Correlation between Adiponectin Receptor (AdipoR) with Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), Peroxisome Proliferator-Activated Receptor Gamma (PPAR-ϒ), and p38-mitogen-activated protein kinases (MAPK) in Type 2 Diabetic Rats Treated with Puguntano (*Turanga feel-terrae* Lour.) Leaves Extract

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Abstract: Adiponectin has a role in regulating metabolic processes, anti-inflammation, and insulin sensitizers. This study aimed to examine the correlation between adiponectin receptor (AdipoR) with FPG, insulin, HOMA-IR, PPAR-ϒ, and p38MAPK in rats model of T2DM. A total of 48 Wistar rats aged 8-10 weeks with a weight of 180-200 grams were selected. The Wistar rats were induced into T2DM by feeding high-fat (HFD) diet and injecting small doses of streptozotocin (STZ) 30 mg/kg.bw. The Wistar rats were then divided randomly into 2 groups: control group (n=24) and treatment group (n=24). The treatment group was given puguntano leaves extract at a dose of 200 mg/kg. BW once daily for 10 days. After that, AdipoR, FPG, insulin, HOMA-IR, PPAR-ϒ, and p38MAPK levels were examined. FPG, insulin, and HOMA-IR levels were significantly lower, but p38MAPK and AdipoR levels were significantly higher in the treatment group than in the control group. In the group of all subjects, there was a significant correlation between AdipoR with FPG, insulin, HOMA-IR, PPAR-ϒ and p38MAPK (r=-0.536, p=0.000**, r=-0.416, p=0.003**; r=-0.478, p=0.001**; r=0.587, p=0.000**; respectively). There was a significant correlation between AdipoR with the characteristic of insulin resistance (HOMA-IR) and pathways of post-receptor insulin (PPAR-ϒ and p38MAPK).

1 INTRODUCTION

Adiponectin is widely known as an insulin sensitizer based on its identification, characterization, and underlying mechanisms. Adiponectin can reduce plasma glucose level of rats by suppressing liver glucose production or increasing glucose uptake in the peripheral which is independent of insulin (Berg et al, 2001) inhibiting phosphoenolpyruvate carboxykinase, glucose-6-phosphatase expression (Yamauchi et al, 2002), 5'-AMP-activated protein kinase (AMPK), thereby improving insulin resistance and preventing hepatosteatosis (Liu et al, 2012). Based on other studies, giving adiponectin will increase fatty acid oxidation in the skeletal muscle thereby reducing the content of triglycerides in muscles and liver and increasing insulin sensitivity in vivo (Yamauchi et al, 2001). Adiponectin suppresses the inflammatory response to macrophages in the tissue (Iannitti et al, 2015).

The effect of adiponectin is mediated by adiponectin receptors namely AdipoR1 and AdipoR2 by regulating the expression of metabolic genes and insulin sensitivity in the target tissue of insulin (Yamauchi et al, 2007). The expression of both adiponectin receptors increases fatty acid oxidation, decreases liver triglyceride levels, improves insulin resistance, modulates food intake and energy expenditure, and reduces inflammation. There is a correlation between mRNA expression from adiponectin receptors and adiponectin (Yamauchi et al, 2007; Yamauchi and Kadowaki,

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2008). According to previous studies, administration of the ethanol extract of puguntano leaves *(C. feel-terrae [Lour.])* can significantly improve glucose metabolism and insulin resistance and increase adiponectin (Lindarto et al, 2016) and adiponectin receptor (Lindarto et al, 2019), p38 mitogen-activated protein kinase (MAPK) levels and GLUT-4 expression (Syafril et al, 2019) in the treatment group than in the control group.

The aim of this study was to investigate the correlation between adiponectin receptor with fasting plasma glucose (FPG), insulin, homeostatic model assessment for insulin resistance (HOMA-IR), peroxisome proliferator-activated receptor gamma (PPAR-ϒ), and p38 MAPK in T2DM Wistar rats treated with puguntano leaves extract.

2 METHODS

The study used 48 male Wistar rats aged 8-10 weeks with a weight of 180-200 grams. Rats were placed under the natural light cycle at a temperature of 22-25°C. The Wistar rats were induced T2DM by feeding HFD for 5 weeks, followed by intraperitoneal STZ injections 30 mg/kg.BW (Sigma-Aldrich, Munich, Germany). FPG was measured from the blood of the lateral tail vein using a glucometer in which FPG > 200 mg/dL was considered T2DM (Zhang et al, 2008). Rats were divided randomly into two groups (control group, n=24, and treatment group, n=24). The treatment group was given ethanol extract of puguntano leaves 200 mg/kg.bw/day for 10 days using an orogastric cannula. The control group was sacrificed when was diagnosed with T2DM, while the treatment group was sacrificed after 10 days of treatment. The sacrifice was done by administering anesthesia (ketamine), and the head of the rats was beheaded. After the blood was collected from the left ventricle, FPG (spectrophotometry) and fasting insulin (sandwich ELISA) were examined.

Skeletal muscle samples were processed by homogenization in a cold homogenizing buffer (-80°C) which was used to determine the level of p38-mitogen-activated protein kinases (MAPK), PPAR-ϒ, and AdipoR with Qayeebio kit (China).

The research was conducted at the Molecular Genetics Laboratory, Faculty of Medicine, Universitas Padjadjaran Bandung. The ethanol extract of puguntano leaves was obtained by maceration method in the Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia (Kemenkes RI, 2013). This research has been approved by the Ethics Committee of the Universitas Sumatera Utara, Medan, Indonesia (Reference 42 / TGL / KPEK FK USU-RSUP HAM / 2018).

Statistical analysis was performed using SPSS 22.0 software. All data are expressed as a mean±standard deviation. The Wilcoxon test was used to compare non-normally distributed groups, while the Pearson's or Spearman's test was used for the correlation test. A p-value < 0.05 indicated a statistically significant difference.

3 RESULTS

In Table 1, the FPG, Insulin, HOMA-IR, PPAR, and p38-MAPK levels in the control group had a significant difference with the treatment group.

Table 1. Characteristic Baselines of Subjects

Characteristic	Group			p[a]
	All subjects (n=48) Mean±SD	**Control** (n=24) (Mean±SD)	**Treatment** (n=24) (Mean±SD)	
FPG (mg/dl)	256.10±153.68	375.58±29.15	136.63±33.62	0.000**
Insulin	54.64±5.58	57.36±6.28	52.32±3.32	0.001**
HOMA-IR	1.95±1.17	3.05±0.51	0.86±0.20	0.000**
PPAR-ϒ(ng/mL)	35.18±7.46	29.56±1.06	40.80±6.83	0.000**
p38-MAPK (ng/mL)	22.25±3.82	20.81±3.02	23.70±4.04	0.005**
AdipoR (ng/mL)	15.22±3.21	13.79±1.47	16.64±3.83	0.000**

Data are expressed as a mean ± standard deviation; Wilcoxon test. [a]: Control Group vs Treatment Group; FPG: fasting plasma glucose; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; PPAR-ϒ; Peroxisome Proliferator-Activated Receptor-ϒ; p38-MAPK: p38 mitogen-activated protein kinase.
*: < 0.05; **: <0.01.

Table 2. shows that AdipoR in all subject group significantly correlated with insulin, HOMA-IR, and PPAR-ϒ, whereas there was no correlation found in the control and treatment groups, except for p38-MAPK.

Table 2. Correlation between AdipoR with HOMA-IR, PPAR-ϒ, and p38-MAPK in all groups

Characteristic	Group					
	All subjects (n=48)		Control (n=24)		Treatment (n=24)	
	r	p	r	p	r	p
FPG (mg/dl)	-0.536	0.000**	-0.274	0.196	-0.264	0.212
Insulin	-0.416	0.003**	0.024	0.912	-0.402	0.052
HOMA-IR	-0.478	0.001**	-0.033	0.878	-0.254	0.231
PPAR-ϒ (ng/mL)	0.587	0.000**	0.088	0.680	0.578	0.003**
p38-MAPK (ng/mL)	-0.063	0.670	-0.334	0.106	-0.273	0.197

Data are expressed as mean ± standard deviation; Wilcoxon test. [a]: Group 1 vs Group 2; FPG: fasting plasma glucose; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; PPAR-ϒ; Peroxisome Proliferator Activated Receptor-ϒ; p38-MAPK: p38-mitogen-activated protein kinase. *: < 0.05; **: <0.01.

4 DISCUSSION

Secondary metabolites of the ethanol extract of puguntano leaves identified were glycosides (Zhou, 2005), flavonoids (Huang, 1998), saponins (Fang et al, 2009), and terpenoids (Wang et al, 2006), which can decrease blood sugar levels by stimulating the production and secretion of insulin. Tannin increases glucose uptake via insulin signaling pathways such as phosphoinositide 3-kinase (PI3K), p38-MAPK, and GLUT-4 translocation (Kumari and Tannins, 2012). AdipoR improves glucose metabolism through mechanisms such as increases fatty acid oxidation in the muscles and suppresses lipid cumulation in the liver by activating AMPK so that the content of triglycerides in the liver and muscles decreases and insulin sensitivity is improved (Yamauchi et al,2001).

Adiponectin can stimulate the improvement of the p38-MAPK pathway (Mao et al, 2006) as an anti-inflammatory effect (Xin et al, 2011) and the enhancement of PPAR-ϒ expression through increasing 3T3-L1 cells associated with differentiation of adipocytes (Fu et al, 2005). Adiponectin in diabetic patients had a significant negative correlation with BMI and positive correlation with systolic blood pressure and microalbuminuria (El Dayem et al, 2015).

In this study, treatment with puguntano significantly improved FPG, insulin, HOMA-IR, PPAR-ϒ, p38-MAPK, and AdipoR. The increase in adiponectin receptor correlates significantly with insulin, HOMA-IR, and PPAR-ϒ in all subjects groups.

Conclusion: Puguntano treatment reduces the risk of cardiovascular diseases, while an increase in adiponectin receptor is associated with improved insulin resistance (HOMA-IR) and post-receptor insulin (PPAR-ϒ and p38-MAPK). In addition to insulin sensitivity and post-receptor insulin, adiponectin and AdipoR has various working mechanisms.

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CONFLICT OF INTEREST

The author stated that there is no conflict of interest regarding the publication of this article

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