IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 9 Ver. 10 (September. 2018), PP 51-56
www.iosrjournals.org

The Effect of Puguntano Extract (*Curanga fel-terrae*Merr.) Towards *Peroxisome Proliferator Activated Receptor- ϒ* (PPAR-ϒ) Levels Intype 2 Diabetes Mellitus Wistar Rat Model

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Abstract: *Type 2 diabetes mellitus (T2DM) is a group of diseases that require continuous treatment with a strategy for decreasing blood sugar levels. Peroxisome Proliferator Activated Receptor- ϒ (PPAR-ϒ) is a receptor that may effect the insulin signaling to enhance the insulin sensitivity.Puguntano is a plant of Sumatera Utara used hereditary as anti diabetic.Our study aimsto investigate the effect of Puguntano extract (Curangafel-terrae* Merr.*) towardsPPAR-ϒ levels inT2DM Wistar rat model. It is an experimental study with posttest-only control group design, was conducted in April to August 2018 at Laboratorium Unit PenelitianFakultasKedokteranUniversitasPadjadjaran, Bandung. The samples wereT2DM Wistar rat model was divided into 2 groups: control group and experiment group. Experiment group was given puguntano extract(200mg/Kg). Changes that occurred were assessed using Wilcoxon test with p value of <0.05 was considered statistically significant.This study did for 48 T2DMWistar rat model divided into 2 groups: control group and experiment group. The average PPAR-ϒ levels of the control group was 29,78 (27,71-31,16)ng/ ml while the experiment group was 37,53 (32,35-54,69)ng / ml. There were significant differences in PPAR-ϒ levels between control and experiment groups in T2DMWistar rat model (p<0.001).There were significant increasing in PPAR-ϒ levels in T2DMWistar rat model without and with givePuguntano extract therefore it can be considered in management T2DM, advance study is needed for that achievement.*

Date of Submission: 10-09-2018 Date of acceptance: 27-09-2018

I. Introduction

Diabetes mellitus (DM) is a group of diseases that require continuous treatment with a strategy for decreasing blood sugar levels. Good management education required of patients and strong support to prevent the onset of complications[1].Several epidemiologic studies showed a trend of increasing incidence and prevalence type 2 diabetes mellitus (T2DM) throughout the world[2]. There are two main causes of hyperglycemia on T2DM, disorders of the insulin secretion and increased insulin resistance (IR). Evaluation of IR (sensitivity) and beta cell function are important to know the status of disease and the selection of the pharmacological treatment. IR is very important on a DM, not only as a strong predictor of the development of diabetes, but also as a therapeutic target in hyperglycemia which is already happening[3]. Several studies reported that IR is closely related to obesity and genetics, and several of them shows that the molecular mechanism is a trigger of resistance. A few years ago, it is said that there are more than 50 genetic loci associated with IR and T2DM, and 15% of whom are associated with hereditary disorders. One of the genes related to IR is Peroxisome Proliferator Activated Receptor-ϒ (PPAR-ϒ) [4].PPAR-ϒ is potent receptor which have effects on carbohydrate metabolism as well as lipid[5]. It increases the concentration of plasma adiponectin in individuals with IR and T2DM through increased activity of pancreatic β cells and improves peripheral glucose [6]. PPAR-ϒ will decrease TNF-α, resistin,leptin, and free fatty acids but it will increase the adiponectin levels in an attempt to improve IR. The activation of PPAR-ϒ will increase the activity and expression of GLUT-4 and GLUT-1 translocation, which increases the effect of glucose removal from circulation and lowers blood glucose levels, as well as improve insulin sensitivity[7]. Nowadays, utilization of traditional medicine is growing rapidly in the community. In many areas, the utilization of traditional medicine is a hereditary heritage based on experience/empirical subsequently evolved through scientific proof through the pre-testing clinic and clinical trial [8]. One of the plants that are efficacious drugs are Puguntano (*Curangafel-terrae*Merr.) that grows in the region such as China, India, Indonesia, the Philippines, Malaysia and Myanmar. The village community in TigaLingga village, Dairi of Sumatera, Indonesia has been cultivating and using Puguntano leaves as anti-diabetes [2].Sitoruset al., have found that ethanol extracts of leaves of Puguntano contain phytochemicals in the form of flavonoids, saponins, tannins, glycosides, terpenoids and steroids [9]. Lindarto et al., comparing Puguntano extract with metformin for 12 weeks

on newly diagnosed patients of T2DM and reported that the extract of puguntano effective in improving the HOMA-IR, blood glucose level and Hemoglobin A1C (HbA1c) as well as improve adiponectin significantly, while metformin only significantly lowering blood glucose level and HbA1c [2].

II. Material And Methods

It is an experimental study with posttest-only control group design,was carried out on wistar rat induced T2DM by give high fat diet (HFD) and low-dose Streptozotosin (STZ) injection at *Laboratorium Unit PenelitianFakultasKedokteranUniversitasPadjadjaran, Bandung* from April 2018 toAugust 2018. A total 48 Wistar rat as subjects (Male) of aged 8 weeks old, were for in this study.

Sample size calculation: The sample size was estimated by Federer formula. We assumed that the confidence interval of 10% and confidencelevel of 95%. The sample size actually obtained for this study was 16 Wistar rat for each group. We planned to include 48 Wistar rat (Group 1- Control, Group 2- Experiment).

Subjects & selection method:This research aims to look at the levels of PPAR-ϒ in skeletal muscle tissue, so it is not ethically be done in humans. Therefore this research was conducted in Wistar rat (*rattusnorvegicussp*)

Inclusion criteria:
1. Wistar rat (*rattusnorvegicussp*)
2. Male
3. Aged 8 weeks old
4. Body weight 180-200 grams
5. Does not have other disease
6. The Rat of T2DM model must have HOMA-IR $\geq$ 2,6 and Fasting blood glucose(FBG) must be >200 mg/dl

Exclusion criteria:
1. The rat with other disease
2. HOMA-IR < 2,6
3. The rat dies when the study still running

Procedure methodology

Study begins with making the Wistar rat of T2DM model by modify procedure of Zhang et al.[10]. Male Wistar rat aged 8 weeks oldadapted in a standard cage for 7 days (2 rats/ cage) and maintained at 25^0C with 12/12-hour light-dark cycle. The Wistar rat had free access to water. The Wistar rat fed with standard chow ad libitum via oral gastric tube (OGT) consisting of 12% fat, 60% carbohydrate and 28% protein, with total calorific value is 25 KJ/Kg body weight (BW).After 1 week, Wistar rat fed with HFD consisting of 41% fat, 41% carbohydrate and 18% protein with calorific value 45Kj/Kg BW for 5 weeks. After 5 weeks, rats were fasted for 12 hours in the night and they were injected intraperitoneally (IP) with low-doses STZ, 30mg/kg BW in 0,1 cc buffer citrate (pH 4,5), repeated after 1 week. 1 week after the second injection, all the wistar rats were fasted for 12 hours and measured FBG, fasting blood insulinand HOMA-IR was calculated. The rats were selected with inclusion and exclusion criteria appropriately.

There are 48 selected rats, were divided in to 2 groups, they are control group (G1) and experiment group (G2). G1 was the group did not get Puguntano, and G2 was the group got Puguntano extract 200 mg/kg BW for 10 days by or OGT. After 10 days, the rats were executed with injection ketamine 75 mg/Kg BW IP. PPAR-ϒ was analyzed from rat's musculus gastrocnemius with enzyme-linked immunosorbent assay (ELISA) method.

Puguntano extract was prepared by maceration process with etanol 70% as solvent. 300mg dry leave of puguntano powder inserted to macerator Iand moistened with a liquid solvent ethanol 70% for 6 hours. After 6 hours, stir the solution and wait for 18 hours, it is mass I, move the mass I from macerator I to macerator II and repeated the process until get mass II. Combine mass I and mass II and evaporate the solvent with heating in water 90^0C until get a thick extract then dried in the freeze dryer. Dried extract stored in parchment paper and labeled in the form of the name, creation date and weight, then conducted storage. Good storage is done at a temperature of 15^0-30^0C with average air humidity 80% [8].

The Group citeria:
Group 1 (G1)- did not get Puguntano extract
Group 2 (G2)- got Puguntano extract 200mg/Kg BW ad libitum via OGT for 10 days

FBG was determined by usingAccu-Check Active Kit glucose meter (Roche, Indonesia) after 12 hours of overnight fasting. The fasting blood insulin was determined by using ELISA method. A fasting venous sample was collected from vena on the tail's rat.

All biochemical assays was carried out by the same team of laboratory technicians using the same method, throughout the study period. The samples were assayed for FBG, fasting blood insulin, PPAR-ϒ.

PPAR-ϒ was assayed with ELISA method by using Rat PPAR-ϒ ELISA kit from Qayee Bio-technology, Shanghai.

Statistical analysis

Data was analyzed using SPSS. Independent*t*-test was used to analyze the significance of differences between mean values of two groups (G1 and G2). Wilcoxon test was used to determine the difference of PPAR-ϒ between G1 and G2. The level $P < 0.05$ was considered as the cutoff value or significance.

III. Result

We success to induce 48 Wistar rat to be T2DM model. The sample divided in to 2 groups, control group (n=24) and experiment group (n=24).

Table no 1 Shows FBG and BW of the two groups before T2DM. FBG in control group, 77 ± 7 mg/dL and in experiment group is 75 ± 7 mg/dL, BW in control group 209 (200-221) grams and in experiment group is 209 (200-390) grams. The difference in the values of all parameters in respect of two groups was not statistically significant (p>0.05).

Table no 1:FBG and BW of Wistar rat before T2DM.

	G1 (control group)	G2 (experiment group)	P value
FBG, mg/dL	(77 + 7)	(75 + 7)	0,198
BW, grams	209 (200-221)	209 (200-390)	0,634

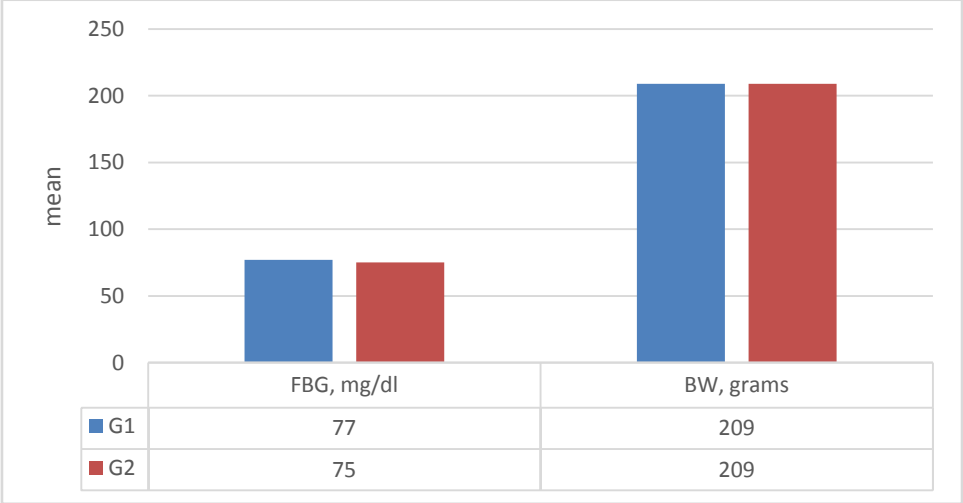


Table no 2:Show FBG and BW after experiment group got the Puguntano extract 200 mg/Kg BW for 10 days. FBG in control group, 384 (207-490) mg/dL and in experiment group is 122 (95-213) mg/dL, BW in control group, 395 (350-430) grams and in experiment group is 247 (201-304) grams. There had been reduction in FBG level and BW, were highly statistically significant, P<0.001.

Table no2: Shows the changing of FBW and BW after puguntano extract given.

	G1(without Puguntano)	G2 (with Puguntano)	P value
FBW, mg/dL	384 (207-490)	122 (95-213)	<0,001
BW, grams	395 (350-430)	247 (201-304)	<0.001

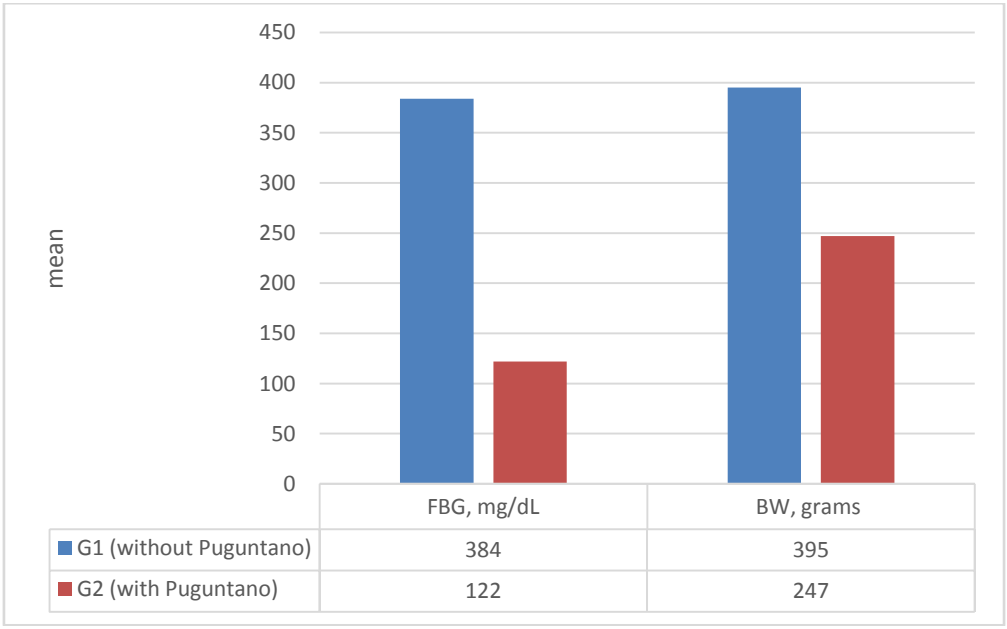
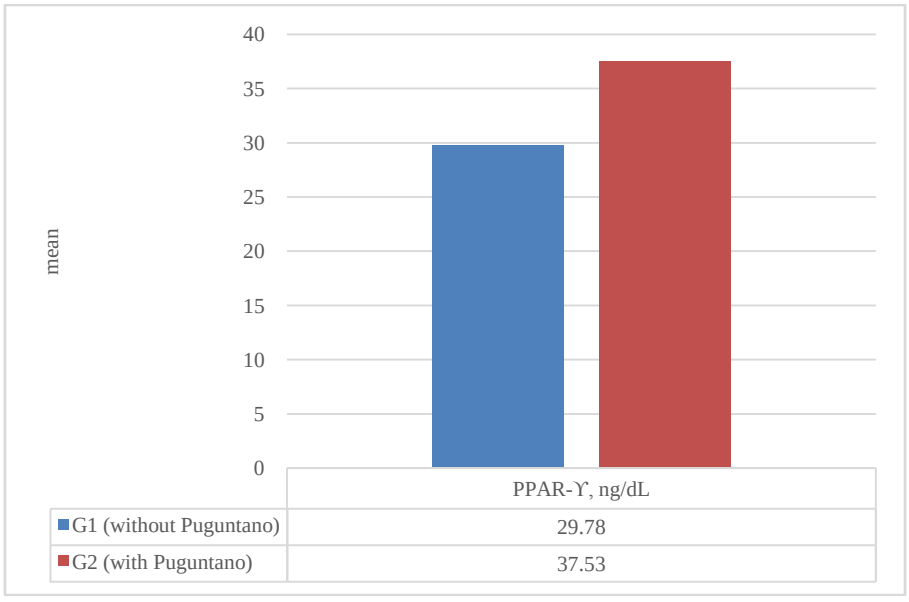


Table no3: Shows PPAR-ϒ levels (ng/mL) between control group and experiment group. In control group is 29,78 (27,71-31,16) ng/mL and in experiment group is 37,53 (32,35-54,69) ng/mL. There had been an increasing of PPAR-ϒ level in experiment group, were statistically significant, P<0,001.

Table no3 : Shows Change in PPAR-ϒ level,(mg/dL) after Puguntano extract given **.**

	G1 (without Puguntano)	G2 (with Puguntano)	P value
PPAR-ϒ, ng/dL	29,78 (27,71-31,16)	37,53 (32,35-54,69)	<0,001



IV. Discussion

Utilization of traditional medicine increase and growing rapidly. People inspired by "back to nature".Sitorus et al., have found that ethanol extracts of leaves of Puguntano contain phytochemicals in the form of flavonoids, saponins, tannins, glycosides, terpenoids and steroids [9].Terpenoids, one substance observed on Puguntano, have been investigated have the effect of anti-diabetic. Saha et al., noted that this substance has pleotropic effect like PPAR-ϒ transactivation and activation of NF-kB[10].

Statistical analysis begins with the analysis of the similarity of the comparison group, the control group and experiment group. From the results obtained by the calculation that there was no significant difference in FBG and BW between them, so that both groups worthy of paired matching process based on criteria.

This study got there were a reducing FBG in group had given Puguntano extract. This reducing was consistent with study's result from Lindarto et al., that comparing Puguntano extract with metformin for 12 weeks on newly diagnosed patients of T2DM and reported that the extract of puguntano effective in improving the HOMA-IR, FBG and HbA1c as well as improve adiponectin significantly, while metformin only significantly lowering blood glucose level and HbA1c [2]. Experiment group had been reducing BW, it can be mediated by PPAR-ϒ activation mechanism.PPAR-ϒ activation can also increaseleptin gene expression in fatty tissues and underlying obesity treatment in vitro with the results of the body fat mass loss [11].

The changing of PPAR-ϒ level can detect by measured it on tissue, because PPAR-ϒ is specific in tissue. PPAR-ϒ have important role to adipogenesis process, glucose homeostatic, lipid metabolism, insulin sensitizing, reducing inflammation, even growth of neoplasm[12]. The activation of PPAR-ϒ will increase the activity and expression of GLUT-4 and GLUT-1 translocation, which increases the effect of glucose removal from circulation and lowering blood glucose levels, as well as improve insulin sensitivityas well as improving insulin sensitivity through increased NAADP-pathway through up-regulation transcription of CD38-dependent pathway[7,13]. Improvement of PPAR-ϒ level is caused by the reaction of PPAR-ϒ with its' ligan likeTerpenoids[14,15]. Wang et al. have noted that some of the plant and its products such as tea (*Camellia sinensis*), soybean (*Glycine max*), coconut oil (*Elaisguinensis*), Ginger (*Zingiberofficinale*), Rosmeri leaf (*Rosmarinusofficinale*) contain ofTerpenoids and can activate PPAR-ϒ[16]. There had been a significant increasing in PPAR-ϒ level between G1 and G2 ($p<0,001$) in our study, because of terpenoids from Puguntano extract. It is supported by study's result fromHandayani et al, explaining that soy milk can lower insulin resistance through activation of PPAR-ϒ because soy milk contains these Terpenoids[17].Similar things are also obtained by Tandrasasmita et al.,thatTerpenoids in Lagerstroemia speciosa can activate PPAR-ϒ and increase insulin sensitivity, lowering FBG in mice induced into DM[18].

V. Conclusion

Puguntano extract 200mg/Kg BW for 10 days can increase PPAR-ϒ level in T2DM Wistar rat model.

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Risnahayati Siregar1, "'The Effect Of Puguntano Extract (*Curangafel-Terrae*merr.) Towards *Peroxisome Proliferator Activated Receptor- ϒ* (Ppar-ϒ) Levels Intype 2diabetes Mellitus Wistar Rat Model."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 9, 2018, pp 51-56.