Red Dragon Fruit Peel Yogurt and Tempe Flour (Soygurt) Effectively Improve Oxidative Stress on a STZ-induced Diabetic Model in Rats

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Abstract: Chronic hyperglycemia can lead to a variety of complications such as cardiovascular disease. Probiotic yogurt is suggested as a nutritional approach in hyperglycemia. The aim of this research was to investigate the impact of probiotics red dragon fruit peel yogurt and tempeh flour on oxidative stress of hyperglycemic rats. This was experimental research with pre and post-test control group design. A total of 36 male Sprague-Dawley rats were randomly divided into six groups. Hyperglycemic conditions were made by administering STZ and NA for 72 hours. All groups received standard feed. The groupings were P1 (negative control); P2 (positive control); P3 (hyperglycemia; 3.6 ml/kgBW/day of standard soygurt); P4 (hyperglycemia; 3.6 ml/kgBW/day of commercial soygurt); P5 (hyperglycemia; 1.8 ml/kgBW/day of standard soygurt); P6 (hyperglycemia; 1.8 ml/kgBW/day of commercial soygurt). MDA was measured twice, before and after the 28 days of intervention. Data were analyzed using paired t test and one-way ANOVA. The group that received 3.6 ml/kgBW/day of standard soygurt (P3) showed the highest reduction in MDA compared to other groups. Red dragon fruit peel and tempeh flour yogurt is effective in improving stress oxidative of hyperglycemic rats.

Keywords: hyperglycemia rats, MDA, stress oxidative, yogurt

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is associated with hyperglycemia, insulin resistance, and gut dysbiosis (Sengupta et al., 2019). The condition of T2DM will result in the body cells gradually failing to absorb and use insulin produced by the pancreas. The contribution of T2DM patients is almost 90% of the total diabetes mellitus patients. The International Diabetes Federation predicts 700 million people with T2DM by 2045 (Tabrizi et al., 2019).

Oxidative stress plays a major role in the pathogenesis and progression of diabetes. Diabetes is associated with increased free radical production and impaired antioxidant defense. This condition will trigger cellular damage, enzyme dysfunction and disruption of paraoxonase-1 binding to HDL (Ejtahed et al., 2012). Patients with T2DM show disturbances in the gut microbiota.

The population of lactic acid bacteria such as bifidobacterial has a lower population than the concentration of enterococci and Escherichia coli (Chen et al., 2019). The composition of gastrointestinal bacteria may increase inflammation which will affect glucose and lipid metabolism mechanisms and insulin action (Tazakori et al., 2017).

Probiotics, prebiotics and synbiotics are reported to have therapeutic effects for insulin resistance and glucose repair through modulating the gut microbiota. Functional foods based on probiotics and prebiotics can be an alternative food therapy for the prevention or treatment of T2DM. Products made from prebiotics and prebiotics can improve the condition of T2DM through several mechanisms. Certain species that act as probiotics, such as bifidobacterial and lactobacilli, have a role in maintaining the balance of intestinal microflora (Baspinar & Güldaş, 2021).

Research shows that the use of lactic acid bacteria strains has a role as an antioxidant. The antioxidant mechanism in probiotics is possible through the capture of reactive oxygen species, metal ion chelation, enzyme inhibition and reduction activity and inhibition of autooxidation of vitamin C. The use of prebiotic such as dragon fruit peel and tempe flour helps the fermentation process of lactic acid bacteria in yogurt products to be maximized. This study aims to determine the potential of dragon fruit peel and tempe yogurt to improve oxidative stress in hyperglycemia conditioned experimental animals.

METHOD

This study used a true experiment design and was conducted at the House of Experimental Rats CNFS, Gajah Mada University, Yogyakarta, Indonesia. Sprague Dawley rats used had inclusion criteria of age 8-12 weeks, weighed 150-240 grams with fasting blood glucose levels <110 mg/dL, healthy and had no physical abnormalities, active during the adaptation period and had no weight loss during the study of >10%. Sprague Dawley rats were adapted for seven days before the intervention. 36 Sprague Dawley rats were randomly divided into 6 groups so that each group consisted of 6 rats. The treatment used gastric sonde 1x/day for 28 days. The treatment in question is giving tempeh yogurt (soygurt) and added red dragon fruit peel. The division of groups includes

P1 (negative control/ not in hyperglycemia); P2 (positive control group/ hyperglycemia and got 3.6 ml of LAB standard yogurt); P3 (hyperglycemia rats treated with 3.6 ml soygurt LAB); P4 (Hyperglycemia rats treated with soygurt commercial 3.6 ml); P5 (Hyperglycemia rats treated with soygurt LAB 1.8 ml); P6 (Hyperglycemia rats treated with soygurt commercial 1.8 ml). Hyperglycemia conditions in Sprague Dawley rats through the administration of streptozotocin 45

mg/kgBB and Nicotinamide (NA) 110 mg/kgBB for 72 hours. Modified soygurt with red dragon fruit peel was produced based on Bintari's research (Bintari et al., 2020) dan Mardiana (Mardiana et al., 2020). The composition of yogurt consists of milk, red dragon fruit peel, sugar and lactic acid bacteria. Whole blood samples were obtained from retroorbital vein and centrifuged at 3000 rpm for 15 minutes to obtain blood serum before and after the study. Oxidative stress was measured using MDA. The MDA measurement method uses Thiobarbituric acid reactive substances (TBARS) (Barańska et al., 2021).

The data of each group was shown as mean ± standard deviation (SD). Paired t test was used to determine the differences of each group before and after treatment. One-way analysis of variance (ANOVA) was used to determine differences in all groups and continued with the post hoc test to determine differences between groups. Values were considered statistically significant when $p<0.05$.

RESULTS

Table one shows that there was a decrease in MDA levels in the group given red dragon fruit skin yogurt and tempeh flour. The highest decrease in MDA levels occurred in the group given yogurt using Lactobacillus bulgaricus and Streptococcus thermophilus strains compared to yogurt using standard products. Table one also shows the difference in oxidative stress before and after the intervention. Table one also shows that there is a difference in reducing oxidative stress in each treatment group.

Table 1. Oxidative stress in hyperglycemia rats

MDA	P1[a]	P2[b]	P3[c]	P4[d]	P5[e]	P6[f]
pre	1.42±0.18	9.50±0.19	9.85±0.36	9.46±0.32	9.75±0.39	9.84±0.51
post	1.58±0.13	9.16±0.20	2.33±0.42	2.53±0.17	3.45±0.22	3.85±0.25
Δ	0.16±0.07[c,d,e,f]	-0.34±0.03[c,d,e,f]	-7.52±0.58[a,b,c,d,e,f]	-6.93±0.32[a,b,c,e,f]	-6.30±0.46[a,b,c,d]	-5.99±0.71[a,b,c,d]
p	0.006	0.001	0.001	0.001	0.001*	0.001

*Sampling was done 72 hours after induction of STZ+NA and 28 days after start of treatment.

P1: Negative control group; P2: Positive control group/ Hyperglycemia and receiving 3.6 ml of standard yogurt; P3: Hyperglycemia, treated with soygurt BAL 3.6 ml/kg b.wt/day; P4: Hyperglycemia, treated with commercial soygurt 3.6 ml/kg b.wt/day; P5: Hyperglycemia, treated with soygurt BAL 1.8 ml/kg b.wt/day; P6: Hyperglycemia, treated with commercial soygurt 1.8 ml/kg b.wt/day

Values represent the mean ± SD for observation mode on six rats in each group.

Units: milligrams per deciliter

Statistical analysis: p*; paired t- test, significant difference ($p<0.05$); One-way analysis for variance (ANOVA), where significant, post hoc testing (least significant difference) was done for intergroup comparisons.

[a]Statistically significant difference ($p<0.05$) when compared with P1 values

[b]Statistically significant difference ($p<0.05$) when compared with P2 values

[c]Statistically significant difference ($p<0.05$) when compared with P3 values

[d]Statistically significant difference ($p<0.05$) when compared with P4 values
[e]Statistically significant difference ($p<0.05$) when compared with P5 values
[f]Statistically significant difference ($p<0.05$) when compared with P6 values

DISCUSSION

Hyperglycemia triggers massive free radical changes. These free radicals will cause lipid peroxides and MDA generation. The activities of SOD, GPx, CAT and reactive oxygen species capture in hyperglycemia conditions are decreased (Tazakori et al., 2017). Improvement of oxidative stress status can contribute to the management of diabetes. Modification of probiotic products showed an improvement in oxidative stress in rats with hyperglycemia (Ahmadi et al., 2019). This study was conducted for 28 days by giving red dragon fruit skin yogurt modified with tempe flour. The results showed that the concentration of MDA in the group given red dragon fruit peel yogurt with modified tempe flour significantly decreased.

The composition of gut microbiota in patients with T2DM always changes. Research conducted by Chin showed that there was an imbalance of microflora in the digestive tract of people with T2DM. Research conducted by Larsen also showed the presence of gram negative bacteria such as phyla Bacteroidetes and Proteobacteria in the intestinal microbiota of T2DM patients. The proportion of Firmicutes compared to Bacteroidetes significantly decreased in T2DM patients when compared to patients who did not have diabetes (Lasker et al., 2019).

This study is in accordance with research conducted by Harisa. Harisa mentioned that the administration of L. acidophilus can significantly reduce the concentration of MDA in diabetic rats. Fermented products such as dahi have similar functions to yogurt (Harisa et al., 2009). Dahi is fermented milk incubated using L. acidophilus and L. casei. The result of research using this fermented product is the presence of antioxidative effects in hepatic and pancreatic tissues in rats conditioned with diabetes using high-fructose induction.

Red dragon fruit peel yogurt modified with tempe flour was able to inhibit oxidative damage in pancreatic tissue of STZ-induced rats. This inhibition is done through the mechanism of preventing lipid peroxidation and maintaining the activity of SOD, GPx, and CAT. Lactobacillus probiotics used in this study have a role in inhibiting the production of proinflammatory cytokines. The anti-inflammatory effect caused by the consumption of fermented products may be the presence of probiotic compounds that can mediate a decrease in oxidative stress. The process of modulating the immune system and the anti-inflammatory effect of probiotics can modify the intestinal microflora through the mechanism of inhibiting ascorbate autoxidation, metal ion chelation and decreasing the activity and scavenging superoxide anion radicals. Lactic acid bacteria that can

be found in the digestive tract can trigger improvements in intestinal function, increase immunity and have a role as an antioxidant and even antitumor (Damasceno et al., 2014).

The decrease in MDA concentration in the yogurt group was due to the antioxidative effect of releasing bioactive peptides during the yogurt fermentation process by proteolytic lactic acid bacteria. Bioactive peptides derived from casein have the ability to capture free radicals and inhibit enzymatic or non-enzymatic reactions in lipid peroxidation events. Antioxidant peptides derived from whey protein can help synthesize glutathione as an intracellular antioxidant derived from protein cysteine extraction (Punaro et al., 2014).

CONCLUSION

Consumption of red dragon fruit peel and tempeh flour yogurt can reduce stress oxidative levels in hyperglycemic rats.

Conflict of Interest

The authors declare that they have no conflict of interest.

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