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EFFECT OF BLACK GLUTINOUS TAPE BEVERAGE ON MALONDIALDEHYDE IN METABOLIC SYNDROME RATS

Efek Sari Tape Ketan Hitam terhadap Malondiadehid pada Tikus Sindrom Metabolik

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ABSTRAK

Sindrom Metabolik (SM) dikenal sebagai sindrom resistensi insulin, juga dikenal sebagai faktor risiko DMT2. Diperlukan kombinasi perubahan gaya hidup dan pengobatan dalam penanganan SM. Sari tape ketan hitam (BGTB) sebagai pangan lokal mengandung tinggi kandungan antosianin sebagai sumber antioksidan dan Lactobacillus spp., yang berpotensi sebagai sumber probiotik yang dapat memperbaiki stres oksidatif pada pasien SM. Studi ini dirancang untuk mengevaluasi efek BGTB dalam menangani stres oksidatif, dengan melakukan pengukuran pada kadar MDA. Sebanyak 30 tikus Sprague Dawley digunakan dalam penelitian ini dan mereka dibagi menjadi lima kelompok. Kelompok normal (KN) akan diberikan diet standar, sedangkan keempat kelompok akan diberikan high-fat diet (HFD) selama 2 minggu dan kemudian diinduksi menggunakan Streptozotocin (STZ) dan Nicotinamide (NA); kontrol negatif (KN) akan diberikan standar diet; kontrol positif (KP) akan diberikan metformin 9 mg; kelompok perlakuan 1 (P1) dan perlakuan 2 (P2) akan diberikan BGTB sebanyak 0,9 ml 200 gr^{-1} BB dan 1,8 ml 200 gr^{-1} BB, diberikan selama 28 hari. Hasil menunjukan adanya perbedaan sginifikan (p=0.000) pada kadar malondialdehid, dengan perbandingan tertinggi pada kelompok P2. Penelitian ini menunjukkan BGTB dapat menjadi makanan fungsional dan berpotensi menjadi sumber probiotik untuk pasien SM.

Kata kunci: *sindrom metabolik, pangan fungsional, antosianin, tape ketan hitam, malondialdehid*

ABSTRACT

Metabolic syndrome (MetS), also referred to as insulin resistance syndrome, poses a risk for Type 2 Diabetes Mellitus (T2DM). The management strategy for MetS usually involves changes in lifestyle and drug therapy. A local food known as Black Glutinous Tape Beverage (BGTB) is rich in anthocyanin, an antioxidant that can potentially alleviate oxidative stress in those with MetS. It also contains Lactobacillus spp., making it a potential source of probiotics. The purpose of this study was to examine the impact of BGTB on reducing oxidative stress, which was done by measuring MDA levels. In the study, thirty male Sprague Dawley rats were divided into five groups. One group was given a normal diet (N), while the other four groups were put on a high-fat diet for two weeks, induced by Streptozotocin (STZ) and Nicotinamide (NA). The negative control group (KN) continued with the standard diet, the positive control group (KP) was administered 9 mg of metformin, and the two treatment groups (P1 and P2) were given BGTB at doses of 0.9 ml 200 gr-1 rat BW and 1.8 ml 200 gr-1 rat BW for 28 days respectively. The study found a significant difference (p=0.000) in malondialdehyde levels, with the P2 group showing the highest levels. This suggests that BGTB could be used as a functional food and might be a potential source of probiotics for patients with MetS.

Keywords: metabolic syndrome, functional food, anthocyanins, black glutinous tape beverage, malondialdehyde

INTRODUCTION

Metabolic syndrome (MetS) is a set of metabolic abnormalities known as insulin resistance syndrome. Obesity and insulin resistance have been significantly implicated in metabolic syndrome [1], [2]. The prevalence of MetS in Indonesia reached 28% in men and 46.2% in women. Hyperglycemia (51%) ranks second as a component of MetS after hypertension (61%) [3]. Metabolic syndrome is five times more likely to develop into diabetes mellitus, with 4% of deaths caused by diabetes mellitus [4].

Hyperglycemia conditions can result in glucose oxidation, protein glycation, and activation of polyol metabolic pathways that can accelerate the formation of molecularly modified reactive oxygen, resulting in an imbalance of antioxidants and free radicals commonly known as oxidative stress [5], [6]. In addition, obesity causes an increase in adipocyte size and adipose tissue mass, which damages adipocytes and initiates the inflammatory process, which stimulates the release of pro-inflammatory cytokines, adipokines, and free fatty acids into the bloodstream and causes oxidative stress [7], [8]. Malondialdehyde, the end product of lipid peroxidation, affects the activity of enzymes that are key in the mitochondrial respiratory chain and can exacerbate membrane damage [9]. Yokota et al.'s study [10] found in metabolic syndrome patients, malondialdehyde levels were significantly higher compared to antioxidant levels.

Management of MetS involves a dual approach, combining lifestyle changes and pharmacological interventions [2], [11]. There is no single drug therapy for Mets; multiple drugs are required over a long period to improve each component. Developing nutraceuticals and functional foods can help reduce the use of drugs and adverse side effects [2], [12].

Functional foods reduce the risk, slow down or prevent certain diseases, and improve immunity, not including drugs or supplements [13]. Black glutinous tape is one of the functional foods obtained from the fermentation of black glutinous rice. Black sticky rice contains anthocyanins, phenols, and antioxidant activity that can be a healthy snack for people with SM. Consumption of black glutinous rice >11.5 grams per day has a protective benefit against MetS by 16 times compared to individuals who consume <11.5 grams[14], [15].

The purple color of black sticky rice shows the anthocyanin content, which is essential in protecting cell damage from oxidation stress [16]. The direct mechanism of antioxidants will donate hydrogen electrons, which can then react with malondialdehyde [17]. Consumption of foods containing anthocyanins at 2-400 mg/Kg body weight can protect against oxidative stress[18]. It is a polyhydroxyl or polymethoxy derivative of 2-phenyl-benzopyrylium; anthocyanins cannot be metabolized, so the body excretes them in this form. Hydrolysis of anthocyanin glycosides facilitates the degradation and absorption of anthocyanins in the body, and fermentation helps the process of hydrolysis of anthocyanin glycosides [19], [20]. It has hydrophilic properties, making it easier for anthocyanins to dissolve in water [17]. In Trinovani's research [21], the anthocyanin content in black sticky rice on day three of fermentation amounted to 132.490 μg with an antioxidant activity of 127.558%.

Black glutinous tape is an excellent probiotic carrier because it does not need to go through processing again, which can reduce the number of probiotics before consumption [22]. According to Panjaiatan's research [23], the lactic acid bacteria L. fermentum 1 BK2-5 found in tape ketan hitam has the most significant potential as a probiotic candidate. Lactobacillus, Bifidobacteria, and some lactic acid bacteria have enzymes that can break down the structure of anthocyanins, thus helping to increase the availability and absorption process in the body [24], [25].

On the third day of fermentation, it was reported that the water content that resulted from the fermentation of black glutinous rice had reached 55.18% [14]. Black glutinous tape beverage (BGTB) is the term used to describe this water content. BGTB is categorized as alcoholic fermentation, given that the process results in the production of ethanol. Black glutinous tapai beverage utilized in this process contains 0.013% ethanol, as per MUI's fatwa on food and beverage containing alkohool, BGTB is not considered khamr [26]. There is currently no research on the effects of black glutinous rice juice on malondialdehyde levels in metabolic syndrome conditions. The author is therefore interested in learning how BGTB influences malondialdehyde using the metabolic syndrome model of Sprague Dawley rats.

METHODS

Making black glutinous rice juice starts with separating black glutinous rice from foreign objects and washing it. 200 grams of black glutinous rice are soaked for eight hours or overnight to aid in softening and enhance their permeability, thereby accelerating the fermentation process. Next, the black glutinous rice is steamed for 1 hour. After that, prepare hot water that has just been brought to a boil in a ratio of 1:1.2, then put the steamed black glutinous rice into the boiling water and stir again until evenly distributed. Cover the pot and let it sit for 30 minutes. Prepare the steaming pot again, then steam the black sticky rice for ± 1 hour until cooked. Remove and cool the black sticky rice. After cooling, mix the sticky rice with1,5 mg mashed yeast, then stir until smooth. Store the tape in a closed container and let it sit for ± 3 days. After standing for three days at room temperature, the black glutinous rice is squeezed and filtered, and the black glutinous liquid, called BGTB, is taken and stored in a sterilized glass bottle.

The study took place from March to May 2023 in the Laboratory of the Center for Food and Nutrition Studies at Gajah Mada University. The research, approved by the KEPK Faculty of Medicine UNS (ethical clearance number 61/UN27.06.11/KEP/EC/2023), employed a randomized posttest-controlled group design for a lab experiment. The subjects were 30 healthy male Sprague-Dawley (SD) rats, aged between 8-12 weeks, each weighing approximately 200 grams. Throughout the study, the rats were housed in cages with unrestricted access to both food and water, maintaining controlled environmental conditions (22 °C ± 2 °C, humidity 55% ± 5%, and a 12-hour light/dark cycle). Any rat that experienced diarrhea refused to eat, lost up to 10% of its weight, or died during the experiment, was removed from the study.

The Sprague Dawley rats were split into five groups: a normal control group (KN), a negative control group (K-), a positive control group (K+) which was administered 9 mg of metformin, a treatment group 1 (P1) which received 0.9 ml/200KgWB of BGTB, and a treatment group 2 (P2) which was given 1.8 ml/200KgWB of BGTB. All treatment groups were provided with standard feed and unlimited distilled water. The intervention lasted for 28 days.

To create metabolic syndrome in the rats, they were fed a high-fat diet (HFD) and given unlimited distilled water for two weeks, except for the rats in the normal group (KN). Following this, the rats were induced using Streptozotocin (STZ) + Nicotinamide (NA). The STZ was dissolved in a citrate buffer (pH 4.5), and the NA was dissolved in normal physiological saline. The rats were first induced with NA (110 mg/kg BW) in the intraperitoneal section and then, 15 minutes later, induced with STZ (45 mg/kg BW).

Blood will be drawn through the retro orbital plexus. The concentration of Malondialdehyde was determined using the TBARS method, which assesses the level of Thiobarbituric Acid Reactive Substance. To start, 750 μl of phosphoric acid was transferred into a 13 ml polypropylene tube. Next, 50 μl of a standard TEP, quality control, plasma sample, or distilled water was added to the tube. This mixture was then shaken until it was uniformly mixed. Following this, 250 μl of a 40 mM TBA solution and

450 µl of distilled water were introduced into the tube, which was then sealed. The mixture underwent heating for an hour. After the heating process, the tube was immediately cooled in an ice bath. The cooled sample was then processed through the C18 Column set-pack. The absorbance of the sample was measured using a spectrophotometer at a wavelength of 532 nm [27].

The data will be evaluated using One-way ANOVA, followed by the Post Hoc Tukey HSD test. In cases where the data does not follow a normal distribution, non parametric tests such as the Kruskal Wallis and Games-Howell tests will be employed.

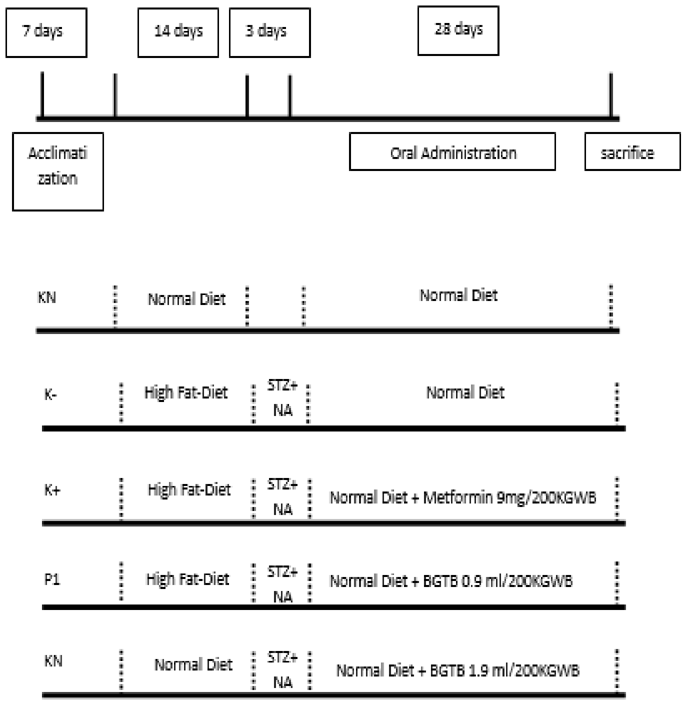

Figure 1. Consort of experimental protocol

RESULT

The two weeks of HFD and STZ+NA induction results in Table 1 show that the three components of NCEP-ATP III have been fulfilled. Rats can be used as experimental animals.

Table 1 Components of Metabolic Syndrome after HDF Administration and STZ+NA Induction

Components	Mean (mg/dl)		
	FBG	HDL	Trigliserid
KN	69,08	80,28	78,22
K-	270,66	25,84	141,24
K+	269,70	25,03	141,61
P1	269,76	25,84	144,04
P2	267,39	24,45	143,31

Table 1 shows the mean of metabolic syndrome components. The results of the groups given HFD for 2 weeks and STZ+NA induction showed changes in both GDP, HDL, and triglycerides. Each component showed significant differences (p=0.000) between the KN group and the groups given HFD and STZ+NA. It can be concluded, that the administration of HFD for 2 weeks and the induction of STZ+NA at a low dose successfully made rats with a metabolic syndrome model.

Table 2 Effect Of Black Glutinous Tape Beverage On Malondialdehyde In Metabolic Syndrome Rats

Kelompok	Malondialdehyde		Percentage of Change *
	Mean (nmol/ml)	SD	
KN	1,54[a]	0,25	85,18
K-	10,49[b]	0,43	0,00
K+	3,10[c]	0,16	70,16
P1	4,91[d]	0,18	52,74
P2	2,14[e]	0,30	79.40
P	0,000**		

*Comparison of malondialdehyde value with negative control group
**One-way ANOVA statistical test
[a,b,c,d,e]) Numbers followed by the same letter indicate no significant difference (Tukey HSD Post Hoc Test, α=95%)

The results of giving BGTB to malondialdehyde levels in metabolic syndrome rats can be seen in Table 2, that all groups have lower malondialdehyde levels compared to the K- group. This clarifies the impact of administering black glutinous rice juice or metformin as an intervention. Black glutinous tape beverage significantly alters malondialdehyde levels between groups, according to the One way ANOVA test. A significant difference ($P < 0.05$) was found between the intervention groups' doses in the Post Hoc Tukey HSD test. The P2 group had a higher effect of 79.40% compared to the K-group in the intervention group.

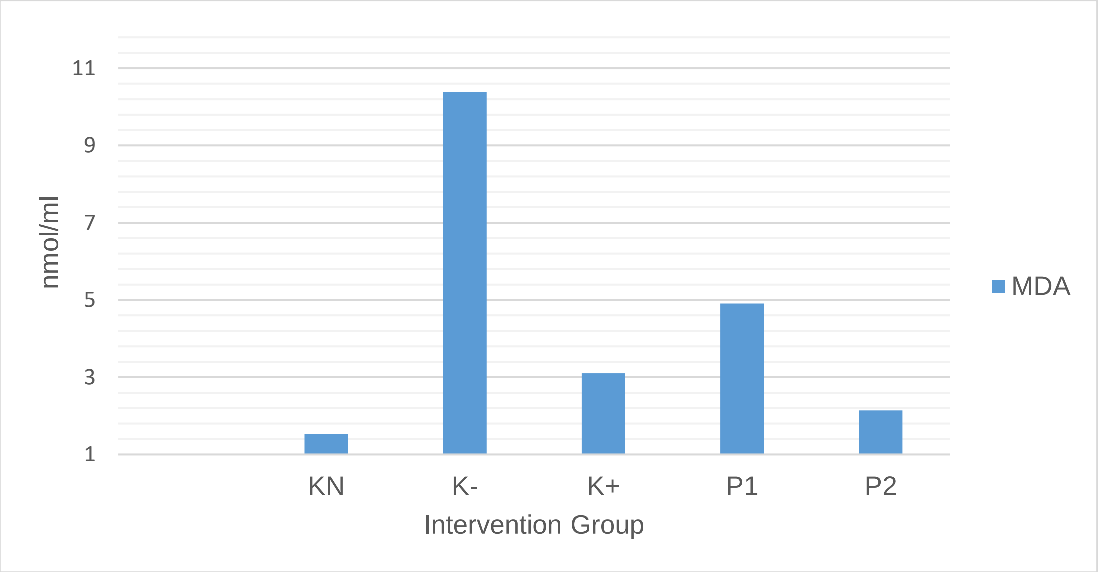

Figure 2 Graphic Effect of Black Glutinous Tape Beverage on Malondialdehyde in Metabolic Syndrom Rats
Note: KN (Normal), K- (Negative Control), K+ (Positive Control, Metformin 9 g), P1 (BGTB 0,9 ml), P2 (BGTB 1,8 ml)

DISCUSSION

The high-fat diet (HFD) involves the addition of a fat component comprising 40%-50% of total calories for a duration of 4-8 weeks [28]. High-fat diets reflect the characteristics of the modern human diet, which currently exceeds the recommended fat intake of 20-25% [29]. The outcomes associated with HFD consumption include obesity, impaired glucose tolerance, and insulin resistance [30]. It has been established that high-fat diet (HFD) consumption can cause gut dysbiosis by oxidative stress on the gut mucosa, which reduces the number of bacteria that protect the gut barrier and increases the number of bacteria that produce endotoxins. The gut microbiota is very sensitive to dietary changes [31]. The administration of STZ at low doses can sufficiently damage β-cells, leading to constant hyperglycemia in the model. Conversely, the pre-administration of NA before STZ protects β-cells from STZ-induced damage, thus facilitating the creation of a rat model of metabolic syndrome [30], [32].

The most effective administration of BGTB in reducing malondialdehyde levels was observed in group P2, where the average malondialdehyde level was 2.14 nmol/ml. However, it did not reach the intermediate level seen in the normal group (1.54 nmol/ml). The results indicate that 5 mg/Kg WB combination of black glutinous rice and dill effectively reduce MDA levels (p=0.0001) and perhaps can enhance mitochondrial function by decreasing oxidative stress production [33]. Additionally, certain probiotic strains such as L. rhamnosus, L. helveticus, and L. casei have demonstrated the ability to reduce lipid peroxidation production and malondialdehyde levels [34]. Furthermore, the administration of L. plantarum KSFY06 to mice induced by D-galactose oxidation has shown the potential to lower malondialdehyde levels and enhance the antioxidant defense system [35].

The probiotic strains exhibited a notable reduction in MDA levels by enhancing the activities of SOD and GST, along with increasing sulfhydryl content (for non-enzymatic antioxidant defense) in the colon mucosa of rats subjected to a high-fat diet (HFD). This suggests the presence of antioxidant capacity in the examined rat model due to the effective action of the probiotics. [36]. The mechanisms involved in the antioxidant capacity of some probiotic strains are still under investigation. However, it has been suggested that the antioxidant capacity of L. fermentum strains may be attributed to a fully functional GSH system formed by GSH peroxidase and GSH reductase, which serve to protect cells from oxidative stress [9]. Enhanced antioxidant capacity in colonic mucosa promoted by administration of a mixed formulation of L. fermentum was associated with downregulation in systemic mild inflammation, and reduced oxidative stress in heart tissue of HFD-fed rats [37].

The bioavailability of anthocyanins is relatively low, necessitating the assistance of gut microbes to optimize their absorption within the body. The majority of anthocyanins are absorbed in their intact form within the colon and subsequently metabolized by the gut microbiota [20]. Notable members of the gut microbiota involved in anthocyanin metabolism include Bifidobacteria spp, Lactobacillus spp, and various other lactic acid bacteria (LABs), which possess enzymes capable of simplifying the structural composition of anthocyanins [25]. Bacteria equipped with β-D-glucosidase and α-L-rhamnosidase enzymes deglycosylate anthocyanins upon reaching the colon. This enzymatic action results in the breakdown of the compound's sugars, yielding aglycones that are more readily absorbed by the body [24]. Furthermore, the aglycone form of anthocyanins undergoes further degradation by enteric bacteria, yielding phenolic compounds. Specific anthocyanin types, such as cyanidin-glucoside, are converted into protocatechuic acid, while malvidin, pelargonidin, delphinidin, and peonidin are converted into syringic acid, 4-hydroxybenzoic acid, gallic acid, and vanillic acid. The fermentation process facilitates the hydrolysis of anthocyanin glycosides, rendering them more bioavailable to the body and enhancing the overall availability of anthocyanins [20], [25].

Anthocyanins indirectly contribute to the elevation of endogenous antioxidants, thereby enhancing the activity of the enzyme Superoxide Dismutase (SOD) by activating the gene responsible for encoding the SOD enzyme. This upregulation of SOD activity subsequently leads to a reduction in malondialdehyde formation by inhibiting the activity of NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidase and xanthine oxidase [38]. Antioxidants can directly remove oxidative stress by donating hydrogen atoms, so that malondialdehyde levels can be suppressed [39]. Furthermore, antioxidants can exert protective effects on endothelial cells by suppressing the production of TNF-α, a key initiator of inflammation [38]. Consequently, the administration of BGTB emerges as a viable alternative for mitigating MDA levels due to its rich anthocyanin content and the presence of probiotics resulting from the fermentation process.

This study further demonstrated the antioxidant and anti-inflammatory attributes of metformin, as evidenced by the average malondialdehyde level in K+ group was 3.10 nmol/ml. Metformin exerts its effects by diminishing the expression of NF-kB, a factor in inflammatory transcription, subsequently inhibiting inflammatory cytokines such as IL-8 and IL-Iα, leading to a reduction in oxidative stress levels. Similar to the probiotic mechanism, metformin can lower hydrogen peroxide levels by curtailing the transcription of NADPH oxidase [33], [40].

CONCLUSION

The administration of BGTB for a duration of 28 days has a significant impact on the serum malondialdehyde levels in rats with metabolic syndrome (p=0.000). Specifically, the group receiving a 1.8 ml dose of BGTB exhibited lower malondialdehyde levels compared to the other treatment groups. This suggests that black glutinous tape beverage may serve as a promising functional food option for reducing malondialdehyde levels in individuals with metabolic syndrome.

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