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THE CORRELATION BETWEEN PROINSULIN/ INSULIN RATIO AND ADIPONECTIN RECEPTOR LEVEL AFTER GIVING THE PUGUNTANO LEAF EXTRACT (Curanga fel-terrae Merr.) IN TYPE 2 DIABETES WISTAR RAT

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Abstract
Introduction: Diabetes mellitus (DM) is a chronic condition characterized by high blood glucose concentration resulting from insufficient insulin production and/ or ineffective insulin action. Proinsulin was synthesized and secreted as a insulin precursor and marker predictor of type 2 DM. Adiponectin has a contributory role in insulin sensitivity through decrease of hepatic gluconeogenesis and increased glucose transport in muscles.

Method: An experimental study, conducted from April to August 2018 at unit of research laboratory of Faculty of medicine in Padjadjaran University, Bandung. The samples were Rat Wistar Strain of type 2 diabetes mellitus (T2DM) model was divided into 2 groups: control group and experimental group. Experimental group was given Puguntano extract. Correlation between proinsulin/ insulin ratio and adiponectin receptor after giving Puguntano leaf extract was analysed with using Spearman correlation. Changes that occurred were assessed with p value of <0.05 was considered statistically significant.

Result: This study did for 48 Rat Wistar Strain of T2DM model divided into 2 groups: control group dan experimental group. The correlation between Proinsulin/ insulin ratio and adiponectin receptor is negative correlation with correlation coefficient r=-0,254 and p value p=0,231. This result shown that non significant in statistical analysis on this study (p>0,05).

Conclusion: There is no significant correlation between proinsulin/ insulin ratio and adiponectin receptor after giving the Puguntano leaf extract (Curanga fel-terrae Merr.) in type 2 Diabetes Mellitus Wistar rat.

Introduction
Type 2 diabetes mellitus is a heterogeneous disease characterized by insulin resistance and defective insulin secretion[1].

Proinsulin is synthesized and secreted as the precursor form of insulin. Proinsulin level is a predictor of type 2 diabetes mellitus (T2DM), obesity,and cardiovascular disease. The PI/I ratio reflects β- cell dysfunction associated with the onset and progression of T2DM[2,3]. An elevated PI/I ratio is attributable to increased secretory demand on β-cells. One study reported sulfonylurea-treated subjects had a significant elevation in proinsulin/IRI ratio compared with diet-treated subjects, whereas non sulfonylurea hypoglycemic agent-treated subjects (metformin, alpha-glucosidase inhibitor, troglitazone) did not[4]. the defect in islet beta-cell processing of the proinsulin molecule that directly reflects the degree of beta-cell dysfunction. Impaired beta-cell secretory capacity induces disproportionately elevated serum proinsulin levels, such as found in subjects with T2DM and impaired glucose tolerance[5].

Adipose tissue was found to produce a variety of adipocytokines including leptin, adipsin and tumor necrosis factor[6-11]. Adiponectin is the recently identified most abundant among of them – is a 30. kDa protein[12-14].

Mechanisms of regulation of adiponectin proposed to be multifactorial. Involvements of genetic factors, glucocorticosteroids, body fat distribution and insulin have been shown in different studies[12,15,16]. Adiponectin serum levels were found to be lower in type 2 diabetes.

Puguntano (*Curangafel-terrae* Merr), a medicinal plant from Scrophulariaceae family, grows in Asia especially in China, India, Indonesia, Philippines, Malaysia and Myanmar. Puguntano leaves from the Dairi area of North Sumatera Province have long been used empirically to control blood glucose levels. Puguntano leaves contain flavonoids, saponins, tannins, and steroids/terpenoids, which have anti-diabetic activity[17,18].

Flavonoids are known to be able to work directly against pancreatic β cells, by triggering the activation of the cAMP signal cascade in strengthening glucose secretion which is sensitized by glucose[19]. Tannins increase glucose uptake through mediators from insulin signaling pathways such as PI3K (phosphoinositide 3-kinase) and activation of p38 MAPK (Mitogen-Activated Protein Kinase) and GLUT-4 translocation. Tannins also inhibit adipogenesis and increase insulin activity so that it becomes a potential drug for the treatment of Non-Insulin Dependent Diabetes Mellitus (NIDDM)[20]. Terpenoids, one of the substances observed in this plant, have also been investigated as having anti-diabetic effects. Where this substance is known through clinical trials has pleiotropic effects such as PPARγ transactivation and NF-kB activation[21].

The cucurbitacin compound in glycosides contained in the Puguntano simplicia powder is thought to provide a decreased blood sugar level effect by stimulating insulin secretion so that a lot of insulin production is released to control blood sugar levels to be normal. In addition, there are phytosterols in the form of β-sitosterol which also play a role in stimulating insulin sensitivity, increasing insulin production, and as an antioxidant to reduce damage that occurs in cells in Langerhans[22].

Saponins act as antioxidants by capturing superoxide and forming hydroxperoxides which prevent biomolecular damage caused by free radicals. The hypoglycemic effect of saponins is through changes in insulin signaling, increased insulin secretion by pancreatic β cells, inhibition of disaccharide activity, activation of glycogen synthesis, inhibition of gluconeogenesis, inhibition of □-glucosidase activity, inhibition of mRNA expression of glycogen phosphorylase and glucose 6-phosphate and increase in GLUT-4 expression[23].

The present study aimed to determine the correlation between proinsulin/ insulin ratio and adiponectin receptor after giving the Puguntano leaf extract (Curanga fel-terrae Merr.) in type 2 Diabetes Mellitus Wistar rat.

Method

Data collection

An experimental study, conducted from April to August 2018 at unit of research laboratory of Faculty of medicine in Padjadjaran University, Bandung. This study was conducted on 48 white Wistar rats (Rattus norvergicus, sp) 8-week-old males who had met the inclusion and exclusion criteria and were divided into 2 groups, namely the control group (K1) and the treatment group (K2).

Inclusion criteria:
a. White rat 8 weeks old
b. Male rat
c. Weight 180-200 grams
d. Rats in good health

Exclusion Criteria:
Mice that experienced other diseases or injuries during the study
The data were analyzed in univariate analysis by analyzing the frequency distribution of independent and dependent variables, while bivariate analysis is an analysis of the variables studied (independent) which are thought to have a relationship with the dependent (dependent) variable. For the correlation analysis of each

group we used Pearson correlation for normally distributed data and Spearman for data that were not normally distributed. The desired significance level is p <0.05. Normality test uses Saphiro-wilk because the number of samples used is less than 50.All data were analyzed with SPSS for window version 22.

Result

This study was conducted on 48 white Wistar rats (Rattus norvergicus, sp) 8-week-old males who had met the inclusion and exclusion criteria and were divided into 2 groups, namely the control group (K1) and the treatment group (K2).

Table 1. Mean blood glucose (BG) levels and body weight (BW) of animals tested Wistar strain rats before and after DMT2

Characteristics	K1 (n=24)	K2 (n=24)	P
Mean BG (mg/dl)			
• Pre DMT2	(77 ± 7)[a]	(75 ± 7)[a]	0,742
• Post DMT2	384 (207-490)[b]	250 (201-385)[b]	0,001
Mean BW (gram)			
• Pre DMT2	209 (200-221)[b]	209 (200-390)[b]	0,634
• Post DMT2	395 (305-430)[b]	375 (308-400)[b]	0,033

[a]normal distribution: mean ± SD
[b] abnormal ditribution: median (min-max)
[*]p value < 0,05 : significant

Table 1 shows the mean (mean), standard deviation (SD), median, minimum and maximum values of Blood Glucose (BG) and body weight (BW) of experimental animals Wistar strain rats before and after T2DM. The mean BG before T2DM in the control group was 77 ± 7 mg / dl and in the treatment group was 75 ± 7 mg / dl. The difference in BG in the two groups before T2DM showed a value of p = 0.742 which means there was no statistically significant difference between the two groups. Whereas the mean BG after T2DM in the control group was 384 (207–490) mg / dl and the treatment group was 250 (201–385) mg / dl. The difference in BG in the two groups after T2DM was found to be p = 0.001 which means there was a significant difference between the two groups namely higher BG levels in the control group compared to the treatment group. The mean BW value of the control group before T2DM was 209 (200–221) grams and in the treatment group there were 209 (200–390) grams. The difference between the two groups shows the value of p = 0.634 which means there is no significant difference between the two groups. While the mean value of the control group after T2DM was 395 (305–430) grams and in the treatment group 375 (308–400) grams were obtained. The difference between the two groups shows the value of p = 0.033 which means that there is a statistically significant difference between the two groups namely BW decreases in the treatment group.

Table 2 shows the mean (mean), standard deviation (SD), median, minimum and maximum values of the levels of adiponectin, proinsulin, insulin and proinsulin / insulin (PI / I) receptors in both groups, the control group (K1) and the treatment group (K2). In the control group, the average value of the adiponectin receptor level was 13.82 (10.88-16.53) µg / ml, whereas in the treatment group it was 16.07 (10.09-28.25) µg / ml. Both groups showed a value of p = 0.001 which means there was a statistically significant difference between the two groups. The mean value of proinsulin in the control group was 192.89 ± 11.44 pg / ml, while in the treatment group it was 188.26 ± 8.69 pg / ml. A value of p = 0.121 indicates there was no statistically significant difference between the two groups. The mean value of insulin levels in the control group was 54.37 (47.77-70.81) IU / ml, whereas in the treatment group it was obtained 55.41 (48.81-73.67) IU / ml. A value of p = 0.404 indicates there was no statistically significant difference between the two groups. Proinsulin / insulin ratio values in the control group were 3.47 ± 0.26%, while in the treatment group obtained 3.39 ± 0.38%. Value of p = 0.398 indicates there was no statistically significant difference between the two groups.

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Table 2. Data on average levels of adiponectin (AdipoR) receptors, proinsulin, insulin and proinsulin / insulin ratio (PI / I) in the control group (K1) and treatment group (K2)

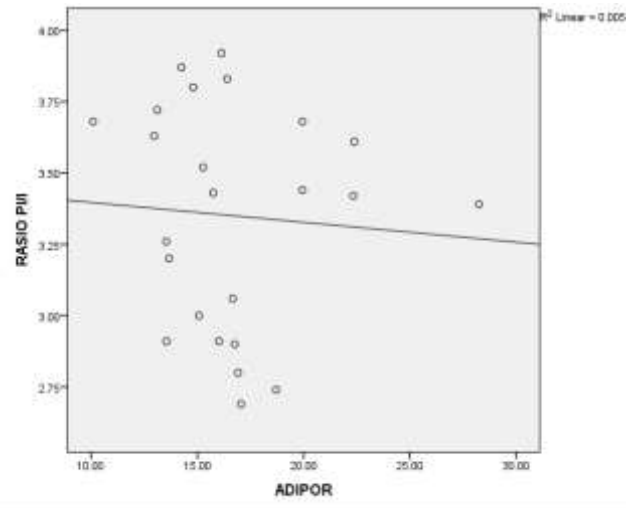
Characteristics	K1 (n=24)	K2 (n=24)	p
AdipoR (µg/ml)	13,82 (10,88-16,53)[b]	16,07 (10,09-28,25)[b]	0,001
Proinsulin (pg/ml)	(192,89 ± 11,44)[a]	(188,26 ± 8,69)[a]	0,121
Insulin (IU/ml)	54,37 (47,77-70,81)[b]	55,41 (48,81-73,67)[b]	0,404
PI/I Ratio	(3,47 ± 0,26)[a]	(3,39 ± 0,38)[a]	0,398

[a]normal distribution: mean ± SD
[b] abnormal ditribution: median (min-max)
[*]p value < 0,05 : significant

Table 3. Correlation of adiponectin receptors (AdipoR) with proinsulin, insulin and proinsulin / insulin ratio after administration of Puguntano leaf extract (Curanga fel-terrae Merr.)

Variables	r	p
Proinsulin (pg/ml)	-0,252	0,235
Insulin (IU/ml)	0,137	0,522
PI/I Ratio (%)	-0,254	0,231

After conducting statistical tests using Spearman correlation (abnormally distributed data) with a sample size of n = 24, the correlation of adiponectin receptors with proinsulin obtained correlation coefficient r = -0.252 and p = 0.235. This shows that the correlation between proinsulin and adiponectin receptors after administration of Puguntano (Curanga fel-terrae Merr.) Leaf extract is negatively correlated with statistical tests that are not significant. Correlation between adiponectin receptors with insulin obtained correlation coefficient r = 0.137 and p = 0.522. This shows the correlation between the two is directly proportional to the statistical test is not significant. Correlation of adiponectin receptors with proinsulin / insulin ratio obtained the value of the correlation coefficient r = -0.254 and p = 0.231. This shows that both of them are negatively correlated with statistically insignificant test (p> 0.05).



Picture 1. Scatter plots AdipoR levels to proinsulin/ insulin ratio

Discussion

Insulin resistance (IR) is a condition that underlies the occurrence of T2DM. IR is characterized by hyperglycemia and hyperinsulinemia. The condition of hyperglycemia is characterized by an increase in blood glucose levels exceeding the normal value. This research has succeeded in inducing white wistar rats into T2DM animal models. From the results of this study it was found that there were significant differences in blood glucose levels (BG) of Wistar strain rats before and after DM induction ($p < 0.001$), where there was an increase in BG in mice that had received DM induction. This happens because DM induction mice type 2 have experienced RI. RI will interfere with glucose uptake by muscle and fat tissue and increase glucose production by the liver. Both of these will cause hyperglycemia[24].

Statistical analysis begins with the analysis of the similarity of the groups being compared, namely the control group and the treatment group. From the calculation results it was found that there were no significant differences in the BG and BW between the control group and the treatment group, so these two groups were suitable to be paired based on the matching process criteria.
In this study, there was a significant difference in the mean body weight of Wistar rats before and after T2DM ($p < 0.001$), it was found that there was an increase in body weight in mice that had been induced into DM. This is because mice that have been induced have undergone lipogenesis (storing excess calories which can be in the form of fat). This has been proven by Kalderon et al. in his research, which explains that hyperglycemia and hyperinsulinemia will increase the activity of central enzymes in lipogenic pathways, such as acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS), NADP-maleate dehydrogenase (NADP-MD) and pyruvate dehydrogenase, which in turn can increase weight[25].

This study found changes in the mean BG and BW of experimental animals Wistar rats and those who did not get treatment Puguntano leaf extract treatment. The BG of experimental animals which had received Puguntano leaf extract had a significant decrease ($p < 0.001$). These results are in line with the study of Lindarto et al., Who compared Puguntano extracts with metformin for 12 weeks and found that Puguntano leaf extract could significantly improve BG[26]. The body weight of experimental animals which had received Puguntano leaf extract also decreased significantly. The reduction in BG and body weight in animals receiving this treatment can be attributed to the extract content of Puguntano leaves.

The reduced insulin response in the first phase is an important inhibitor signal for hepatic glucose release, so it can predict the timing of insulin secretion in early stage T2DM patients. The progression of the disease over time will cause disruption of insulin secretion gradually and irregularly caused by failure of secretion time. Quantitative disorder begins when β cells increase the volume of insulin secretion based on external needs. In the later stages, production capacity will be disrupted and ultimately insulin secretion decreases[27].

Increased levels of proinsulin and the proinsulin / insulin ratio can be strong predictors of β cell dysfunction. Research by Saisho et al said that intact proinsulin levels had a positive correlation with the use of sulfonylureas, BMI, hypertension, glucose, HbA1c levels, insulin and HOMA-IR in univariate analysis[28].

Mean proinsulin and insulin levels in the two groups in this study obtained differences that were not statistically significant ($p > 0.05$). While the mean proinsulin / insulin ratio in Wistar rats in the T2DM model in the control group and the treatment group obtained insignificant differences after being given Puguntano leaf extract ($p > 0.05$). This is in line with research conducted by Harfina et al who said the cucurbitacin compounds in glycosides contained in the Puguntano simplicia powder were thought to have an effect on reducing blood sugar levels by stimulating insulin secretion so that a lot of insulin production is released to control blood glucose levels to be normal. In addition, there are phytosterols in the form of β-sitosterol which also play a role in stimulating insulin sensitivity, increasing insulin production, and as an antioxidant to reduce damage that occurs in cells in Langerhans[22].

Research by Syafril et al in treated T2DM mice (administration of Puguntano extract) showed a significant increase in GLUT-4 expression compared to the control group. This significant increase in GLUT-4 expression is due to the effects of triterpenoid metabolites and saponins from Puguntano leaves which increase glucose

uptake in skeletal muscle tissue in DMT2 mice through translocation and GLUT-4 expression[29]. GLUT-4 plays an important role in maintaining blood glucose homeostasis through translocation and expression in muscle tissue. One defect in post receptor insulin will cause insulin resistance in the target tissue, this includes defects in GLUT-4 function and expression (Gutierrez et al., 2011).

Another study by Lindarto et al. Compared the effects of puguntano extract with metformin for 12 weeks on metabolic and inflammatory parameters in newly diagnosed type 2 DM patients, finding that after metformin administration decreased waist circumference, fasting blood glucose, HbA1c and HOMA-B which is statistically significant, whereas after administration of puguntano extract is effective in reducing fasting blood glucose levels, and HOMA-IR is statistically significant (Lindarto et al., 2016).

Adiponectin (adipoQ / Acrp30) is a protein secreted by mature adipocyte cells into the circulation. In animal and human studies adiponectin has an effect on increasing insulin sensitivity, anti-inflammatory effect, anti-atherogenic effect and affecting lipid profile levels. In this study, differences in the mean value of adiponectin receptors in T2DM Wistar rats in both groups showed significant results ($P < 0.001$) after being given Puguntano leaves.

Study by Lindarto et al in rats induced into T2DM showed that after administration of Puguntano leaf extract there was a significant decrease in body weight, fasting BG and HOMA-IR levels but increased levels of adiponectin receptors compared to the control group. This is due to the content of the leaves of Puguntano can increase glucose metabolism and improve insulin resistance and inflammatory processes through increased adiponectin and adiponectin receptor activity in T2DMsubjects[26].

Study by Ye and Scherer shows that adiponectin has an important role in the metabolism of skeletal and liver muscles. In muscle, the insulin sensitivity function of adiponectin is mediated by AMP kinase (AMPK) and Peroxisome proliferator-activated receptor (PPAR). In the liver, adiponectin activates glucose transport and inhibits gluconeogenesis through AMPK, adiponectin activates fatty acid oxidation and decreases inflammatory reactions through the PPAR pathway. AMPK activation is seen to be mediated mainly by AdipoR1, while PPARa is mediated by AdipoR2. Adiponectin in the liver will increase insulin sensitivity through stimulation of insulin receptor phosphorylation and insulin receptor protein substrate 1 (IRS-1). In the pancreas, adiponectin plays a role in cell proliferation in stimulating insulin secretion. In adipose tissue, adiponectin increases basal glucose uptake and increases insulin stimulated glucose uptake through AMPK activation. In vitro research, explains that adiponectin can regulate lipid metabolism by inhibiting the process of lipolysis. However, a significant increase in lipolysis was observed to occur in both mouse gene adiponectin and primary adipocytes from these mice.

In this study, a correlation test between adiponectin levels with proinsulin, insulin and proinsulin / insulin ratio after administration of Puguntano leaf extract (Curanga fel-terrae Merr.) With the results obtained the correlation between proinsulin and proinsulin / insulin ratio with adiponectin receptors was negatively correlated with statistical tests which is meaningless. While the correlation of adiponectin receptors with insulin levels is directly proportional to the non-statistically significant test.

Adiponectin production is reduced in obesity and causes hepatic insulin resistance. Adiponectin plays an important role in the occurrence of insulin resistance associated with molecular structure and its mechanism of action, namely reducing the content of triglycerides, activating PPAR-α and AMP-Kinase. Low adiponectin levels are one of the risk factors and predictors of T2DM. In addition, some adipocyte and adipokine products stimulate inflammation resulting in an increase in IL-6 and C-reactive protein in T2DM[30].

Conclusion

Based on the results and discussion in this study, it can be concluded that there is no significant correlation between the proinsulin / insulin ratio and adiponectin receptors after administration of Puguntano leaf extract (Curanga fel-terrae Merr.) In Wistar strain T2DM strains.

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