parts of developing countries that have limited access to adequate health facilities (Eleazu *et al.* 2014).

Momordica charantia is commonly known as bitter gourd (BG) and is widely consumed as a vegetable worldwide, including India. Consumption of BG is associated with a variety of health-promoting benefits, including hypoglycaemic properties in both type-1 and type-2 diabetic individuals (Platel and Srinivasan 1997). With a few exceptions, studies have shown BG to have beneficial effects (Karunanayake *et al.* 1993; Platel and Srinivasan 1997). Earlier studies reported that consumption of BG juice increases glucose uptake by tissues *in vitro* (Welihinda *et al.* 1986). In addition, it has been shown to increase glycogen storage in the liver, stimulate secretion of insulin by isolated beta cells of the pancreas (Welihinda *et al.* 1982) and reduce systemic arterial blood pressure. The primary purpose of this study was to assess the effects of freeze-dried BG *in vivo* against PD-induced plasma glucose, insulin and lipid profile alterations in nSTZ-PD model.

Materials and Methods

Animals

Forty five male Sprague-Dawley rat pups weighing 8-9 g (obtained from the National Center for Laboratory Animal Sciences, National Institute of Nutrition Hyderabad, India) were used in this study.

Preparation of bitter gourd powder

Fresh bitter gourd was obtained from the local market and was lyophilized. The dried bitter gourd was

pulverized to powder form and added to AIN-93 diet and was fed to the experimental animals.

Induction of pre-diabetes and experimental groups

Female pregnant rats which were obtained from the NCLAS (National centre for laboratory animal science) were maintained on stock diet and followed till delivery. After 2 days of delivery, male pups were selected for this experiment. These two-day old rat pups (n=35) were injected with a single intraperitoneal injection of streptozotocin (STZ) at a dose of 90 mg/kg body weight which was dissolved in 100mM citrate buffer of pH 4.5. Control pups (n=10) received only citrate buffer. Only nSTZ injected rats having postprandial blood glucose levels more than 140 mg/dL and fasting blood glucose levels between 100-125 mg/dL at 2 months after STZ injection were considered as pre-diabetic and included in the study. Pre-diabetic rats were further divided into two groups; pre-diabetic untreated (PD, n=9) rats maintained on AIN 93 diet and pre-diabetic rats fed with 5% bitter gourd mixed with AIN 93 diet (PD+BG, n=9). Control rats were maintained on AIN 93 diet. All rats were maintained in the temperature and humidity controlled rooms with 12 hours light-dark cycle. All rats had free access to water. Body weights of rats were monitored once in a month throughout the study.

Oral glucose tolerance test (OGTT)

OGTT was performed after 2nd and 10th month of STZ injection on overnight fasted rats by administering glucose orally as a bolus, at a dose of 2.0 g/kg of body weight. Blood samples were collected at 0, 30, 60 and 120 minutes time intervals for estimating plasma glucose and insulin concentrations to assess impaired glucose tolerance (IGT) and insulin resistance.

Clinical parameters

Plasma glucose was estimated in OGTT samples by the GOD-POD method with a commercially available kit (Biosystems). Plasma Insulin levels were estimated by RIA kit (BRIT-DAE, Mumbai, India).

Estimation of triglycerides

Plasma triglyceride was estimated enzymatically by using commercially available kit (Biosystems, Barcelona, Spain) following the instructions of the manufacturer.

Estimation of total cholesterol

Total cholesterol was estimated in the plasma by using commercially available kit (Biosystems, Barcelona, Spain).

Estimation plasma free fatty acids (FFA)

Free fatty acids estimated in plasma samples at the

end of the experiment using a commercially available kit (BioAssay Systems, Hayward, USA).

Statistical analysis

By using SPSS 19.0 software all statistical analyses were performed. All quantitative data were presented as mean \pm standard deviation (SD). Differences among means were analyzed by one-way ANOVA test, followed by Tukey HSD and student test. Statistical significance was set at $p < 0.05$.

Results

OGTT after 2nd and 10th month and pre-diabetes

In the present study, after 2nd and 10th month of STZ injection, OGTT graph showed higher plasma glucose and decreased insulin levels in all the time points except 0 min in nSTZ injected rats when compared to control (FIG 1A and 1B) indicating development of IGT or pre-diabetes by two months and maintained pre-diabetes upto 11 months. Moreover after 10 months of STZ injection, BG treated PD rats showed a slightly lower plasma glucose and improve insulin levels when compared to PD rats in all the time points except at 0 min fasting plasma glucose (fig 1).

Plasma Lipid Profiles

Hypercholesterolaemia and hypertriglyceridemia were observed in the nSTZ-PD rats, but not the control rats. Plasma cholesterol and triglyceride levels were not significantly different among the treatment groups (table 1).

Table 1: Plasma lipid profiles. Triglycerides and cholesterol were analysed at the end of the experiment.

Parameters	Control	PD	PD+BG
Triglycerides (mg/dl)	32.94 \pm 3.46	45.83 \pm 18.79	44.15 \pm 6.25
Total cholesterol	66.23 \pm 16.18	81.00 \pm 14.65	80.40 \pm 7.35
Free fatty acids	336 \pm 151	339 \pm 184	340.17 \pm 150.98

Values are means \pm SD, n = 9. PD, prediabetes; BG, bitter gourd

Discussion

Several animal models have been developed to investigate PD or IGT-associated pathophysiology, including the frequently used nSTZ model (Iwase *et al.* 1986, Grill *et al.* 1987, Welsh and Hellerstrom 1990, Hemmings and Spafford 2000, Arulmozhi, Veeranjanyulu and Bodhankar 2004). In the present study, we evaluated

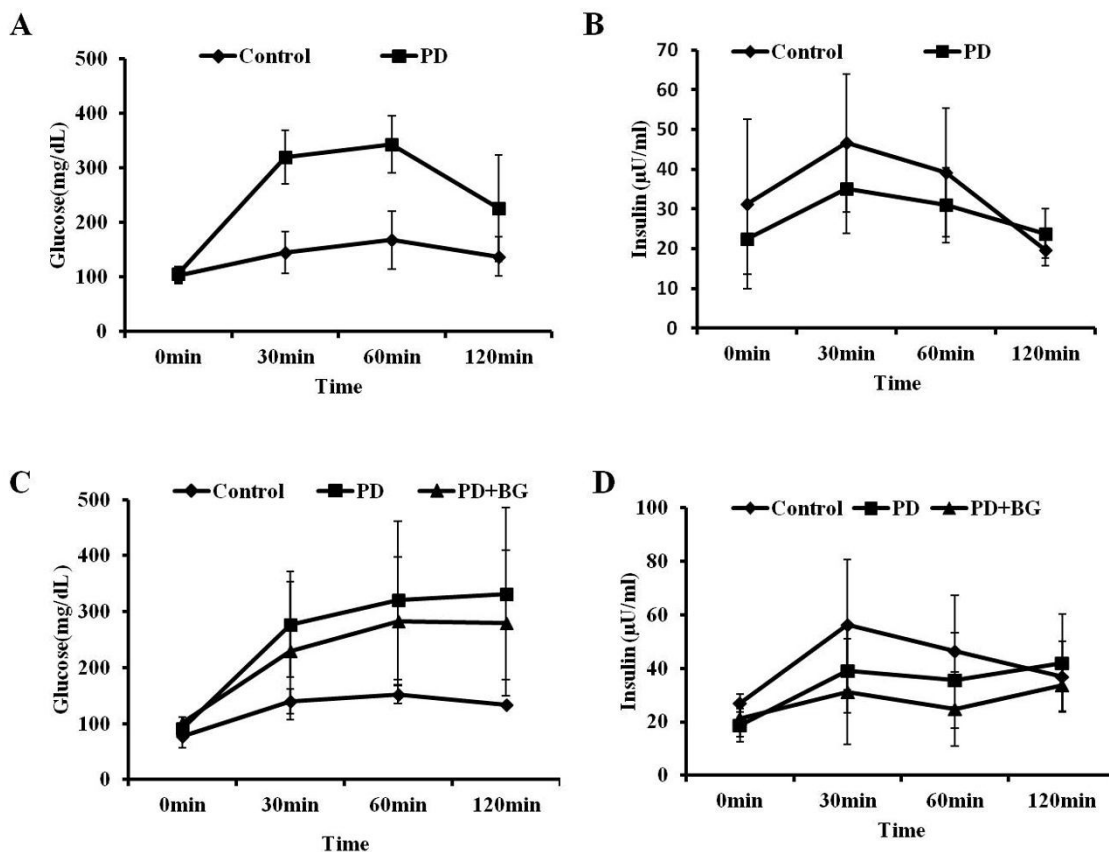


Fig. 1: Glucose and insulin response at various time points (0, 30, 60, and 120 min) during an OGTT in overnight-fasted rats. OGTT was conducted in control, PD, and PD+BG group rats 2 and 10 months after feeding their respective diets (PD or PD+BG). Values are means \pm SD, n = 6–9. OGTT, oral glucose tolerance test; PD, prediabetes; BG, bitter gourd

the nSTZ induced PD rat model for development of retinopathy, cataracts, and nephropathy, as well as the biochemical alterations responsible for these complications. According to previous reports, STZ-treated neonatal rats exhibit slightly elevated plasma glucose levels and low pancreatic insulin content at adulthood (Arulmozhi *et al.* 2004, Inoue *et al.* 1994, Tourrel *et al.* 2001, Giroix *et al.* 2002). Previously, we did not observe the development of diabetic characteristics in nSTZ-induced Wistar-NIN rats (Suryanarayana *et al.* 2011); however, in the present study we used Sprague Dawley (SD) rats and observed the development of IGT in most (70%) animals. Interestingly, though all the PD rats exhibited lower fasting insulin levels than that of normal rats, their fasting glucose levels remained normal throughout the experimental period.

The development of IGT, insulin resistance, or diabetic characteristics in rats depends on the STZ dose, age, duration, and strain of the rats (Suryanarayana *et al.* 2011, Schaffer and Wilson 1993, Arulmozhi *et al.* 2004, Shinde and Goyal 2003, Giroix *et al.* 2012, Goyal *et al.* 2011, Ribeiro *et al.* 2005, Sartoretto *et al.* 2005, Schaffer *et al.* 1997). Previously, it was reported that an intraperitoneal injection of STZ (90 mg/kg) in 2-day old SD rat pups resulted in hyperglycaemia and insulin resistance upon maturity (Goyal *et al.* 2011). In the present study, while none of the animals developed insulin resistance, most of the animals developed IGT with frank hyperglycaemia. These results agree with previous reports reports (Arulmozhi *et al.* 2004, Inoue *et al.* 1994, Tourrel *et al.* 2001, Giroix *et al.* 2002). In this study, 70% of the nSTZ-treated rats developed IGT and remained in an IGT state until the end of the experiment without developing hyperglycaemia.

In this present paper, we find out that long term PD accumulates total cholesterol, triglycerides in plasma leading to early phase cardiac problems in rats. The increased plasma lipids (TG and TC) are partly due to the lower cost of lipid deposition during long term PD. Lose of body weights in PD rats probale low levels of insulin levels because insulin is anabolic hormone. In this present work we find out low levels of HOMA-IR in PD rats compared to control rats. BG marginally prevent these alterations such as glucose, HOMA-IR and lipid profile.

Conclusion

In conclusion, STZ injection to neonatal SD rat pups had developed IGT/pre-diabetes at two months. Prolonged exposure of these rats to pre-diabetic state had increase plasma glucose levels, decreased insulin and dislipidemia. Feeding of BG to pre-diabetic rats prevented these

abnormalities probably through its hypoglycemic and antioxidant nature. This nSTZ induced IGT/pre-diabetic models can be useful for IGT/pre-diabetes associated complications.

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Conflict of Interest: None declared

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