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The Alleviation Effect of Combination of Tempeh and Red Ginger Flour towards Insulin Sensitivity in High-Fat Diet Rats

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Abstract High-fat diet is considered as a factor reducing insulin sensitivity. The purpose of this study was to evaluate the alleviation effect of the combination of tempeh and red ginger flour towards the insulin sensitivity represented by triglyceride-high density lipoprotein ratio in rats treated high-fat diet. Sprague Dawley male rats (n=30; body weight 150-200 g) were randomly divided by 5 groups (n=6), consisted K(-) group: group fed by normal diet (laboratory standard diet) as a control; K(+) group: group fed by high-fat diet; P1 group: group fed by high-fat diet and treated by tempeh flour/200 g body weight 1.9 g; P2 group: group fed by high-fat diet and treated by red ginger flour/200 g body weight 0.036 g; and P3 group: group fed by high-fat diet and treated by a combination tempeh flour/200 g body weight 0.95 g and red ginger 0.018 g. The treatments were given for 21 days. There was a significantly difference of the blood glucose and triglyceride-high density lipoprotein ratio ($P<0.001$), and P3 group was also showed the lowest level of glucose (96.21 mg/dL) and triglyceride-high density lipoprotein ratio (1.41) compared to K(+) (158.552±6.02 mg/dL; 6.02±0.36), P1 (103.71±1.79 mg/dL; 1.66±0.08), and P2 (108.46±2.61 mg/dL; 2.2±0.07). The combination of tempeh and red ginger flour promoted the improvement of blood glucose level and triglyceride-high density lipoprotein ratio better than the sole treatment.

Keywords: *glucose level, insulin sensitivity, red ginger, tempeh*

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1. Introduction

Type 2 diabetes mellitus (T2DM) has become a catastrophic disease as it has driven mortality 115.3 per 100,000 for age more than 20 years old in South-East Asia [1]. Westernized lifestyle which is featured by saturated fat becomes the main cause for T2DM through fat accumulation primarily in the abdomen [2]. Besides, insulin resistance is one of the major factors contributing to T2DM [3]. The exact molecular mechanism leading to insulin resistance is still unexplained. Nevertheless, in brief, there are two mechanisms elucidating insulin resistance: lack of suppression of glucose production and lack of glucose uptake by peripheral tissue, ultimately muscle [4].

At present, there are some methods for assessing insulin resistance, such as hyperinsulinemic euglicemic clamp test (HEC test) as a gold standard, homesostasis model assessment of insulin resistnace (HOMA-IR), and triglycerides/HDL ratio. As a gold standard, HEC test is still expensive and time consuming. While, HOMA-IR appears lack of standardized even though it has been used widely in the study of metabolic syndrome. Therefore, we used a simple and more accessible marker to identify insulin resistance in early state, namely TG/HDL ratio[5]. Besides, the previous study concluded that TG/HDL ratio is a significant and sensitive predictor of insulin resistance [6,7].

Tempeh is one of fermented products consumed worldwide which originated from Indonesia [8]. It is well known that fermentation exerted by bacteria improves the biological ingrendients of tempeh leading to attenuating the absorbtion of isoflavones in tempeh [9]. Tempeh gains much attention due to its roles as lipid-lowering, anti-diabetic, blood lpwering pressure, cardiac disorder, and anti-cancer agent [10].

In other perspective, ginger rhizome (*Zingiber officinale* Roscoe) is widely used as spice in culinary purposes, and has proven biologically active component containing in ginger, whose most active contents are known as gingerols and shogaols [11]. The previous study revealed that extract of ginger successfully attenuated

oxidative stress, inflammation and apoptosis, and enhanced antiozidant in the diabetic kidney rats [12].

There has no study revealed the effect of combination of tempeh and red ginger flour on insulin sensitivity attenuation. Therefore, this study aimed to examine whether tempeh flour combined by red ginger flour possessed an increasing effect on insulin sensitivity instead of the sole treatment. We used TG/HDL ratio as a marker of insulin sensitivity.

2. Material and Methods

2.1. Reagent and Materials

Tempeh was obtained from Tembalang, Semarang, while red ginger (RG) was obtained from Sendangmulyo, Semarang, Indonesia. GPO-PAP kit for TG level, and GOD-PAP kit for glucose assay were purchased from Elabscience.

2.2. Preparation of Tempeh and Red Ginger Flour

Before steamed, tempeh was sliced in the dice-form and be remained in the cabinet dryer. Then, dried-tempeh was ground using a sieve mesh size 100 mm. The same procedure was conducted to make red ginger flour.

2.3. Animal Experimental Design

Sprague Dawley male rats (n=30; body weight 150-200 g) were obtained from the Study Center of Food and Nutrition Laboratory, Gadjah Mada University, Yogyakarta. The rats were housed individually under a 12 h light/dark cycle with a regulated temperature (21^0C) and humidity (50% ± 5%). The study was conducted in accordance with the Animal Laboratory Guideline of the Study Center of Food and Nutrition Laboratory, Gadjah Mada University, Yogyakarta. The study design was reviewed and approved by the Ethical Clearance Committee of Public Health Faculty, Universitas Muhammadiyah Semarang with certificate number 162/KEPK-FKM/UNIMUS/2019.

After a week of acclimatization, the rats were randomly divided by 5 groups (n=6), consist of K(-) group: group fed by normal diet (laboratory standard diet AD II, 4.35 kcal/g, 0% fat) as a control; K(+) group: group fed by high-fat diet; P1 group: group fed by high-fat diet and treated by TF 1.9 g/200 g body weight; P2 group: group fed by high-fat diet and treated by RG flour 0.036 g/200 g body weight; and P3 group: group fed by high-fat diet and treated by a combination TF 0.95 g/200 g body weight and RG 0.018 g/200 g body weight. The high-fat diet was made by the addition of duck egg yolk in laboratory standard diet AD II (5.28 kcal/g, 11.1% fat). All rats were given diets in the pellet form in 20 g/day and were supplied by *ad-libitum* water. TF and RG flour were added to the pellet. The interventions were given for 21 days. At the end of the study, the overnight fasted blood rats were collected through retroorbital. To get plasma, 3 mL blood was centrifuged at 3500 rpm for 5 minutes and was stored at -80°C for future use.

2.4. Assessment of Food Intake and Body Weight

Food intake was assessed daily by subtraction of the amount of food given daily (20 g) by leftover using the **Ozeri ZK14-S Pronto Digital Food Scale**. Furthermore, bodyweight was assessed weekly static weight (Biosep-In Vivo Research Instrument, USA).

2.5. Assessment of Biochemical Parameter

Triglyceride level (TG)

TG level was assessed using 10 µL serum which was added by 1000 µL working solution then was mix thoroughly. After incubated at 37^0C for 10 minutes, the OD was read at 510 nm with a 0.5 cm diameter cuvette. TC was calculated by the following formula:

$$TG\ level\ (mmol/L) = \frac{\text{OD sample} - \text{OD blank}}{\text{OD standard} - \text{OD blank}} \text{x} 2.26.$$

HDL level

HDL level was assessed using a 10 µL sample which was added by 750 µL Reagent 1 (consist of Good's Buffer, Toos, $MgCl_2$ $6H_2O$, fat oxidase, and peroxidase). The solution was mixed and was incubated at 37°C for 5 minutes. The OD value (A1) was measured at 546 nm. Reagent 2 (consist of Good's Buffer, 4-ampyrones, $MgCl_2$ $6H_2O$, fat esterase, and surfactant) as many as 250 µL was added to the solution and was mixed. After incubated at 37°C for 5 minutes, the OD value (A2) was measured at 546 nm. HDL level was calculated by the following formula:

$$HDL\ level\ (mmol/L) = \frac{(\text{A2sample} - \text{A1sample}) - (A2blank - A1blank)}{(\text{A2standard} - \text{A1standard}) - (A2blank - A1blank)} \text{ x concentration of standard.}$$

Glucose level

Blood sample 1 ml was taken in the Eppendorf tube which has been dropped by anticoagulant EDTA. The sample then was centrifuged in 3000 rpm for 3 minutes. Of the sample, 20 µl was taken and was added in 2000 µl enzyme working solution tube. The same steps were done to standard and distilled water. The tubes were incubated in 37°C water bath for 25 minutes. The spectrophotometer was set to zero with a blank tube and was measured in the OD values and in 505 nm wavelength. Glucose level was calculated using the formula:

$$Glucose\ (mmol/l) = \frac{\text{OD sample} - \text{OD blank}}{\text{OD standard} - \text{OD blank}} \text{x} 5 \text{ x dillution factor of sample before tested.}$$

$$Glucose\ (mg/dl) = mmol/l\ x18.$$

2.6. Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and were analyzed by one-way ANOVA followed by

Tukey's post hoc test. All data were performed using the Statistical Product and Service Solution (IBM SPSS 21.0). The data were regarded as significant at P<0.05 and a confidence interval 95%.

3. Results

Bodyweight and food intake

Along with the study, there was no significant difference in food intake between all groups (Figure 1A). All groups had a food intake ranging from 17.2 to 18 g per day during the study. On the other hand, a significant differences appeared on body weight primarily in the third week (P<0.05). The significant different experienced on P3 and K(-) groups compared with the K(+) group which can be seen in Figure 1B.

Glucose and TG/HDL ratio

Blood glucose levels of the treatment groups were significantly different (P<0.05) compared to K(+). Of the treatment groups in Figure 1A, the P3 group possessed the lowest blood glucose level followed by P1 and P2 respectively. Between the treatment groups, P3 had significantly different (P<0.05).

The same pattern showed in Figure 1B occurred to the insulin sensitivity which was represented by TG/HDL ratio. TG/HDL ratio in P3 group revealed the lowest ratio compared to all the groups followed by P1 and P2 respectively. P3 group had significantly lower compared to the P2 group (P<0.05). In the same way, P1 showed significantly a higher TG/HDL ratio than P2 (P<0.05).

4. Discussion

Melatonin is a hormone regulating sleep/wake cycle and also is considered as an antioxidant which progresses through non-receptor processes [13]. Some foods contain melatonin levels which not only originates from the main ingredients, like grapes but also through the process, like cultural foods from Japan which mostly processed by fermentation (such as *natto*, *miso*, *tofu*, *shoyu*) [13,14]. Melatonin could be synthesized in the fermentation process due to yeast growth [15]. In this animal experimental study, tempeh flour treatments exerted the bodyweight level lower than red ginger treatment which might indicate that tempeh flour contains melatonin components. This is our first hypothesize and it potentiates to be further explored since tempeh is one of the fermented products which quite similar to the basic ingredient of *tofu*. A theory corroborating our finding is the previous study conducted in animal laboratory supplemented by melatonin which successfully reduced lipogenesis and acted to increase the lipolytic capacity and oxygen rate consumption of adipocytes through PPAR-γ and CEBP-α by 3T3-L1 cells differentiation [16]. The total bodyweight of the group treated by the combination of tempeh and red ginger flour showed lower compared to the total body weight in the group treated by sole tempeh flour. It is speculated that there is a synergistic component in tempeh and red ginger leading to increase beneficial effects towards body weight.

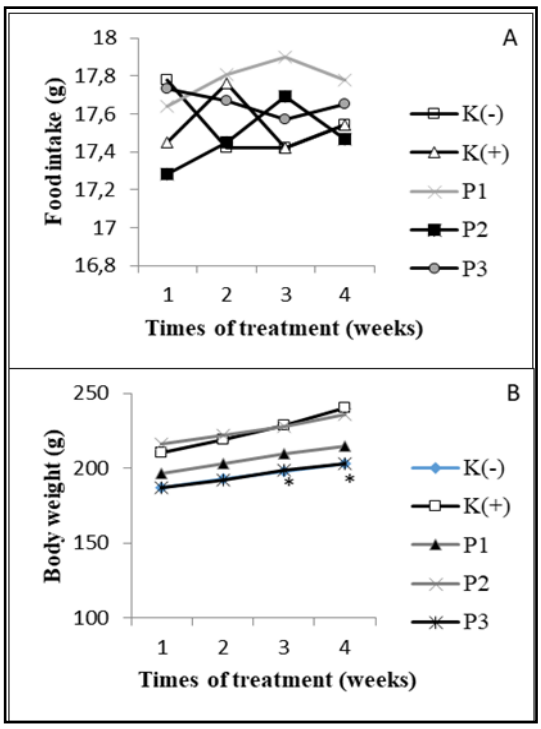

Figure 1. Effect of tempeh and red ginger flour, and the combination of tempeh and red ginger flour on food intake (A) and body weight (B). Values high-fat as means ± SD (n=30). *P<0.05 versus K(+) group

High-fat diet prescription for 21 days successfully decreased insulin sensitivity in this study. There are some possibilities that can explain the mechanism: 1) decreases in binding affinity of cell membrane towards insulin action which eventually decreases the cell membrane responsiveness; 2) increases in inflammatory cytokines [3].

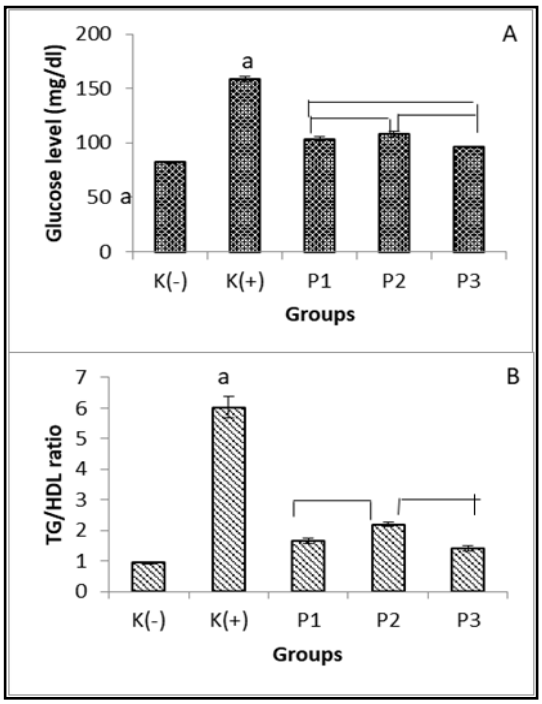

Figure 2. Effect of tempeh and red ginger flour and the combination of tempeh and red ginger flour for 21 days. (A) glucose level. (B) TG/HDL ratio. (a) represents a significant difference compared to all groups represents a significant difference towards a related group. Value is expressed as mean ± SD. Data were considered significant at P<0.05

Tempeh is one of the fermentation products originated from Indonesia which is considered a low-cost protein source [14]. The fermentation process in tempeh has long been known to hydrolyze isoflavone glucosides content in soybean to aglycones form, therefore isoflavone content including genistein, daidzein, and glycitein are absorbed at higher rate than their glucosides [8,9,14]. Tempeh contains approximately 43.5 ± 8.3 mg%/100 g isoflavone that The group treated by tempeh had a significant difference in blood glucose level and insulin sensitivity compared to the positive control group. Soybean isoflavones in tempeh are featured by diphenolic compounds that are similar to estrogen and bind to estrogen receptors[8]. Estrogen is an essential factor for glucose-stimulated insulin secretion and the expansion and maintenance of β-cell mass. Even though the exact mechanism is still unclear, but estrogen is able to close K_{ATP} channels through a cyclic guanosine monophosphate-dependent phosphorylation leading to stimulates insulin secretion [9,17].

Furthermore, the sole treatment of red ginger flour alleviated glucose level and insulin sensitivity of rat fed by a high-fat diet. The hypoglycemic effect of red ginger powder is because of its phenols, polyphenols, and flavonoids content which decrease blood glucose level by antagonistic activity agonists serotonin receptors and its blockage. Ginger also potentiates to reduce glucose absorption by inhibiting intestinal glucoside and amylase enzyme activity [18]. Another mechanism that can support the ginger powder administration to improve insulin sensitivity is a component so-called 6-Gingerol in ginger stimulates glucose metabolism through AMPKalpha2-mediated AS160-Rab5 pathway and via regulation of insulin-mediated glucose [19]. The other components in ginger such as 6-shogaol and 6-paradol promote glucose utilization in both adipocytes and muscle cells [20]. Ginger roles as a suppressor for NF-κB due to S-[6]-gingerol effect, as the most abundant component in ginger, to ameliorate diabetes-induced upregulation of TNF-α, IL-1, and IL-6 and to suppress ROS-activated NF-κB/COX2 [21,22,23].

The effect of the combination of the tempeh and red ginger has the biggest reduction in blood glucose levels and the highest improvement of insulin sensitivity. This postulates that the combination of those has a synergistic effect to alleviate insulin sensitivity. However, as this is speculation, further investigation is needed to determine the most active component of ginger that responsible for the isoflavones in tempeh to improve insulin sensitivity.

5. Conclusions

In conclusion, the combination of tempeh and red ginger flour on a high-fat diet rat showed a beneficial effect better than the sole treatment on body weight, blood glucose level, and insulin sensitivity.

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Statement of Competing Interests

There is no conflict of interest.

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