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ASSESSING THE PHYSIOLOGICAL EFFECTS OF PURPLE SWEET POTATO-ENRICHED YOGURT ON BODY WEIGHT, LIPID PROFILES, AND ANTIOXIDANT STATUS IN AN EXPERIMENTAL RAT MODEL

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Abstract

This study investigated the effects of purple sweet potato yogurt (PSPY) and green tea yogurt (GTY) on body weight, lipid profiles, and total antioxidant status in a high-fat diet-induced rat model. Thirty male white mice were divided into five groups and subjected to different dietary interventions. After 12 weeks, the results showed that PSPY and GTY significantly reduced body weight gain compared to the positive control group. Moreover, PSPY exhibited a notable improvement in total antioxidant status and lower total cholesterol and triglyceride levels, suggesting potential health benefits in managing lipid metabolism and oxidative stress. No significant changes in low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were observed. Histological examination revealed protective effects against hepatic cell apoptosis in the PSPY group. These findings highlight the potential of PSPY and GTY as functional foods with cardio metabolic benefits.

Keywords: Purple Sweet Potato Yogurt; Green Tea Yogurt; High-Fat Diet; Lipid Profiles; Total Antioxidant Status

INTRODUCTION

Dyslipidemia is one of the most important cardiovascular risk factors (CRF) in triggering coronary heart disease (CHD) events and is still the most important modifiable risk factor.[1] [2]. Probiotics are believed to modulate various factors that may play an important role in the occurrence of CHD. Probiotics can lower cholesterol levels by increasing bile salt synthesis and bile acid deconjugation, and enable protection from cardiovascular disease. Additionally, probiotics have been found to have anti-oxidative, and anti-inflammatory properties.

The epidemiological and clinical studies conducted by Reis et al. (2016) found that anthocyanins, a polyphenolic compound, have been associated with an improved cardiovascular risk profile and reduced comorbidities. Intervention studies in humans using berries, vegetables, plant parts and cereals (either fresh or as juice) or purified anthocyanin-rich extracts have shown significant in improvement of low-density lipoprotein oxidation, lipid peroxidation, total plasma antioxidant capacity, and dyslipidemia and reduced molecular biomarker level of CVD.[3]

The study conducted by Hartley et al. (2014) concluded that the antioxidant effect of green tea could be suggested to protect against CHD. The reduction in CVD risk with tea may be largely due to the high levels of polyphenols, particularly flavonoids, which are contained in green and black tea. Some of the potential mechanisms include reducing body weight, increasing insulin sensitivity, improving dyslipidemia, improving endothelial function by reducing oxidative stress, platelet inhibition and anti-inflammatory effects. In addition, tea and its flavonoids have antioxidant properties that help reduce the risk of CHD.[4]

MATERIAL AND METHOD

Purple sweet potato is washed, peeled then steamed and mashed then sieved. Yogurt is made from pure cow's milk which has been tested for the appropriateness of milk. Pasteurization is carried out until it reaches a temperature of 70-80ºC, cooled to 40ºC, then added a starter culture mixture of *Lactobacillus bulgaricus* and *Streotpcoccus thermophyllus* bacteria with a 1:1 ratio of 3-5% of the volume of milk. Fermented for 8-24 hours. Then added purple sweet potato mashed 10% and fermented for 8-24 hours.

This research used 30 male white mice (Rattus norvegicus Wistar strain) aged 8 weeks with body weight of 150-200 grams acclimated in cages for 7 days. The treatment was conducted at the Animal Laboratory of the Food and Nutrition Study Center, Gadjahnmada University Yogyakarta. This research has received a Certificate of Ethical Properness from KEPK Poltekkes Kemenkes Yogyakarta Number. E-KEPK/ POLKESYO/0389/IV/2022.

RESULTS AND DISCUSSION

In the current study, the aim was to evaluate the effects of several treatments on rat body weight, lipid profile, and total antioxidant status. Five groups of rats were studied with different treatments, namely: C- (negative control), C+ (positive control), PSPY (purple sweet potato yogurt), GTY (greentea yogurt), and Sim (simvastatin).

This research was conducted for twelve weeks, first week for adaptation. Thirty mice were grouped into five groups, group I was given standard feed (SD) as a negative control (C-). Group II was fed HFD as a positive control (C+), group III was fed HFD and 3 mL/ kg bwt of purple sweet potato yogurt (PSPY), group IV was fed HFD and 3 mL/ kg bwt of green tea yogurt (GTY), and group V was given HFD and simvastatin 2 mg/kg body weight. Each treatment was given by oral sonde into the mice's stomachs.

At the end of the treatment period, the mice were sacrificed with the cervical dislocation method. The liver and pancreas were taken and fixed with formalin, then processed and stained with HE. The liver tissue was observed for the level of steatosis, inflammation, necrosis and fibrosis. Pancreatic tissue was also stained with anti-insulin immune-histochemical stain to reveal islet cells of Langerhans.

Table 1: Body weight of mouse

group	n	Body weight (gram)						
		pre			*post*			*p-value*
C-	6	180.50	±	2.18	246.00	±	3.41	<0.05
C+	6	176.17	±	3.82	324.67*	±	5.28	
PSPY	6	178.00	±	3.16	251.17	±	2.79	
GTY	6	174.83	±	3.54	244.67	±	5.09	
Sim	6	176.33	±	4.18	243.17	±	5.46	

*(p<0.005)

From Table 1, the C+ group had the most significant increase in body weight. This could be due to several factors, including diet, physical activity, or other factors that may have influenced the positive control group. The PSPY, GTY, and Sim groups showed a more moderate increase in body weight compared to C+. This suggests the potential of these treatments in controlling weight gain or even having a protective effect. The substantial increase in body weight observed in the negative control group

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(C-) following the treatment period is consistent with well-documented findings in the field of nutrition and rodent physiology. Mice in this group experienced a statistically significant weight gain, which may be attributed to factors such as ad libitum access to food and a lack of dietary restrictions. This outcome aligns with previous research emphasizing the importance of calorie intake regulation for maintaining healthy body weight. Numerous studies have demonstrated that unrestricted dietary access can lead to overconsumption and weight gain in mice, serving as a relevant model for understanding human obesity. These findings underscore the critical role of dietary control in body weight management.

Table 2: Lipid profile and total antioxidant status

Meas	N			C+			PSPY			GTY			Sim			*p*
TC	72,3	±	14,5	133,0*	±	13,2	66,33	±	13,9	63,8	±	13,9	62,3	±	8,43	<0.05
TG	157,0	±	44,1	172,2*	±	73,2	153,8	±	39,3	159,2	±	17,2	84,0	±	49,4	<0.05
LDL-C	54,1	±	12,9	45,7	±	5,17	44,5	±	17,5	43,2	±	8,56	41,0	±	8,62	>0.05
HDL-C	8,77	±	2,92	8,47	±	1,78	10,7	±	4,28	9,78	±	2,37	9,75	±	1,47	>0.05
TAS	274,6	±	40,1	265,3	±	51,6	294,4	±	78,1	270,3	±	45,5	279,8	±	40,8	<0.05

N=negative control group, C+= positive control; PSPY = purple sweet potato yogurt; GTY= greentea yogurt, Sim= simvastatin; TC= total cholesterol; TG=triglyceride; TAS=total antioxidant status

TC and TG levels increased significantly in the C+ group. This increase suggests that there are factors in the positive control group that affect the lipid metabolism of rats. The PSPY group showed lower TC and TG levels compared to C+, suggesting that purple sweet potato yoghurt may have a positive effect in controlling blood lipid levels. GTY and Sim also showed similar results to PSPY, indicating their potential in maintaining lipid balance. LDL-C and HDL-C did not show significant differences between the groups, so the effects of the treatments on these cholesterol fractions still need to be further investigated.

The positive control group (C+), in contrast, displayed elevated levels of total cholesterol (TC) and triglycerides (TG) after the treatment period. This is consistent with well-established research regarding the effects of high-fat or high-cholesterol diets on lipid profiles. High dietary fat intake has been linked to an increase in TC and TG levels, contributing to hyperlipidemia—a recognized risk factor for cardiovascular diseases. These results emphasize the direct impact of dietary composition on lipid metabolism, with the excessive consumption of dietary fats and cholesterol leading to adverse lipid profile changes. Understanding these relationships is critical for elucidating the role of diet in cardiovascular health.

The significant increase in total antioxidant status (TAS) observed in the purple sweet potato yogurt (PSPY) group is noteworthy and consistent with the growing body of research on the health benefits of antioxidants. Purple sweet potatoes are known for their rich antioxidant content, particularly anthocyanins and carotenoids, which confer their vibrant color. Antioxidants play a crucial role in mitigating oxidative stress, which is linked to various chronic diseases, including cardiovascular diseases and cancer. Research has indicated that regular consumption of foods rich in antioxidants can positively impact overall antioxidant status in the body. The observed increase in TAS in the PSPY group suggests that the treatment may have led to enhanced antioxidant defenses, potentially protecting against oxidative damage.

The PSPY group had the highest TAS levels, which suggests that purple sweet potato yoghurt may be rich in antioxidant compounds. Antioxidants are known to have many health benefits, including protecting the body from free radical damage and preventing chronic diseases. The other groups, including C+, GTY, and Sim, had similar TAS levels, suggesting that they may not have the same powerful effects in terms of antioxidant status as PSPY. The provision of a high-fat diet for 12 weeks resulted in significant weight gain in the positive control group compared to the negative control group of rats which were given a standard diet. The mean weight of the negative control rats was 246.00 grams ± 3.41, while the positive control group was 324.67 grams ± 5.28. Giving yogurt, both purple sweet potato and green tea yogurt, has been shown to significantly inhibit weight gain. These results are in line with Shalaby's research (2021) which proves that consumption of yogurt plus green tea extract is effective for reducing obesity. The mechanism of reducing obesity is via the lipolytic route by inhibiting catechol-O-methyltransferase by catechins.[18]

High-fat diet increased total cholesterol and triglyceride levels in the positive control group with an average of 133.0 mg/dL ±73.2 and 172.20 mg/dL ±73.2. The group of rats that were treated with high-fat feed and given purple sweet potato yogurt (YU) showed lower total cholesterol levels than the positive control group (K+). This shows that giving purple sweet potato yogurt can reduce total cholesterol in rats with high-fat feed. This research is in line with the results of research conducted by Lestari et al (2020) that purple sweet potato yogurt contains several bioactive components that can reduce cholesterol levels such as anthocyanins, dietary fiber, and lactic acid bacteria [5][6]. This is also in accordance with a review article by Wallace, Slavin and Frankenfeld (2016) which states that nine of the 10 studies included LDL as an outcome evaluated for statistical significance in the intervention group compared with the control [7]. Possible mechanism for cholesterol-lowering effects of anthocyanins could be the inhibition of cholesterol synthesis. It has been shown that anthocyanins can activate AMP-activated protein kinase (AMPK) [8] which is involved in the regulation of energy homeostasis and influences the activity of many enzymes. One enzyme that is inhibited by AMPK is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Because HMG-CoA reductase is the limiting enzyme of cholesterol synthesis, increased AMPK activity would inhibit cholesterol synthesis and consequently lead to lower cholesterol levels. Furthermore, AMPK inhibits the activity of acetyl-CoA carboxylases ACC1 and ACC2, which leads to increased fatty acid oxidation and decreased fatty acid synthesis and, accordingly, lower triglyceride concentrations [9]. Whereas that green tea and its catechins effectively lower the intestinal absorption of lipids. Among the green tea catechins, EGCG is the most potent inhibitor of lipid absorption. The potent inhibitory effect of EGCG appears to be associated with its ability to form complexes with lipids and lipolytic enzymes, thereby interfering with the luminal processes of emulsification, hydrolysis, micellar solubilization, and subsequent uptake of lipids [10]

Studies show that certain probiotics secrete insulin analogues and enhance the desired biologic effect on adipocytes targeting both humans and animal subjects [11][12]. The results of previous studies indicate that probiotic and prebiotic supplementation can have a preventive and therapeutic effect on CHD by reducing total serum cholesterol, low density lipoprotein (LDL cholesterol) [13]. This strengthens the evidence for the role of probiotics and prebiotics in modulating the metabolic activity of the human gut microbiota [14][15]. The gut microbiome communicates with distant organs, including the heart, in a variety of ways. Among them are the production of trimethylamine (TMA) / trimethylamine N-oxide (TMAO), short chain fatty acids, bile acids, lipopolysaccharide (LPS), and peptidoglycan[16].

The mechanism of reducing serum cholesterol levels by lactic acid bacteria in purple sweet potato yogurt can be through the assimilation of bacteria in the intestine, binding of cholesterol to the bacterial cell wall, and deconjugation of bile salts, as well as production of lactic acid and short-chain fatty acids [5].

Total cholesterol and triglyceride levels also showed a decrease in the group of mice that were given a high-fat diet and green tea yogurt. This is in line with a study conducted by Shalaby (2020) which showed that mice given yogurt plus green tea showed a decrease in total serum cholesterol, LDL-C, V LDL-C level and an increase in HDL-C level [17]. In line with the results of research by Wang et al (2020) that showing, green tea leaf powder significantly reduced TC and LDL-C levels, and significantly increased HDL-C levels in a dose-dependent manner and also found a reduction in TG; however, the difference was not statistically significant [18].

The results of the study in the positive control rat group showed the lowest antioxidant status compared to the other groups. This shows that a high-fat diet can reduce the antioxidant system in the body. The measurement of total antioxidant status provides information about all the antioxidants in an organism.

Interestingly, there were no statistically significant differences in low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels among the experimental groups. This result suggests that the treatments did not significantly affect the balance of LDL-C and HDL-C, two critical components of lipid profiles. Previous research has shown that dietary interventions, such as specific dietary patterns or cholesterol-lowering medications, can influence these lipid fractions. The lack of significant changes in LDL-C and HDL-C in this study may imply that the treatments did not have a substantial impact on cholesterol transport and metabolism.

The antioxidant system in the body can be classified as enzymatic antioxidants and non-enzymatic antioxidants. The main antioxidant enzymes directly involved in the neutralization of reactive oxygen species (ROS) and reactive nitrogen species RNS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx). Non-enzymatic antioxidants are also divided into metabolic antioxidants and nutritional antioxidants. Metabolic antioxidants which are classified as endogenous antioxidants are produced by metabolism in the body, such as lipoid acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal chelating proteins, transferrin, etc. Meanwhile, nutrient antioxidants that are classified as exogenous antioxidants are compounds that cannot be produced in the body and must be provided through food or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and fatty acids omega-6 fats, etc.[19]

The results of Shalaby's research (2021) also show that the phenolic component of green tea added to yogurt has antioxidant activity. But from the results of this study, yogurt enriched with purple sweet potato showed a better increase in antioxidant status than yogurt enriched with green tea. The enzymes alanine transferase and aspartate transferase are hepatic cell intracellular enzymes, so they are commonly used to determine the level of hepatic cell damage. An increase in blood levels indicates an increase in hepatic cell damage. In this study, there was no significant increase in AST and ALT enzyme activity in all groups. This shows that the increase in triglyceride and cholesterol levels did not cause an increase in hepatic cell necrosis, even though there was fatty liver in the group of rats that were given a high-fat diet without being treated with yogurt. Research by Su et al. (2020) proved that there was very high expression of AC-P53 protein (Lys373, Lys382) and total P53, which resulted in increased expression of protein P21 in the livers of rats fed a high-fat diet. Interestingly, in the group that was treated with purple sweet potato dye, there was a significant decrease in AC-P53, T-P53 and P21 protein levels in the livers of rats that were fed a high-fat diet. Based on this, the study concluded that dyes from purple sweet potatoes protect liver cells induced by a high-fat diet from apoptosis by increasing inhibition of the Sirt1-dependent p53 apoptotic pathway and facilitating the Akt survival pathway [20].

Liver cell damage in this case is not always caused by inflammation but by apoptosis. Apoptosis, a major form of programmed cell death, is considered a fundamental component in the pathogenesis of various liver diseases including Non-Alcoholic Fatty Liver Disease (NAFLD). It has been proven that a high-fat diet can increase the development of NAFLD through the induction of liver cell apoptosis [17]. HFD caused the hepatocyte hypertrophy and vacuolization and inflam- matory cell infiltration in mouse livers, further confirming the occurrence of NAFLD. Significant increases in serum alanine aminotransferase and hepatic triglyceride levels were found in HFD-treated rats compared to controls. These results indicated that color of purple sweet potato protected against hepatocyte apopto- sis in HFD-induced NAFLD mouse model [20]. Research by Zhu et al. (2020) found that giving yogurt on a high-fat diet can reduce serum AST and ALT enzymes, as well as reduce cholesterol and triglyceride levels. [21].

While these findings provide valuable insights into the effects of different treatments on body weight and lipid profiles in mice, further research is needed to elucidate the precise mechanisms underlying these outcomes and to assess their long-term health implications. Investigating the gene expression, metabolic pathways, and hormonal changes associated with these treatments could shed light on the observed effects. Moreover, extrapolating these findings to human populations should be done cautiously, as mouse models may not fully replicate human responses. Nevertheless, this study underscores the critical importance of dietary interventions and their potential impact on body weight and lipid profiles, highlighting the ongoing relevance of nutrition research in promoting health and preventing diseases.

CONCLUSION

The results of this study indicated that the group of rats that were fed a high-fat diet and treated with green tea yogurt, increased the AST enzyme although not statistically significant. Meanwhile, in the group of rats that were fed a high-fat diet and treated with purple sweet potato yogurt, the enzyme activity was lower. This proves that this yogurt is safe for consumption. Safety tests were also carried out by cytotoxicity tests using Vero intestinal cells using the MTT method. The results of the cytotoxicity test showed the living cells were 99%, meaning that giving purple sweet potato yogurt and green tea did not increase the occurrence of apoptosis in Vero's intestinal cells.

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