Puguntano Extract Supplementation Enhances Insulin Secretion and Decreases Insulin Resistance in High Fat Diet/Streptozotocin-induced Type 2 Diabetic Rats

Santi Syafril[1*], Dharma Lindarto[1]
[1]*Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara-H. Adam Malik General Hospital, Medan, Indonesia*
Santi Syafril. Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara-H. Adam Malik Hospital, Taman Setiabudi Indah Blok L no.18 Medan, 20122, Indonesia.

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Abstract: BACKGROUND: Secondary metabolites of puguntano (*Curangafel-terrae* [Merr.]) which contains flavonoids, saponins, tannins, and steroids/ terpenoids, increased insulin secretion and decreased insulin resistance in type 2 diabetes mellitus (T2DM). AIM: This study investigated the effect of puguntano leaf extract to insulin secretion and insulin resistance in high-fat diet/streptozotocin-induced type 2 diabetic rats. METHODS: A combination of high-fat diet-feeding (HFD) and multiple low dose intraperitoneal injections of streptozotocin(STZ) was used to induced T2DM in 48 male Wistar rats, which were then randomly divided into control and treatment groups (n = 24 per group). Puguntano leaf extract was administered to the treatment group once daily (200 mg/kg.bw) for 10 days. Insulin secretion (HOMA-β) and insulin resistance (HOMA-IR) were measured from fasting glucose and fasting serum insulin concentration by homeostasis model assessment (HOMA) in control group after becoming T2DM and in the treatment group after 10 days of puguntano treatment. Data were analyzed using the Wilcoxon test. RESULTS: HOMA-β was significantly higher in the treatment group than in the control group, while HOMA-IR was significantly lower in the treatment group than in the control group ($p<0.05$). CONCLUSION: This study demonstrated that puguntano leaf extract treatment may significantly enhance insulin secretion and decreased insulin resistance in T2DM rats.

1 INTRODUCTION

Impaired insulin secretion from β-cells of the pancreas, resistance to tissue actions of insulin, or a combination of both is the pathophysiology of type 2 diabetes mellitus (T2DM). The spectrum of the disease originally is arising from tissue insulin resistance and gradually progressing to a state characterized by complete loss of secretory activity of the beta cells of the pancreas (Chaudhury et al., 2017). In T2DM, both its action and secretion are impaired (Irsad et al., 2011). Homeostatic model assessment (HOMA) is a method for assessing β cell function (HOMA-β) and insulin resistance (HOMA-IR) from basal (fasting) glucose and insulin concentrations (Wallace et al., 2004)

The incidence of diabetes is alarmingly increasing throughout the world. One of the worst affected areas appears to be Asia where diabetes could rise two- to three-fold in the near future. It is crucial to identify effective and low-cost medications for treating diabetes considering the economic constraints. Alternative strategies to the current pharmacotherapy of diabetes such as herbal medicine are urgently needed because of the enormous cost and limited access to modern therapies for many rural populations in developing countries(Hafizur et al., 2012)

Puguntano (*Curangafel-terrae* [Merr.]) is a plant belonging to the Scrophulariaceae family found in TigaLingga village, Dairi regency, Sumatera Utara province, Indonesia (Sitorus et al., 2014). Puguntano traditionally has been used as a drug of colic, malaria, diuretic, fever, and skin disease. Modern pharmacological investigations indicated that the extract of puguntano exerts diuretic, antipyretic, hepatoprotective, cardioprotective, antidiabetic,

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antioxidant, anti-inflammatory, anthelmintic, antiasthma, and analgesic activities (Sitorus et al., 2017).

Phytochemical analysis of puguntano showed that the major phytochemicals are flavonoids, saponins, tannins, and steroids/terpenoids, which have anti-diabetic activity (Harahap et al., 2013).. Tannins decrease insulin resistance through enhancing the glucose uptake via mediators of the insulin-signaling pathways (Kumari et al., 2012). and enhance insulin secretion through inducing β-cell regeneration (Kim et al., 2017). Flavonoids enhance insulin secretion by preventing β-cell apoptosis and promoting β-cell proliferation and enhance insulin activity (Vinagayan et al., 2015). Terpenoidsdecrease insulin resistance through increasing *glucose transporter-4* (GLUT-4) expression and translocation via*proliferator-activated receptor gamma* (PPAR- Y) activation (Goto et al., 2010;Song et al., 2004;Monsalve et al., 2013). Saponindecrease insulin resistance through increasing insulin signaling via increasing GLUT-4 expression and increase insulin secretion from the beta cell islets (Elbarky et al., 2017).

Lindarto et al., reported that insulin resistance is decreased in newly diagnosed T2DM patients after treatment with puguntano leaf extract for 12 weeks, showed by the significant a significant decrease in fasting blood glucose (FBG) levels and HOMA-IR, a significant increase in adiponectin levels, while there is the increase in HOMA-β but is not statistically significant (Lindarto et al., 2016).

In this study, we investigate the effect of puguntano leaf extract to insulin secretion and insulin resistance in high-fat diet/streptozotocin-induced type 2 diabetic rats.

2 MATERIAL AND METHODS

Male Wistar rats (180–200 g) were used throughout the study. The rats were kept and maintained in laboratory experimental animal units under standard conditions of temperature and humidity with a 12h light:12 h darkness cycle. The rats were maintained in clean cages with ad libitum access to water and food. All animals were treated according to the guideline for care and use of laboratory animals with the approval of the Ethics Committee of Universitas Sumatera Utara, Medan, Indonesia (Reference 42/TGL/KPEK FK USU-RSUP HAM/2018). A high-fat diet (HFD) feeding for 5 weeks, followed by two intraperitoneal injections of streptozotocin (STZ) (30 mg/kg; Sigma-Aldrich, Munich, Germany) was given to induced T2DM. After this, FBG levels were determined in blood obtained from a lateral tail vein using a glucometer (Roche Diagnostics). The rats with FBG levels of more than 200 MD/dL were used for this study (Zhang et al., 2008)

Forty-eight diabetic rats were randomly divided into two groups (a control group and a treatment group) of twenty-four rats each. The treatment group was treated with 200 mg/kg/day ethanolic extract of puguntano leaves using an orogastric cannula for 10 days. Control rats were sacrificed on the day their diabetes was confirmed, while the puguntano-treated rats were sacrificed after 10 days treatment period was complete.

After anesthesia with ketamine, the rats were decapitated, and blood was obtained from the left ventricle for the measurement of FBG by spectrophotometry and fasting insulin using a sandwich ELISA. Insulin secretion was assessed using HOMA-β equation and insulin resistance was assessed using HOMA-IR equation, which is calculated using fasting insulin and glucose concentrations. The HOMA-IR and HOMA-β were calculated using the formula given below [3]:

HOMA-IR= [(fasting plasma insulin in μU/L) x FBG in mg/dL)/405]

HOMA-β = [(360 x fasting plasma insulin in μU/L) / (FBG in mg/dL - 63)].

The study was conducted in the Molecular Genetics Laboratory, Medical Faculty of Universitas Padjajaran. The ethanolic extract of puguntano leaves was obtained by maceration methods in the Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia (Kemenkes RI,2013)

2.1 Statistical Analysis

Statistical analyses were performed by using the SPSS 22.0. All values were expressed as the median (minimum-maximum). Statistical difference between the groups was assessed by the Wilcoxon test. P values <0.05 were considered significant.

3 RESULTS

As shown in Table 1, FBG, fasting insulin was significantly lower in the treatment group than in the control group.

Table 1. FBG and fasting insulin in the control and treatment group

	Group		p
	Control (n=24) Med (min-max	Treatment (n=24) Med (min-max)	
FBG (mg/dl)	384 (207-490)	122 (95-213)	0.001*
Fasting insulin (µlU/ml)	56.56 (49.63 – 73.67)	51.31 (47.77-59.00)	0.001*

Wilcoxontest. *Significantif $p < 0.05$

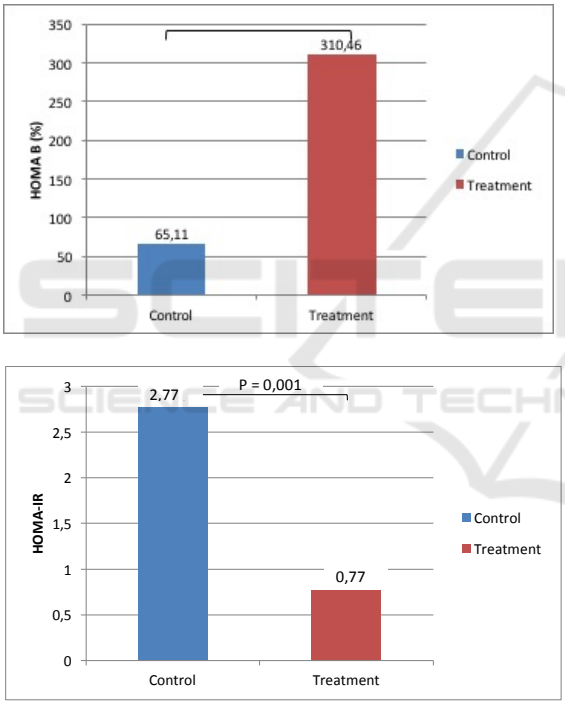

Figure 1.HOMA-β in the control and treatment group Figure

As shown in Figure 1, HOMA- β was significantly higher in the treatment group than in the control group, while HOMA-IR was significantly lower in the treatment group than in the control group.

4 DISCUSSION

An HFD combined with multiple low doses of streptozotocin with the dose 30 mg/kg at weekly intervals for 2 weeks, proved to be a better way for developing an animal model of T2DM which closely simulates the natural pathogenesis of T2DM and is widely used in studies of the efficacy of anti-diabetic drugs. HFD is a better way to initiate the insulin resistance which is one of the important features of T2DM and the low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of T2DM. Feeding the animal with HFD following low-dose STZ would closely mimic the natural history of the disease events (from insulin resistance to β cell dysfunction) as well as metabolic characteristics of human T2DM (Okita et al., 2013)

The secondary metabolites of puguntano leaf have the anti-diabetic activity, which tannins decrease insulin resistance and enhance insulin secretion, flavonoids enhance insulin secretion and enhance insulin activity, terpenoids decrease insulin resistance and saponin decrease insulin resistance and increase insulin secretion from the beta cell islets. Because of these in vitro results, we were tempted to investigate the scientific basis of the use of puguntano leaf for the management of T2DM by the TigaLingga people. This was performed by looking at the antidiabetic activity of puguntano leaf extract on HFD/STZ-induced T2DM rats.

The two main causes of hyperglycemia in T2DM are impaired insulin secretion and increased insulin resistance. Evaluation of insulin resistance (or sensitivity) and β-cell function is important for understanding the disease status and selection of pharmacologic treatment (Akbarzaedah et al., 2018) The insulin resistance was calculated using the HOMA-IR and the steady state of pancreatic β-cell function was measured by calculating the HOMA-β(Sumantri et al., 2017).

This present study has demonstrated significantly lower FBG, fasting insulin levels, and HOMA-IR, and significantly higher HOMA-β in the treatment group with puguntano leaf extract than in the control group. This may be explained by an effect of one or more secondary metabolites the tannins, flavonoids, triterpenoids, and saponins present in puguntano leaf extract to insulin secretion and insulin sensitivity.

A previous study showed that suspension of puguntano extract of 100 mg/kg bb decreased blood glucose level similar to the 10 mg/kg bwglibenclamide suspension (Thakare et al., 2017). Glibenclamide is a second generation of sulfonylureas with the mechanism of action to increase insulin secretion primarily augment the 2nd phase of insulin secretion with little effect on the 1st phase . Another study reported that after 12 weeks

treatment with puguntano leaf extract in newly diagnosed T2DM patients, there was the decrease of insulin resistance as shown as the significant reduction in FBG levels and HOMA-IR (p=0.012, p=0.033; respectively) but there was no significant increase of insulin secretion (HOMA-β)(p=0.262) (Lindart et al., 2016).

This study is the first study to evaluate the effect of puguntano leaf extract *(Turanga feel- terrae* Merr*.)* on insulin secretion and insulin resistance in such a rat model of T2DM.

In conclusion, this study revealed, for the first time, the antidiabetic activity of puguntano leaf extract to insulin secretion and insulin resistance in an animal model of T2DM. Our data strongly suggest that the antidiabetic activity is due to enhanced insulin secretion and improvement in insulin resistance.

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