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The Effect of Rosella (*Hibiscus sabdariffa* Linn) on Insulin Resistance in Patients with Type 2 Diabetes Mellitus: A Randomized Clinical Trial

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Abstract

Insulin resistance in Type 2 Diabetes Mellitus (T2DM) is indicated with high fasting blood glucose level, fasting insulin and HOMA-IR. The long-time consume of diabetes drugs would bring harm. Rosella can be used as a complementary drug to improve insulin resistance and prevent T2DM complications. This study seeks the effect of consuming Rosella on fasting blood glucose, fasting insulin and HOMA-IR in T2DM patients.

The study design used double-blinded & placebo-controlled randomized clinical trial with intervention (placebo and Rosella) for 8 weeks. The sample consisted of 52 T2DM outpatients at Health Office Yogyakarta City. Measurement of fasting blood glucose was conducted through GOD-PAP method, fasting insulin was measured by MEIA, HOMA-IR was calculated with HOMA Calculator 2.2.3 Version. Analysis of the influence of Rosella on fasting blood glucose, fasting insulin and HOMA-IR level in one group were tested with the Wilcoxon Signed Test and the effect between groups were tested through Mann Whitney with a significance level of 95%.

Rosella consumption can reduce fasting blood glucose, fasting insulin and HOMA-IR levels. There was a significant effect of Rosella administration on decreasing fasting blood glucose level in T2DM patients (p=0.001) but there were no significant effect on decreasing fasting plasma insulin level and HOMA-IR levels (p=0.932 and p=0.368). Rosella can improve insulin resistance by reducing fasting blood glucose levels, fasting insulin levels and HOMA-IR values.

Keywords: type 2 diabetes mellitus, glucose, *Hibiscus sabdariffa* Linn, HOMA-IR, insulin, Rosella

INTRODUCTION

The International Diabetes Federation states that the number of people with diabetes in the world has reached 415 million and is expected to continuously increase in 2040 to around 642 million (55%). The prevalence rate of Diabetes Mellitus (DM) in Indonesia in 2013 was 2.1% which experienced an increase of 1.1% compared to 2007 from total population of 250 million (National Institute of Health Research and Development, 2013). Nearly 80-90% of the prevalence of diabetes mellitus is T2DM with world's prevalence rate of 90-95%. Indonesia ranks third country with the highest number of people with T2DM in the world behind India, China and the United States (World Health Organization, 2016).

The causes of T2DM are insufficiency of insulin secretion, insulin resistance, or both (ADA, 2018). T2DM is a chronic disease that has not been cured yet due to a disruption in the mechanism of blood sugar regulation in which the pancreas is unable to produce insulin. In addition, DM T2 may occur because the target cell is unable to respond to the insulin so hyperglycemia emerges or insulin resistance presents (Whiting *et.al.*, 2011).

Insulin resistance is resistance to the effects of insulin on glucose uptake, metabolism, and storage. Clinical manifestations of insulin resistance, glucose intolerance and hyperinsulinemia are consequences of the inability of insulin to stimulate glucose absorption in insulin target tissues, such as muscle and fat. Insulin resistance interferes with glucose uptake in peripheral tissues and results in excessive glucose production by the liver. This affects the occurrence of hyperglycemia in T2DM. In addition, insulin resistance causes impaired insulin secretion by sensitive tissues and an increase in liver glucose secretion which is distinguished by an increase in fasting blood sugar. The disruption may also occur in the formation of glycogen (Tangvarasittichai, 2015).

Studies on insulin resistance in T2DM have been widely conducted. In North Indian, the prevalence is 37.8% where 58.46% happens in men & 41.53% in women (Kumar *et.al.*, 2005). About 17% of children are obese in the US, 50% have insulin resistance and 10-25% have glucose abnormalities (Mizokami *et al.*, 2015). Whereas in Peshawar is 78% where 77% is in men & 79% in women over the age of 30 years (Shah, *et al.*, 2008). Another study in Korea shows that the prevalence of insulin resistance is 70.6%, whereas the number is higher in impaired insulin secretion patients with 59.5% vs. 22.0% (Kim & Lee, 2016). A cross-sectional study in Kenya shows 82.6% out of 167 T2DM patients suffer insulin resistance (Gulam, *et al.*, 2017). In general, the prevalence of insulin resistance in T2DM is 18%, with ratio of 4:1. Research in Indonesia shows a strong correlation ($r = 0.939$; p-value <0.001) between insulin resistance and HOMA-IR index and T2DM in Indonesia (Srihardyastutie *et al.*, 2014).

Impaired insulin secretion and insulin resistance in T2DM are shown by higher of fasting blood glucose, fasting insulin and HOMA-IR value compared to normal individuals. (Vimaleswaran *et al.*, 2011). Shah *et al.*, (2008) observed in T2DM patients with insulin resistance that indicated by 60% uncontrolled glucose level. Hyperglycemia due to insulin resistance in T2DM may cause complications such as microvascular complications (retinopathy, nephropathy, neuropathy) & macrovascular (cardiovascular & cerebrovascular).

Due to expensive DM treatment costs and clinical complications, herbal medicines as complementary therapy (adjuvant) is required for treatment and prevention of T2DM complications. Herbal medicines that contain antioxidants are proven to boost and prevent the progression of T2DM. Treatment with herbal ingredients has several advantages, for instance, patients feel more comfortable because of the relatively fewer side effects compared to synthetic drugs. The use of oral diabetes drugs (OAD) for a long time causes resistance (Atiqoh *et al.*, 2011; Lin *et al.*, 2016). One of the tropical plants consumed to prevent complications and as T2DM adjuvant drugs is Rosella (*Hibiscus sabdariffa* Linn). Rosella is proven as an antidiabetic by reducing blood glucose levels, boosting insulin secretion and improving insulin resistance (Andraini & Yolanda, 2014).

The Rosella polyphenols content, especially the anthocyanin group (*delphinidin 3-sambubioside, delphinidin 3-glucoside, cyanidin 3-sambubosyde, cyanidin 3 glucoside*), alkaloids (*quercetin, protocatechuic acid*), and some organic acids (vitamin C) have antioxidant activities that can suppress oxidative stress from free radical effects on insulin resistance in T2DM (Zarrabal *et al.*, 2012). Studies in experimental animals have shown that Rosella extract boosts insulin resistance by reducing blood glucose level, plasma insulin level, visceral fat tissue weight & HOMA-IR value and increasing adiponectin secretion (Chuenta *et.al.*, 2011; Bunbupha *et.al.*, 2012; Huaysrichan *et al.*, 2016; Singh & Pannangpetch, 2017). Rosella has antihyperinsulinemia effect on T2DM with insulin resistance (Belwal *et al.*, 2017).

The content of anthocyanin Rosella has been shown to reduce systolic and diastolic blood pressure in T2DM patients with in vitro, in vivo and clinical hypertension and can reduce blood glucose level and increase plasma insulin level in diabetic rats (Lin *et al.*, 2016; Ardalani, 2016). The

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inhibition mechanism of Rosella for T2DM through inhibiting the key enzymes of carbohydrate digestion forming glucose is ⍺ -glucosidase and ⍺ -amylase enzymes with in vitro by polyphenol compounds (flavonoids, phenolic acids, and tannins) (Ademiluyi & Oboh, 2013). Another study also shows that phenolic compounds are able to regulate postprandial glucose levels and inhibit glucose intolerance due to insulin response and decrease glucose secretion caused by glucagon insulinotropic polypeptide (GIP) and glucagon like polypeptide-1 (GLP-1). Protocatechuic acid content acts as antidiabetic by reducing plasma glucose level and increasing insulin level in diabetic rats and has the potential to prevent complications in reducing triglyceride level, anticoagglucosation, antioxidant and anti-inflammatory (Lin *et al.*, 2016; Farombi, 2007).

There is a significant effect of various concentrations in Rosella petals infusion on decreasing blood glucose level in glucose-induced mice (Atiqoh *et al.*, 2011) and reinforced by Rosemary *et al.* (2014) and Mardiah *et al.* (2015) in streptozotocin-induced mice, besides able to increase MDA level and maintain bodyweight of alloxan-induced mice and reduce blood glucose level insignificantly (Ojewumi & Kadiri, 2013). Rosella can reduce blood glucose level 1.6±26 mg/dl in T2DM (Mozaffari-Khosravi *et al.*, 2014) and 22.5 mg/dl in pre-diabetes women in Yogyakarta (Rohmah *et al.*, 2018). Andraini & Yolanda (2014) prove that Rosella can reduce or prevent insulin resistance in high-fructose dietary-induced rats, in which decrease in fasting blood glucose level, fasting blood insulin level and HOMA-IR are found. Nerdy (2015) proves that Rosella bioactive compounds (*quercetin, hibiscetin, gossypetin, protocatechuic acid*) have better potential as PEPCK enzyme inhibitors than metformin. The ability of Rosella phenolic acid in glucose uptake is similar to the absorption of *metformin & thiazolidinedione*.

Besides as a good antioxidant, Rosella is also considered safe. The prescription of Rosella extracts 150 to 180 milligram/kg BW/day orally shows no signs of additional effects (LD_{50} between 2000-5000 mg/kg/day) (Hopkins *et al.*, 2013). It is also reported that consumption of Rosella tea has no side effects on the liver and kidneys (except consumption> 5000 mg/kg/day). Rosella can capture ROS and free radicals, reduce reactive O2, metabolize fat peroxidation into non-radical products, and prevent the generation of free radicals by neutralizing free radicals by 44% (Wang *et al.*, 2000). High vitamin C content can reduce blood glucose and HbA1c levels (Wu *et al.*, 2014). Many studies that prove the effectiveness of Rosella for antidiabetic have been conducted both in vitro and in vivo. Mozzafari-Khosravi *et al.* (2009) prove that consumption of 2 gram Rosella calyx tea dissolved in 240 ml of boiling water with 2 times per day consumption (morning and evening) between main meals for a month can significantly reduce systolic blood pressure.

Based on the backgrounds above, further research is required to analyze the effect of Rosella therapy on insulin resistance in T2DM, fasting blood glucose level, fasting insulin level and HOMA-IR with a randomized controlled clinical trial model in T2DM patients.

PATIENTS AND METHODS

Study Design and Participants

This study used experimental design with a double-blinded & placebo-controlled randomized clinical trial (RCT) design conducted in 2018-2019 with total participants of 60 T2DM outpatients.

The population was 5 (five) Public Health Center in the Yogyakarta City which was randomly selected. The chosen public health center was determined by choosing those with T2DM prevalence above the national prevalence (2.6%) from January 2018-June 2018, those were Puskesmas Tegalrejo, Jetis, Gedongtengen, Kotagede 1 and Gondokusuman 1. The inclusion criteria were determined by T2DM patients with fasting blood glucose levels ≥126 mg.dL or random blood glucose level 2JPP

≥ 200 mg/dL, willing to be sample by signing informed consent, able to communicate well, T2DM patients without kidney complication and heart disease, T2DM patients ranged between 35-65 years, and consuming the same drugs with the type, dose/amount and frequency until the study was completed. Exclusion criteria were pregnant & nursing patients. Samples were not included in the data processing and analysis (withdrawn criteria) if mortality occurred during the study, patients withdrawing during data collection, patient compliance consuming Rosella capsules <20% during the study, suffering from infections and abscesses, increasing in diabetes medications (dosage & frequency) during the study, consuming herbal tea or herbal medicine, and the patient defying the study protocols.

After obtaining samples based on inclusion and exclusion criteria of the study, the aims and objectives of the research were explained. Subjects agreed with the research by signing informed consent to become a respondent. Samples were randomly divided based on permuted block randomization by computer using 4 random numbers consisting of 2 (two) groups, the group that received Rosella capsules and the group with no Rosella prescription but received placebo capsules (500 mg lactose). The samples were selected by convenience sampling technique from affordable population by dividing it into 2 groups (control and Rosella groups) and then drawing for each group division. Randomization was performed by *PT Liza Herbal International* and the randomization team.

Researchers, samples and laboratory analysts of the study outcomes were completely unaware of the interventions provided. Rosella and placebo capsules were made in an identical shape, size, color, taste, and bottle package. Rosella and placebo capsules were secured by *PT Liza Herbal International* with quality standards certificate based on National Food and Drug Agency with number of POM TR: 073 371 151. Rosella and placebo packages were labeled using 1-52 numbering, the composition of contents was only recognized by the manufacturer of Rosella capsules. Both study groups consumed the same amount of capsules and direction with 2 capsules a day after meals (morning and evening) for 8 weeks.

Prior to the intervention of Rosella and placebo capsules, the samples were given nutrition counseling or nutrition education by a nutritionist to regulate samples' dietary so the food intake factor did not become a confounding factor in study. During the study, samples were not permitted to take supplements or other herbal medicines. All participants were followed up every 1 week (8 times during the study) to ensure their compliance with capsules consumption through home visits by enumerators. Each participant was given a diary containing a record of compliance with capsule consumption, the remaining capsules, reasons for not consuming capsules and a record of complaints during the capsules consumption that written every day. Participants who experienced digestive & kidney disorders during the study were excluded from the study. PT Liza Herbal International or capsules manufacturer delivered the composition of the capsules when the study was completed.

Measurements

Subject characteristics (sex, age, supplement consumption, herbal/tea consumption, family history, drug consumption, smoking status, infection and abscess presence, occupation, DM duration) were collected through interviews with patients conducted by enumerators using characteristic questionnaires for research subjects. The physical activities of the samples were measured by interviews using International Physical Activity Questionnaire (IPAQ). Food intakes (energy, carbohydrate, protein, and fat) were obtained through interviews by enumerators using Semi Quantitative Food Frequency Questionnaire (SQFFQ) method with the assistance of food

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photo books from the Ministry of Health Republic of Indonesia in 2014. Compliance with the intervention capsule consumption can be seen from the record of the remaining capsules in the compliance book capsule consumption using the formula:

$$\frac{Remaining\ capsules}{Total\ capsules} \times 100$$

Nutritional status was measured using body mass index (BMI). Nutritional status is determined by anthropometric measurements including measurement of weight (kg) and height (cm) in units of kg/m2 using the formula:

$$\frac{Weight\ (kg)}{Height\ squared\ (m^2)}$$

Weight was measured using a digital body scale (Camry brand) with a minimum clothing capacity of 200 kg and accuracy level of 100 g. Weight measurements were taken before and after the intervention. Height was measured by microtoice with a length of 200 cm and level of accuracy of 1 mm. During measuring height, the participants were asked to take off shoes and stand upright straight forward. The nutritional status of the participants was measured using body mass index (BMI).

Blood pressure value is three times the average value of systolic and diastolic blood pressure measurements measured using a stethoscope and mercury sphygmomanometer (Omron brand) before and after the interventions.

Blood samples were used to obtain serum for examination of fasting blood glucose level and fasting insulin level. Before blood draw, patients were asked to fast for 8-10 hours to subsequently be taken for blood samples. Fasting is a condition of no-calorie intake but drinking water is allowed. Blood was drawn through the cubital vein as much as 3 mL and used a 3 mL syringe after disinfection was completed at the collection site. Each venous blood sample taken was collected in a tube and then centrifuged at a rate of 2-3 x 10^3 rpm for 15 minutes to obtain serum. The serum was pipetted using a micropipette and put in an Eppendorf tube and labeled according to the randomization number and then stored in the refrigerator at -40-80^{o}C until the examination was proceeded (fasting blood glucose level and fasting insulin level examination). Blood samples were taken by laboratory staff.

Blood glucose level was measured using the *glucose oxidase-para amino phenazone* (GOD-PAP) method in mg/dL unit using the *Diasys* kit (*Diasys Diagnostic System GmbH Alte Strasse 9 65558 Holzheim, Germany*). Fasting insulin level was measured using the Microparticle Immunoassay Enzyme (MEIA) method in µIU / mL unit. Fasting insulin level was analyzed using ELISA Insulin kit (The Calbiotech Inc., A Life Science company, USA). Measurement of fasting blood glucose and fasting insulin was performed before and after the interventions (*pre-post*).

To find the presence of insulin resistance was determined by using the *Homeostasis Model Assessment-Insulin Resistance* (HOMA-IR) Index to express the measure of insulin activities. HOMA-IR was calculated using the HOMA calculation software (HOMA calculator 2.2.3 version, Diabetes Trial Unit of the University of Oxford).

Ethical Considerations

Researchers had submitted to Universitas Gajah Mada Faculty of Medicine, Public Health and Nursing Research Ethics Commission to obtain Ethical Clearance with Ref: KE FK/00995/EC/2018. Data collection was conducted after obtaining approval from the Ethics Commission

and participants or guardians of participants by signing informed consent by both researchers and participants/ guardians of participants. One doctor was provided to anticipate any unexpected possibilities related to the subjects' health. All research information and data are only used for scientific purposes and confidentiality is maintained.

Statistical analysis

Data on subject characteristics (sex, age, supplement consumption, herbal/tea consumption, family medical history, drug consumption, smoking status, infection and abscess presence, occupation, duration of DM), blood pressure data, fasting blood glucose level data, insulin level data, and HOMA-IR data were processed with the assistance of SPSS 21 version. Anthropometric data (weight and height) were processed through ANTRO 2008. Nutrition intake data were processed with nutrysurvey program by comparing their needs.

Categorical data are described by frequency distribution and percentage, while numerical data are described by means and standard deviations. Data normality test used Student T-Test.

Analysis of the effect of Rosella capsule consumption on systolic blood pressure, diastolic blood pressure, fasting blood glucose level, fasting insulin level & HOMA-IR in group 1 T2DM patients used *Wilcoxon Signed Rank-Test* as the data were not normally distributed. To find differences between groups was conducted using Mann Whitney test because the data were not normally distributed with a significance level of 95% ($p<0.05$).

RESULTS

In the intervention, 60 samples fulfilled the inclusion and exclusion criteria included in the clinical trial, 30 samples for control group and 30 samples for Rosella group. Before the intervention was given, 3 samples withdrew after taking blood for the baseline, 2 samples from the control group and 1 sample from intervention group so there was total of 57 samples received the intervention. The withdrawal reasons were due to family members' permission issues, abundant medicine consumption and out of town for a long time. At the time of data analysis, only there were 52 participants could be analyzed because percentage of compliance with the intervention capsules below 80% (n = 3) and changing in the type and dose of hypoglycemic drugs consumed before the study was completed (n = 1) (Figure 1). The lowest percentage of compliance with capsule consumption for control group was 80% and the highest was 99% with an average of 90.86%, while for Rosella group the lowest was 81% and the highest was 100% with an average of 91.08%. Noncompliance during capsules consumption intervention was due to several factors such as leaving the city for a long time, illness and hospitalized and forgetting.

Variables that affected insulin resistance before intervention are presented in Table 1 and Table 2. Table 1 shows the quantitative variables before the intervention with no difference between the control group and Rosella group, thus, the condition was homogeneous for the characteristics of age, duration of diabetes, weight, height, nutritional status, carbohydrate intake, fat intake, protein intake, physical activity, systolic blood pressure, diastolic pressure and also blood biochemical parameters (fasting blood glucose level, fasting insulin level) and insulin resistance status (HOMA-IR).

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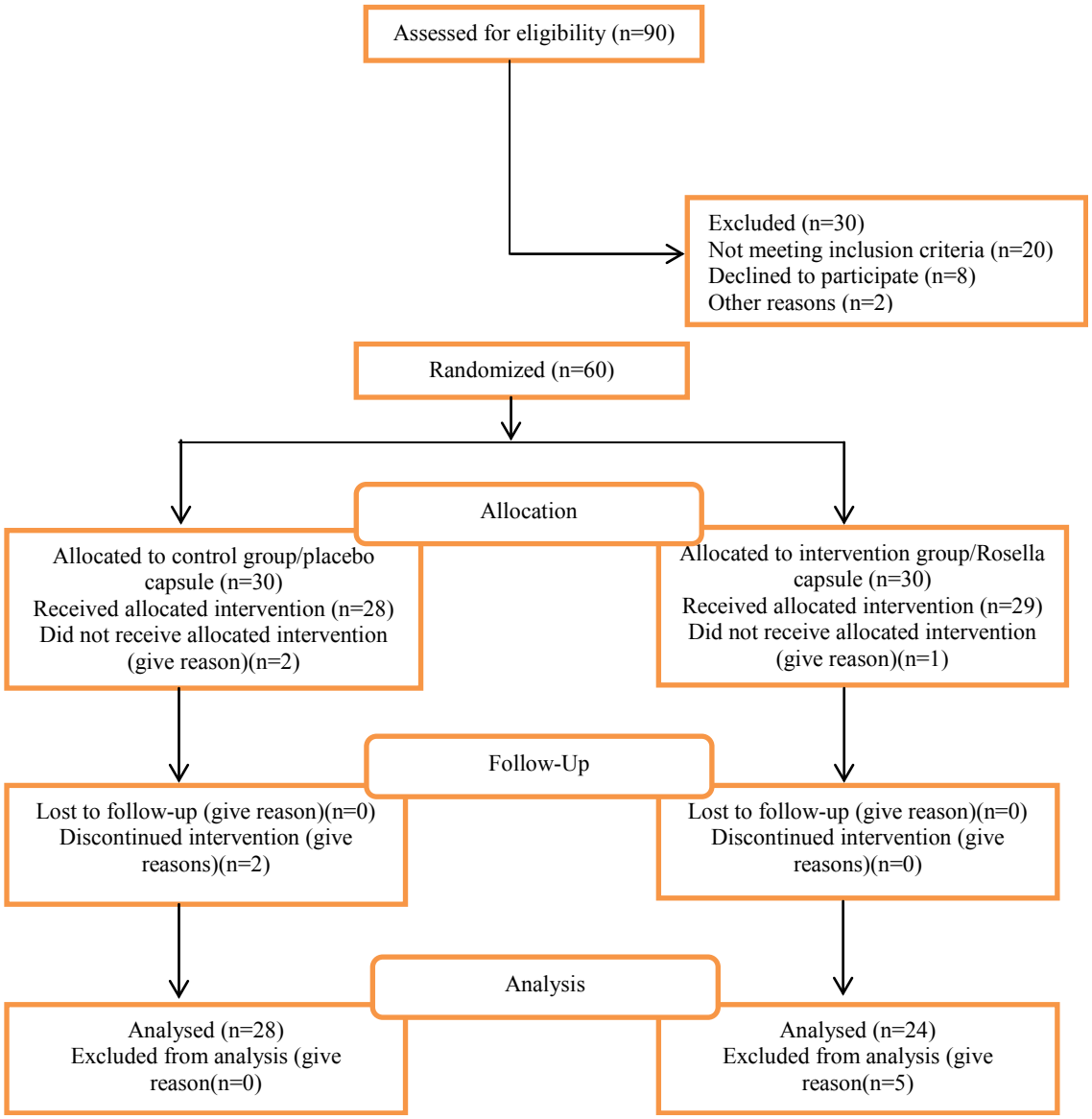

Figure 1. Consort Flow Diagram Intervention Steps

Table 1. Comparison of the Quantitative Variables between the Two Groups before Intervention

Variables	Control group/placebo (n=28)	Intervention group/ Rosella (n=24)	P-value*
Age (year)	56.14±5.11**	54.08±5.89**	0.183
Duration of with diabetes (year)	7.62±5.82	7.25±6.01	0.819
Weight (kg)	62.26±8.88	66.98±11.42	0.100
Height (cm)	152.64±6.82	155.09±7.75	0.231
Body mass index (kg/m^2)	26.69±3.19	27.82±4.20	0.273
Dietary intake			
Energy (Kcal/day)	828.91±394.89	710.77±327.92	0.251
Carbohydrate (g/day)	129.67±61.75	113.42±38.92	0.271
Protein (g/day)	98.00±34.31	27.82±16.67	0.183

Fat (g/day)	75.20±25.07	74.50±19.66	0.253
Systolic blood pressure (mm/Hg)	146.80±20.00	188.00±143.54	0.575
Diastolic blood pressure (mm/Hg)	81.47±9.71	79.14±8.24	0.360
Physical Activity (MVPA/day)	350.95±388.90	819.95±2028.77	0.236
Fasting glucose level (mg/dL)	157.25±54.29	143.95±48.51	0.36
Fasting insulin level (μIU/mL)	11.11±8.34	13.45±12.44	0.423
HOMA-IR	1.71±1.60	3.17±4.76	0.375

*Student t-test, **mean±SD

Table 1 provides an overview of sample characteristics, clinical and biochemical parameters before intervention were homogeneous (p≥0.05). Most of the samples were elderly (50 years and older) with an average of T2DM duration 7 years and more, the average BMI was above 25 kg/m^2 (over body weight), food intake was less than necessary. Systolic blood pressure shows high above 130 mm/Hg, while diastolic blood pressure is good (<80 mm / Hg). Fasting insulin level and HOMA-IR before normal intervention, while fasting blood glucose level is high (above 108 mg/dL).

Table 2 shows that most of the samples were female (82.1% in control group and 70.8% in Rosella Group) and were patients with long duration of T2DM. In control group, the number of patients taking hypoglycemic drugs (60.7%) was bigger than Rosella group, but the Rosella group consumed more blood pressure medications (50%) and lipids (4.2%).

Table 2. Comparison of the Qualitative Variables between the Two Groups before Intervention

Variables	**Control group/placebo (n=28)**	**Rosella group (n=24)**
Gender		
Male	5(17.9)	7(29.2)
Female	23(82.1)	17(70.8)
Taking oral hypoglycemic agents	17(60.7)	11(45.8)
Taking anti-hypertensive drug	11(39.3)	12(50.0)
Taking anti-hyperlipidemia drug	0	1(4.2)
Duration of DM		
Newly	3(10.7)	4(16.7)
Long	25(89.3)	20(83.3)

Table 3 shows that after the intervention there were a decrease in systolic blood pressure, fasting blood glucose, fasting insulin, and HOMA-IR levels but an increase in diastolic blood pressure in both control group and Rosella group. The decreasing of systolic blood pressure and fasting glucose level after the intervention in the control group was higher but in fasting insulin and HOMA-IR values were higher in Rosella group. The administration of Rosella can reduce fasting insulin level by 0.04 mg/dL greater than control group. Meanwhile, the decreasing on HOMA-IR values also was greater by 0.55 μIU/mL than control group.

Statistical tests show no significant difference between control group and Rosella group on systolic blood pressure, diastolic blood pressure, fasting blood glucose level, fasting insulin level & HOMA-IR value (p≤0.05), so there was no effect of consuming Rosella as complementary medicine on insulin resistance in T2DM patients.

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Table 3. Comparing Means of Diferrence (Δ) Clinical and Biochemical Parameters Between Control and Rosella Groups During Intervention

Variables	Control group (mean±SD)	Rosella Group (mean±SD)	P-value*
Systolic blood pressure (mm/Hg)	-5,69±17.28	-4.04±17.69	0.854
Diastolic blood pressure (mm/Hg)	0.13±8,40	4,73±12.77	0.378
Fasting blood sugar (mg/dL)	-24.64±26.01	-24.25±29.69	0.949
Fasting blood insulin (μIU/mL)	-0.73±10.13	**-0.77±9.73**	0.783
HOMA-IR	-0.25±1.75	**-0.80±4.12**	0.497

* Mann Whitney Test, significance rate 95 %

Comparison of average systolic blood pressure, diastolic blood pressure, fasting blood glucose level, fasting insulin level and HOMA-IR before and after intervention in 1 group is shown in Table 4. There was a decrease in all clinical and biochemical blood parameters but only fasting blood glucose level showing a very significant difference between before and after the intervention in both control group and Rosella group (p = 0.001).

Table 4. Comparing Means of Clinical and Biochemical Parameters Between Baseline and End In Two Groups

Variables	**Baseline (mean±SD)**	**End (mean±SD)**	**P-value***
Control Group			
Systolic blood pressure (mm/Hg)	146.80±20.00	141.11±20.63	0.086
Diastolic blood pressure (mm/Hg)	81.47±9.71	81.61±8.29	0.859
Fasting blood sugar (mg/dL)	**157.25±54.29**	**132.61±40.01**	**0.001**
Fasting blood insulin (μIU/mL)	11.11±8.34	10.37±7.22	0.690
HOMA-IR	1.71±1.60	1.45±0.93	0.322
Rosella Group			
Systolic blood pressure (mm/Hg)	188.00±143.54	139.50±18.27	0.305
Diastolic blood pressure (mm/Hg)	79.14±8.24	83.87±10.68	0.219
Fasting blood sugar (mg/dL)	**143.95±48.51**	**119.71±36.54**	**0.001**
Fasting blood insulin (μIU/mL)	13.45±12.44	12.68±8.01	0.932
HOMA-IR	3.17±4.76	1.73±1.05	0.368

* Wilcoxon Signed Rank Test, significance rate 95 %

DISCUSSION

This study shows that consumption of Rosella capsules twice a day for 8 weeks has no effect on insulin resistance in T2DM patients. This is not in accordance with the research by Mozaffari *et.al.* (2014) which has proven that administration of *Hibiscus sabdariffa* Linn tea can improve insulin resistance in T2DM patients. This study used pure Rosella extract of 50 mg to anticipate that all bioactive compounds in Rosella can be well-absorbed in the body with a longer duration than the research by Mozaffari *et.al.* (2014). The absence of significant effects might due to less large extract dose. In addition, the duration of T2DM also affected the IR parameters in which the longer the

T2DM patients suffered, the more likely the patients would experience IR. In research Mozaffari *et.al*. (2014), the average duration of DM was 24 years and more, while the duration of T2DM this study was under 1 year.

This study does not support the research of Chuenta *et al*. (2011), Bunbupha *et al*. (2012), Huaysrichan *et al*. (2016), Singh & Pannangpetch (2017), Belwal *et al*. (2017) proving that Rosella extract consumption boosts insulin resistance in T2DM because the participants in those studies are experimental animals which are easy to control its food intake and physical activity. Researches of Rosella's effect on insulin resistance in experimental animals were also conducted by Lin et al. (2016), Ojewumi & Kadiri (2013), Andraini & Yolanda (2014), Atiqoh *et al*. (2011), Rosemary *et al*. (2014) and Mardiah *et al*. (2015). Although the results of this study are not statistically significant, there is a decrease in systolic blood pressure, fasting blood glucose level, fasting insulin level and HOMA-IR value between control group and Rosella group. Statistically significant fasting glucose level is highly significant between before and after the intervention in each group. The results of this study are in line with the research of Mozaffari-Khosravi *et al*. (2014) explaining there is a decrease in blood glucose levels of 1.6 ± 26 mg/dL in T2DM & 22.5 mg/dL in prediabetes women in Yogyakarta (Rohmah *et al*., 2018).

Rosella's ability to reduce fasting blood glucose level is generated by the enzymes ⍺ -glucosidase and ⍺ -amylase (enzymes inhibiting key enzymes in digestion of carbohydrates to form glucose) and polyphenol compounds (flavonoids, phenolic acids, and tannins) (Ademiluyi & Oboh, 2013). Phenolic compounds are able to regulate postprandial glucose levels and inhibit glucose intolerance due to insulin response and decrease glucose secretion induced by insulinotropic polypeptide (GIP) and glucagon like polypeptide (GLP-1). Antidiabetic Rosella is due to protocatechuic acid which has the ability to reduce plasma glucose levels and increase insulin levels in diabetic rats (Lin *et al*., 2016). *Quercetin, hibiscetin, gossypetin, protocatechuic acid* in Rosella according to Nerdy (2015) are more potent as PEPCK enzyme inhibitors than metformin. The ability of Rosella phenolic acid in glucose uptake is similar to the absorption of metformin & thiazolidinedione. High vitamin C content potentially reduces blood glucose and HbA1c levels (Wu *et al*., 2014).

This study has several limitations, including there was no the third group as a positive control group that consisting of healthy individuals or non-diabetic patients. In healthy individuals, the effect of providing food or natural ingredients or functional food will bring more effective results because healthy individuals contain no drug factors involved so it can precisely illustrate its effectiveness in reducing fasting blood glucose level and fasting insulin level. In this study, all patients took hypoglycemic drugs with different doses, so the effect of Rosella consumption was ineffective. Furthermore, drugs greatly influenced the biochemical parameters of blood in a short time because of large doses. The next drawback is the possibility of a less large capsule dose. The extract dose added in capsules was only 10% of the weight of 500 mg meaning it contained only 50 mg purely extract per capsule. So that this capsules have low antioxidant activity. The effectivity of antioxidant activity of Rosella for recovering insulin resistance in T2DM patient must be compared with other herbal medicine as the fourth group, so the fourth group must be required as the group that is given the intervention of other herbal medicine.

There was no difference between control group and Rosella group in age, duration of diabetes, weight, height, nutritional status, carbohydrate intake, fat intake, protein intake, physical activity, systolic blood pressure, diastolic pressure, fasting blood glucose level, fasting insulin level and insulin resistance (by HOMA-IR) status at the beginning of this study. Those were strengths of this research. Another strength of this research are the samples condition in the initial study were homogeneous so that it could be compared with after treatment (comparable). In terms of the

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number of samples chosen (60 samples), 86.7% could follow the research protocol until the end of the study. In addition, the average percentage of compliance in consuming capsules intervention reached 90.86% for control group and 91.08% for Rosella group. This proves that participants were very committed to following the instructions of this study.

Based on the potential of Rosella as an antidiabetic, it is necessary to study the mechanism of Rosella in inhibiting insulin resistance in T2DM. Consideration to choose capsule form is those with stability in storage & transportation, the shape is more practical & attractive, can be combined with several kinds of oral diabetes medications, mask unpleasant odors & bad flavors and can avoid direct contact with air and sunlight (Syamsuni, 2006).

Statistically, there was no significant effect on insulin resistance in this research but herbal medicines were proven to reduce insulin resistance parameters in T2DM patients, especially fasting blood glucose levels. For this reason, it is recommended for further studies to use a higher dose of Rosella extract (75-100 mg) and can be applied to T2DM patients with complications (hyperlipidemia, hypertension), Type 1 Diabetes Mellitus (insulin-dependent DM) and gestational diabetes patients.

CONCLUSION

This study shows that consumption of Rosella capsules 500 mg 2 times a day can significantly reduce fasting blood glucose levels, also can reduce fasting insulin level dan HOMA-IR value but not significantly in T2DM outpatient at Public Health Center Yogyakarta. Based on the potential of Rosella as an antidiabetic for improving insulin resistance, it is necessary to study the mechanism of Rosella in inhibiting insulin resistance in T2DM.

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CONFLICTS OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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