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Correlation Between Adiponectin Receptor with Indices of Glucose Homeostasis and Mediators Of Insulin Sensitivity in Type 2 Diabetic Rats Treated With Puguntano (*Curanga felterrae* Lour.) Leaf Extract

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ABSTRACT. Objective: Adiponectin receptors (AdipoR) regulates metabolism and has anti-inflammatory and insulin-sensitizing effects. We aimed to determine the relationships between AdipoR with parameters of glucose homeostasis (FPG, insulin, and HOMA-IR), and insulin sensitivity (PPAR-γ and p38MAPK) in T2DM rats treated with puguntano (*Curanga felterrae* Lour.) leaf extract. **Methods**: T2DM was induced in Wistar rats aged 8–10 weeks and weighing 180–200 g by high-fat diet (HFD) feeding and low-dose streptozotocin (30mg/kg.bw) administration. The rats were then allocated randomly to a treatment group and a control group (n=24 each). The treatment group was orally administered puguntano leaf extract (200 mg/kg.bb) once daily for 10 days. Subsequently, FPG and plasma insulin were measured, and HOMA-IR was calculated. **Results**: There was significantly difference between treatment group and control group on AdipoR and parameter of glucose homeostasis (FPG, Insulin, HOMA-IR) and insulin sensitivity (PPAR-γ, p38MAPK (all, $p<0.01$). In both groups, there were no significantly correlations between of AdipoR with all parameter of glucose homeostasis and insulin sensitivity except PPAR-ϒ ($p <0.003$) across the entire cohort of rats. **Conclusion:** Our data suggest that puguntano could improve glucose homeostasis, insulin sensitivity and molecular mediators of insulin sensitivity. There were no significantly correlated between improvement of AdipoR with glucose homeostasis and molecular mediators of insulin sensitivity in T2DM.

Keywords: AdiporR, Type 2 Diabetes Mellitus, glucose homeostasis, Insulin sensitivity, *Curanga fel-terrae* Lour.

***Abstrak. Tujuan**: Reseptor Adiponectin (AdipoR) mengatur metabolisme dan memiliki efek anti-inflamasi dan penginderaan insulin. Tujuan penelitian untuk menentukan hubungan antara AdipoR dengan parameter homeostasis glukosa (GP, insulin, dan HOMA-IR), dan sensitivitas insulin (PPAR-γ dan p38MAPK) pada tikus DMT2 yang diobati dengan ekstrak daun puguntano (Curanga felterrae Lour.). **Metode**: DMT2 diinduksi pada tikus Wistar berusia 8-10 minggu dan berat 180-200 g dengan makanan diet tinggi lemak (HFD) dan pemberian streptozotocin dosis rendah (30mg/kg.bw). Tikus kemudian dipilih secara acak ke kelompok pengobatan dan kelompok kontrol (masing-masing n =24). Kelompok pengobatan diberikan ekstrak daun puguntano (200 mg/kg.bb) sekali sehari selama 10 hari. Selanjutnya, GP dan insulin plasma diukur, dan HOMA-IR dihitung. **Hasil**: Ada perbedaan signifikan*

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antara kelompok pengobatan dan kelompok kontrol pada AdipoR dan parameter homeostasis glukosa (GP, Insulin, HOMA-IR) dan sensitivitas insulin (PPAR-γ, p38MAPK (semua, $p<0,01$). Dalam kedua kelompok, tidak ada korelasi yang signifikan antara AdipoR dengan semua parameter homeostasis glukosa dan sensitivitas insulin kecuali PPAR-Υ ($p <0.003$) di seluruh kelompok tikus. ***Kesimpulan:*** *Data kami menunjukkan bahwa puguntano dapat meningkatkan homeostasis glukosa, sensitivitas insulin dan mediator molekul sensitivitas insulin. Tidak ada yang berkorelasi secara signifikan antara perbaikan AdipoR dengan homeostasis glukosa dan mediator molekul sensitivitas insulin di T2DM.*

Kata kunci*: AdiporR, DMT2, homeostasis glukosa, Sensitivitas insulin, Curanga fel-terrae Lour.*

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1 INTRODUCTION

Adiponectin is an insulin-sensitizing adipokine that reduces the plasma glucose concentration of rats by suppressing liver glucose production and increasing peripheral glucose uptake, independent of insulin [Berg *et al*, 2001]. The mechanisms of these effects involve the downregulation of hepatic phosphoenolpyruvate carboxykinase and glucose-6-phosphatase expression [Yamauchi *et al*, 2002], and the activation of AMP-activated protein kinase (AMPK), which ameliorates insulin resistance and prevents hepatosteatosis [Liu *et al*, 2012].

Adiponectin has also been shown to increase fatty acid oxidation in skeletal muscle *in vivo*, thereby reducing triglyceride storage in muscle and liver, and increasing insulin sensitivity [Yamauchi *et al*, 2001]. Furthermore, adiponectin suppresses inflammation associated with macrophage infiltration into insulin target tissues [Iannitti *et al*, 2015].

The effects of adiponectin are mediated by binding to the adiponectin receptors (AdipoR1 and AdipoR2), and regulation of the expression of metabolic genes and insulin sensitivity in insulin target tissues [Yamauchi *et al*, 2007]. Higher levels of expression of both AdipoRs ameliorates insulin resistance, modulates food intake and energy expenditure, and reduces inflammation. Furthermore, the mRNA expression levels of the AdipoRs correlate with adiponectin concentrations [Yamauchi *et al*, 2007; Yamauchi and Kadowaki, 2008].

Previous studies have shown that administration of an ethanolic extract of puguntano leaf *(Curanga felterrae Lour.)* significantly improves glucose metabolism, ameliorates insulin resistance, increases adiponectin concentration [Lindarto *et al*, 2016], and increases the expression of AdipoR [Lindarto *et al*, 2019], p38 mitogen-activated protein kinase [p38MAPK], and glucose transporter-4 [GLUT-4] (Syafril *et al*, 2019) in type 2 diabetes mellitus (T2DM) rats.

We aimed to determine the relationships between AdipoR with indices of glucose homeostasis: fasting plasma glucose (FPG), insulin, homeostasis model assessment-insulin resistance (HOMA-IR); and molecular mediators of insulin sensitivity: peroxisome proliferator-activated receptor-ɣ (PPAR-ɣ) and p38MAPK in T2DM rats treated with puguntano *(Curanga felterrae Lour.)* leaf extract.

2 METHODS

We studied 48 male Wistar rats aged 8–10 weeks and weighing 180–200 g that were maintained under a natural light cycle at a temperature of 22–25°C. T2DM was induced by feeding a high-fat diet (HFD) for 5 weeks, followed by the intraperitoneal injection of 30 mg/kg streptozotocin [Sigma-Aldrich, Munich, Germany].

The control group was sacrificed when diagnosed with T2DM and the treatment group was sacrificed after the completion of puguntano treatment. This was accomplished by the induction of anesthesia using ketamine, followed by decapitation.

Thereafter, blood was then collected from the left ventricle, and FPG (spectrophotometry) was used to measure glucose concentration, and diabetes was considered to be present when the FPG was > 200 mg/dL [Zhang *et al*, 2008], and fasting insulin (sandwich ELISA) were measured. Skeletal muscles were dissected and then homogenized in a cold homogenization buffer (−80°C). The protein levels of p38-MAPK, PPAR-γ, and AdipoR were determined in these homogenates using a Qayeebio kit (China). The rats were then allocated at random to a treatment group and a control group (n=24 each).

The ethanolic extract of puguntano leaves was obtained by maceration in the Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. The treatment group was administered an ethanolic extract of puguntano leaf in carboxylmethylcellulose-Na (CMC Na; 0.5% solution; 200 mg/kg/day) by gavage daily for 10 days [Ministry of Health Republic of Indonesia, 2013].

This research has been approved by the Ethics Committee of Faculty Medicine and H. Adam Malik Hospital (Reference 42 /TGL/ KPEK FK USU-RSUP HAM/2018).

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 [IBM, Inc., Armonk, NY, USA]. All data are expressed as means ± standard deviations. The Wilcoxon test was used to compare non-normally distributed data, and Pearson's or Spearman's correlations were calculated for pairs of variables. A *p*-value < 0.05 was taken to indicate statistical significance.

3 RESULTS AND DISCUSSION

There were significant differences in FPG, plasma insulin, HOMA-IR, and muscle PPAR-γ, p38 MAPK, and AdipoR protein levels between the treatment and control groups (Table 1).

Table 1 Comparison between treatment and control groups on parameter of glucose homeostasis and insulin sensitivity

Parameter	**Treatment group (n=24)**	**Control group (n=24)**	***p*[a]**
FPG (mg/dl)	136.63±33.62	375.58±29.15	0.001**
Insulin (nU/L)	52.32±3.32	57.36±6.28	0.001**
HOMA-IR	0.86±0.20	3.05±0.51	0.001**
PPAR-γ (ng/mL)	40.80±6.83	29.56±1.06	0.001**
p38MAPK (ng/mL)	23.70±4.04	20.81±3.02	0.005**
AdipoR (ng/mL)	16.64±3.83	13.79±1.47	0.001**

Data are expressed as mean ± standard deviation. [a]The Wilcoxon test was used to evaluate differences between the control and treatment groups. FPG: fasting plasma glucose; HOMA-IR: homeostatic model assessment-insulin resistance; PPAR-γ: peroxisome proliferator-activated receptor-γ; p38 MAPK: p38 mitogen-activated protein kinase

Total muscle AdipoR protein levels significantly correlated with FPG, insulin, HOMA-IR, and muscle PPAR-γ protein across all the rats studied. However, there were no correlations identified separately in the control and treatment groups, except for one between total muscle AdipoR protein and muscle p38MAPK protein in the treatment group (Table 2).

Table 2 Correlations of AdipoR with indices of glucose homeostasis and molecular mediators of insulin sensitivity in each group and across all the rats studied.

Parameter	**Treatment Group (n=24)**		**Control group (n=24)**	
	r	*p*	r	*p*
FPG (mg/dl)	−0.264	0.212	−0.274	0.196
Insulin (nU/L)	−0.402	0.052	0.024	0.912
HOMA-IR	−0.254	0.231	−0.033	0.878
PPAR-ϒ (ng/mL)	0.578	**0.003**	0.088	0.680
p38MAPK (ng/mL)	−0.273	0.197	−0.334	0.106

Data are expressed as means ± standard deviations. FPG: fasting plasma glucose; HOMA-IR: homeostatic model assessment-insulin resistance; PPAR-γ: peroxisome proliferator-activated receptor-γ; p38MAPK: p38 mitogen-activated protein kinase. Statistically significant correlations are shown in bold

4 Discussion

The secondary metabolites derived from ethanolic extracts of puguntano leaf have been identified to be glycosides [Zhou, 2005], flavonoids [Huang, 1998], saponins [Fang *et al*, 2009], terpenoids [Wang *et al*, 2006], and these have been shown to reduce blood glucose by stimulating the synthesis and secretion of insulin. In addition, tannins increase glucose uptake by activating the phosphoinositide 3-kinase (PI3K) and p38MAPK signaling pathways and promoting GLUT-4 translocation [Kumari and Tannins, 2012].

AdipoRs mediate improvements in glucose metabolism through mechanisms such as an increase in muscle fatty acid oxidation and the suppression of lipid accumulation in the muscle and liver, which improves insulin sensitivity. These effects are in large part thought to be mediated through the activation of AMPK [Yamauchi et al, 2001]. However, adiponectin also activates the p38MAPK pathway [Mao et al, 2006], which has anti-inflammatory effects [Xin *et al*, 2011], and increases PPAR-γ expression, thus promoting adipocyte differentiation [Fu *et al*, 2005]. Furthermore, in diabetic patients, adiponectin concentrations demonstrate a significant negative correlation with body mass index and positive correlations with systolic blood pressure and microalbuminuria [El Dayem *et al*, 2015]. Treatment with a combination of 0.2 mg/ml of a water/ethanol extract of *Momordica charantia* fruit and seeds and 0.5 nM insulin significantly increases glucose uptake and adiponectin secretion in 3T3-L1 adipocytes [Roffey *et al*, 2007]. In addition, administration of an American ginseng (*Panax quinquefolius*) extract containing a quantifiable amount of ginsenosides reduces cell growth and lipid accumulation, and increases adiponectin expression in 3T3-L1 cells [Yeo *et al*, 2011]. Adiponectin also has anti-atherogenic effects and its effects are similar, but additive to, the effects of insulin on metabolism and the vascular endothelium. However, the best-known relationships are the inverse relationships between adiponectin concentration and obesity and insulin resistance [Balsan *et al*, 2015]. Finally, AdipoR ameliorates diabetes associated with obesity and increases exercise endurance, which prolongs the shortened lifespan of obese mice fed an HFD.

We don’t have identified significant correlations between muscle AdipoR proteins, indicators of glucose homeostasis, and key molecular mediators of insulin sensitivity except PPAR-ϒ in muscle in T2DM rats. It is expected that the current findings will contribute to the elucidation of AdipoR-mediated signal transduction and further encourage the development and optimization of AdipoR-targeting therapeutics for obesity-related diseases, such as diabetes [Okada-Iwabu *et al*, 2015].

5 Conclusion

The study suggest that puguntano could improve glucose homeostasis, insulin sensitivity and molecular mediators of insulin sensitivity. There were no significantly correlated between improvement of AdipoR with glucose homeostasis and molecular mediators of insulin sensitivity in T2DM.

REFERENCES

[1]. Balsan, G.A., Vieira, J.L.Z, Oliveira, A.M., & Portal, V.L. Relationship between adiponectin, obesity and insulin resistance. *Rev Assoc Med Bras* vol.61,no.1,p:72-80. 2015.

[2]. Berg, A.H., Combs, T.P., Du, X., Brownlee, M., & Scherer, P.E. The adipocyte-secreted proteinAcrp30 enhances hepatic insulin action. *Nat. Med.* Vol.7, no.8,p:947-53. 2001

[3]. El Dayem, S.M.A., Nazif, H.K., EI-Kader, M.A, El-Tawil, M.. Study of Adiponectin Level in Diabetic Adolescent Girls in Relation to Glycemic Control and Complication of Diabetes. *OA Maced J Med Sci.* p.3,no.4,p:613-18. 2015

[4]. Fang, H., Ning, D.S., & Liang, X.Y.,. Studies on Technology Optimization for Extracting Triterpenoid Saponins from Picria felterrae by Multi-Target Grading Method. *Journal of Chinese Medicinal Material.* Vol.32,no.12,p:1902-05. 2009

[5]. Fu, Y., Luo, N., Klein, R.L, & Garvey, W.T,. Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. *J. Lipid Res*. vol.46,p:1369–79.2005

[6]. Huang, Y., de Bruyne, T., Apers, S., Ma, Y., Claeys, M., van, den, et al, Berghe,. Complement-Inhibiting Cucurbitacin Glycosides from Picria felterrae. *Journal of Natural Products*. Vol.61,no.6,p:757-61.1998

[7]. Iannitti, T., Graham, A., & Dolan, S. Adiponectin-mediated analgesia and anti-inflammatory effects in rat. *PLoS One. Vol.*10,no.9,p:e0136819.2015.

[8]. Kumari, M., & Tannins, J.S. An Antinutrient with Positive Effect to Manage Diabetes Res. *J. Recent Sci*. vol.1,no.12,p:1-8. 2012

[9]. Lindarto, D., Machrina, Y., Syafril, S., & Saragih, A. The Effect Of Puguntano *(Curanga Fel-Terrae [Lour.])* Extract On Adiponectin Receptor (Adipor) In Rats With Type 2 Diabetes Mellitus. *Asian J Pharm Clin Res, vol.*12,no.2,p:551-3. 2019

[10].Lindarto, D., Syafril, S., Zein, U., & Saragih, A. The Effect Of Dhawalsan-1 *(Curanga Fel-Terrae [Lour.])* Extract Versus Metformin On The Metabolic And Inflammatory Characteristics Of Patients With Newly Diagnosed Type 2 Diabetes Mellitus. *Asean J Pharm Clin Res. 9 Suppl* vol.1,no.p:225-8. 2016

[11].Liu, M., Xiang, R., Wilk, SA., Zhang, N., Sloane, LB., Azarnoush, K., *et al*. Fat-specific DsbA-L overexpression promotes adiponectin multimerization and protects mice from diet-induced obesity and insulin resistance. *Diabetes.* Vol.61,no.11,p:2776-86.2012

[12].Mao X, Kikani, CK, Riojas RA, Langlais P, Wang L, Ramos FJ, *et al*,. APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat. Cell Biol*. Vol.8,no.5,p:516–23.2006

[13].Ministry of Health, Republic of Indonesia,. Farmakope Herbal Indonesia. Ed.I. Suplemen II. Jakarta: *Kemenkes RI* pp. 106–7. 2013

[14].Okada-Iwabu, M., Iwabu, M., Ueki, K., Yamauchi, T., & Kadowaki, T. Perspective of Small-Molecule AdipoR Agonist for Type 2 Diabetes and Short Life in Obesity. *Diabetes Metab J* vol.39,p:363-72. 2015.

[15]. Roffey, B.W.C., Atwal, A.S., Johns, T., & Kubowa, S. Water extracts from Momordica charantia increase glucose uptake and adiponectin secretion in 3T3-L1 adipose cells. *Journal of Ethnopharmacology vol.*112,p:77–84. 2007

[16].Syafril, S., Lindarto, D., Lelo, A., Sembiring, R.J., Manaf, A., Putra, I.B., *et al*. The Effect of Puguntano Leaf Extract *(Curanga Fel Terrae Merr.)* on p38 Mapk Levels and Glut-4 Expression in Type 2 Diabetic Rat Muscle. *OA Maced J Med Sci*. vol.7,no.4,p:521-5.2019

[17].Wang, L.S., Li, S.H., Zou, J.M., Guo, Y.J., & Sun, H.D. Two New Terpenoids from Picria fel-terrae. *Journal of Asian Natural Product Research*. Vol.8,no.6,p:491-4. 2006.

[18].Xin, X., Zhou, L., Reyes, CM,. Liu, F., & Dong, L.Q. APPL1 mediates adiponectinstimulated p38 MAPK activation by scaffolding the TAK1-MKK3-p38 MAPK pathway. *Am. J. Physiol. Endocrinol. Metab*. Vol.300.no.1,p:E103–E10. 2011

[19].Yamauchi, T., & Kadowaki T. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. *Int J Obes (Lond).* Vol.32 Suppl 7,p:S13-8. 2008

[20].Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., *et al*., 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med*. Vol.8,p:1288–95. 2002

[21].Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara., *et al*. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat. Med*. Vol.7,no.8:941-6. 2001.

[22].Yamauchi, T., Nio, Y., Maki, T., Kobayashi, M., Takazawa, T., & Iwabu, M., *et al*,. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat. Med*. Vol.13,no.3,p:332-9. 2007

[23].Yeo, CR., Lee, SM., & Popovich DG,. *Evidence-Based Complementary and Alternative Medicine* 1-9. 2011

[24].Zhang, M., Lv, X.Y., Li, J., Xu, Z.G., & Chen, L. The Characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp Diabetes Res. P:*1-9.2008

[25].Zhou, J.M., Wang, L.S., Niu, X.M., Sun, H.D., & Guo, Y.J. Phenylethanoid Glycosides from Picria felterrae Lour. *Journal of Integrative Plant Biology* vol.47,no.5,p: 632-6.2005