The Effects of Administering Jicama Concentrate (*Pachyrhizus erosus*) and Kefir Grains as a Synbiotic Drink on Malondialdehyde and Superoxide Dismutase Levels in the Testicles of Hyperlipidemic Rats

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Abstract: Background: More than half of infertility prevalence come from men. There is a correlation between hyperlipidemia and male infertility. Malondialdehyde (MDA) and superoxide dismutase (SOD) are oxidative stress markers which indicate the testicular tissue damage caused by hyperlipidemia. The management of hyperlipidemia continues to develop to date, and synbiotics are potential for hyperlipidemia therapy. Objective: To examine the effects of jicama concentrate and kefir grains as a synbiotic drink on the levels of MDA and SOD of the testicles in hyperlipidemic rats. Methods: Twenty-five male Wistar rats were divided into 5 groups and given quail egg yolk for a month. The intervention group was given a synbiotic drink at a dose of 5ml/200grBW for a month with different combinations of jicama concentrate and kefir. The levels of MDA and SOD activity were checked after intervention. Result: The results showed that the mean MDA levels (nmol/ml) were 12.30 ± 0.28 (K +), 2.83 ± 0.27 (K-), 8.43 ± 0.38 (P1), 6.34 ± 0.29 (P2), and 4.49 ± 0.25 (P3) while the mean SOD activities (%) were 32.86 ± 6.75 (K+), 82.14 ± 7.57 (K-), 43.21 ± 5.56 (P1), 62.14 ± 7.40 (P2), 70.35 ± 4.82 (P3). There were significant differences in the MDA levels among all groups ($p < 0.01$), whereas in SOD activities the differences were found among all groups except between K+ and P1, K- and P3, as well as P2 and P3 ($p > 0.05$). Conclusion: Jicama concentrate and kefir grain as a synbiotic drink significantly decrease MDA and increase SOD in testicular hyperlipidemic rats.

1 INTRODUCTION

Infertility becomes one of the health problems which is estimated to keep increasing in the future. The high rate of infertility is influenced by contamination from the environment and lifestyle changes (Pushpendra & Jain, 2015). The estimated infertility incidence in productive age couples in the world is 8% to 12%, with a global average of 9% (Inhorn & Patrizio, 2015). In fact, more than half of the infertility cases in the world come from male partners (Pushpendra & Jain, 2015). There are a number of both internal and external factors which lead to the emergence of infertility in men. Schisterman stated that there is a correlation between high levels of blood lipids or hyperlipidemia and infertility in men. The concentration of lipids has an effect on semen parameters, especially on the morphological changes in the head of the sperm which affects male fertility (Schisterman et al., 2014).

Hyperlipidemia is an abnormal condition in lipid metabolism which is characterized by an increase in the lipid fractions. Changes in lipid fractions include increased cholesterol and triglyceride levels in the blood to above the normal values (Nirosha et al., 2014). Hypercholesterolemic conditions can damage various organs in the human body (Pushpendra & Jain, 2015), including the heart, liver, brain, and

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testes. An increasing cholesterol level in the testicular tissue results in excessive production of free radicals (ROS) and oxidative stress. In the end, such condition will have cytotoxic effects on spermatozoa (Permatasari & Widhiantara, 2017).

Oxidative stress in the testicular tissue can cause abnormalities in sperm quality and quantity, thus leading to sperm DNA fragmentation and transformation. Abnormal genetic material causes imperfect transmission of genetic information the embryo. Meanwhile, oxidative stress leads to increased DNA damage. Free radicals can also result in lipid peroxidation in the sperm cell membrane, and the damage of which will decrease the ability of the sperm to bind to oocytes (Asadi et al., 2017). The Increasing damage of DNA and cell membrane of spermatozoa results in infertility in men. The lipid peroxidation of cell membrane will form Malondialdehyde (MDA) molecules. The high production of MDA will induce cell death in the presence of a biomolecular damage process in these cells (Ayala et al., 2014). The most effective biomarker for assessing cell damage due to lipid oxidative stress is MDA. It is necessary to note that to date the damage caused by lipid peroxidation becomes the most important factor of testicular dysfunctions (Asadi et al., 2017).

Therapy to overcome hyperlipidemia continues to evolve. The use of pharmacology from the statin class remains a popular choice for patients with hyperlipidemia since the side effects of statins on the urology system rarely occur. However, such rare occurrence should not be ignored by the doctor or other healthcare providers. Linnebur and Hiatt in their case report showed that a man (54 years) with hyperlipidemia who received statins complained of discomfort due to the pain in the testicles after a few months of taking the drug (Linnebur & Hiatt, 2007).

Experts develop a new concept of disease treatment with food as medicine. The concept is rated to become a means to optimize food function as a therapy against a disease. Kefir is made from cow, goat, or sheep milk, which is fermented by a type of starter called kefir grains (Wahdania & Pramono, 2012). Kefir made from cow milk and/or goat milk can inhibit lipid peroxidation more significantly than ordinary dairy of cows and/or goats (Liu et al., 2005).

Jicama or Pachyrhizus erosus which comes from America and then grows widely in Indonesia is among the root crops with consumable tubers. It is a species of yam containing water, flour, vitamin C, B1, and minerals such as calcium, phosphate, and potassium (Widiyanti et al., 2018). Jicama can serve as a prebiotic source with fructooligosaccharide (FOS) and inulin content. Prebiotics are defined as indigestible food components with such properties as being resistant to stomach acid and hydrolysis by the enzymes in the digestive system, being fermentable by the gastrointestinal microflora, and having a selective positive effect to stimulate bacterial growth in the gastrointestinal tract (Bruggencate et al., 2006).

Synbiotics are a product which contains a combination of probiotics and prebiotics (Abed et al., 2016). This combination is useful to protect probiotics when going through various processes in the digestive tract16, thereby increasing the number of intestinal microflora and optimizing its activity in the body (Markowiak & Ślizewska, 2017). Based on the aforementioned background, this study aims to examine the effects of the administration of jicama extract (Pachyrhizus erosus) and kefir grains as a synbiotic drink on the levels of malondialdehyde (MDA) and superoxide dismutase (SOD) activity in the testes of rats with induced hyperlipidemia.

2 MATERIAL AND METHODS

This was a quasi-experimental study with a post-test only controlled group design. The subjects were male Wistar rats (Rattus norvegicus) aged 2-3 months with a bodyweight ranging from 100-300 grams. The number of rats used as the subjects followed the criteria from the World Health Organization (WHO), namely a minimum of five (5) rats in each treatment group or using the Federer's formula.

The combination of jicama and kefir grain synbiotic drink was prepared through several processes. First, to prepare the kefir milk, kefir grains were mixed with pasteurized fresh milk in a bottle at the ratio of 1:20 (100 g kefir:2 liters of milk) and fermented for 12 hours at a room temperature (25^0C – 27^0C) in a dark state.

After 12 hours, the kefir grains were strained to obtain the filtrate as kefir milk resulted from fermentation. The kefir milk was then added to pasteurized jicama juice (at a temperature of 80^0C - 90^0C) at the following ratio for each formulation. The kefir milk and jicama juice from each formulation was then mixed and re-fermented for 12 hours at room temperature (25^0C - 27^0C) in the dark. After 12 hours, the fermentation product was stored in a refrigerator at a temperature of 4°C (Mirdalisa et al., 2016).

A total of 25 rats were divided evenly into 5 groups, including the positive control group (K+), negative control group (K-), and 3 treatment groups of P1 (85% milk, 15% jicama), P2 (75% milk, 25%

jicama), and P3 (65% milk, 35% jicama). The K+ group and treatment groups were induced by quail egg yolk at a dose of 5ml/200grBW given once a day for 4 weeks through a feeding tube (Prabowo & Pramaningtyas, 2018). The blood samples were collected via the eye vein (Plexus retroorbital) for 3-5 ml after each rat was anesthetized with 0.1-0.3 cc ketamine-xylazine injected intraperitoneally.

The synbiotic drink was administered at a dose of 5ml/200grBW to the treatment groups once a day for 4 weeks through a feeding tube (Sudiarto et al., 2018). The rats were then given 0.1-0.3 ml ketamine-xylazine anesthetic intraperitoneally and decapitated. Surgery was performed to remove the testicular organs, which were then cleaned in NaCl solution, wrapped in aluminum foil, and stored in a refrigerator at -4°C.

The measurement of MDA levels used the reaction principle of 0.67% TBA and 20% TCA while SOD activity was measured using the Ransod kit. All of the measurements were carried out at UGM Inter-University Center. The bivariate data analysis was employed to determine the effects of synbiotic drink administration on MDA levels and SOD activity in the testicular tissue.

Table 1. Formulations of synbiotic drink

Formulation	Kefir (ml)	Jicama	Total
P1	85% (255 ml)	15% (45 ml)	300 ml
P2	75% (225 ml)	25% (75 ml)	300 ml
P3	65% (195 ml)	35% (105 ml)	300 ml
Total	675 ml	225 ml	

3 RESULTS

The administration of high-fat diet for 4 weeks has induced hyperlipidemia in the rats. After the egg yolk administration was complete, the rats in K+ group were given only ad libitum food, while the intervention groups were given a synbiotic drink. Four weeks later, the rats were terminated and the levels of MDA and SOD were checked. In K+ and K- groups, there were significant differences in the levels of MDA and SOD. In the second 4 weeks, the hyperlipidemic state is likely to be maintained by the endogenous mechanisms of lipid metabolism.

Table 2. Average levels of Malondialdehyde (MDA) *Superoxide dismutase* (SOD)

Group	MDA (nmol/gr)	*p*	SOD (%)	*p*
K+	12.30 ± 0.28	0,000	32.86 ± 6.75	0,000
K-	2.83 ± 0.27		82.14 ± 7.57	
P1	8.43 ± 0.38		43.21 ± 5.56	
P2	6.34 ± 0.29		62.14 ± 7.40	
P3	4.49 ± 0.25		70.35 ± 4.82	

Based on the bonferoni post-hoc test, it was found that the P3 synbiotic drink formulation gave the best effects as opposed to the P1 and P2 synbiotic drink formulations. The P3 formulation is composed of 35% prebiotics and 65% probiotics. Giving synbiotic drinks can reduce MDA levels in the testicular tissue. This is proved by the One-way ANOVA test with $p < 0.05$.

4 DISCUSSIONS

Quail egg yolk in this research can induce hyperlipidemia. Research conducted by Kusuma proved that induction by quail egg yolk at a dose of 10 ml/KgBW for 14 days increased cholesterol levels. Quail egg yolks contain numerous saturated fatty acids which, through the beta-oxidation reaction, are converted into acetyl CoA as a precursor for cholesterol synthesis. The more acetyl CoA is formed, the more it triggers the endogenous pathway for cholesterol synthesis (Kusuma et al., 2016).

This is in line with the research by Prabowo and Pramaningtyas in which a high-fat diet in the form of quail egg yolk administered for 4 weeks can induce hyperlipidemia in rats (Prabowo & Pramaningtyas, 2018). Hyperlipidemia in rats is characterized by total cholesterol levels >130 mg/dl, LDL >60 mg/dl, triglycerides >100 mg/dl, and HDL <50 mg/dl (Cahyaji, 2017).

Triglycerides and cholesterol will form micellar clots with other substances mediated by bile salts. Triglycerides are hydrolyzed into fatty acids and then absorbed by the intestine via the enterocyte cells. In the enterocyte cell, a lipoprotein in the form of a chylomicron will be produced which is composed of fatty acids, cholesterol esters, and apo B-48 as the main structural apolipoprotein in chylomicrons.

Chylomicrons are formed in the lymph tissue and then pass through the thoracic duct and enter the blood circulation. Most of the cholesterols in chylomicrons will be stored in the liver. High levels of cholesterols in the hepatic tissue inhibit cholesterol synthesis and degrade LDL receptors bound by PCSK9 (Mehta & Bhatt, 2017).

Cholesterols in the liver tissue are released into the blood circulation through VLDL, IDL, and LDL lipoproteins. After the exogenous pathway stops, cholesterols in the hepatic tissue will decrease, thus triggering cholesterol synthesis by the hepatocyte cells. Also, the high cholesterol precursor in the form of acetyl CoA will further trigger the synthesis of cholesterols (Kusuma et al., 2016). Therefore, the oxidative stress and lipid peroxidation in tissues, especially of the testes, remain high with increasing levels of MDA.

The provision of synbiotic drink, a combination of kefir grains and jicama juice, can reduce MDA levels and increase SOD activity (Zhao et al., 2020). Kefir grains contain a number of species of probiotic bacteria, including lactic acid bacteria and acetic acid bacteria. Besides, kefir grains contain yeast and fungi (Pogačić et al., 2013).

In this research, P3 group give the best result in decrease of MDA and increase SOD. This is supported by the research conducted by Al-Sultan which explained that the provision of synbiotic drinks is better than only prebiotics or probiotics to improve the function of the gut microflora in chickens. A combination of synbiotics can help optimize the management of hyperlipidemic conditions. Prebiotics protect probiotics from various enzyme activities in the digestive system. Therefore, the number of probiotics reaching the intestine will increase, resulting in optimal function of the intestinal microflora (Al-Sultan et al., 2016). A combination of synbiotics can help optimize the management of hyperlipidemic conditions. Prebiotics protect probiotics from various enzyme activities in the digestive system. Therefore, the number of probiotics reaching the intestine will increase, resulting in optimal function of the intestinal microflora (Alves et al., 2016).

Prebiotics contain inulin and FOS, which are carbohydrates that cannot be digested by the human intestine. Inulin and FOS have anti-hyperlipidemic effects, but must first be fermented into short-chain fatty acids (SCFA), namely acetate, propionate, and butyrate. The fermentation of inulin and FOS into SCFA can be mediated by the activity of the intestinal microflora. Therefore, the more optimal the intestinal microflora is, the more easily the inulin and FOS will be fermented. The result of fermentation in the form of SCFA can act as a HMGCoA-reductase inhibitor to manage endogenous hyperlipidemia conditions (Alves et al., 2016; Daliri et al., 2016).

The high LDL that accumulates in the testicular tissue increases the production of free radicals (ROS) since LDL is easily oxidized. A ROS chain reaction will induce DNA damage and PUFA peroxidation in testicular tissue cells. Probiotics can help overcome this damage by managing hyperlipidemia, thereby reducing the damage to the testicular tissue due to free radicals. Also, probiotics increase the action of antioxidants such as SOD, GPx, and catalase. These enzymes can help to resolve oxidative stress states by breaking the ROS chain and turning it into nonreactive materials. A decrease in the state of oxidative stress in the testicular tissue will inhibit the abnormal condition of the cells in the tissue. The quality of the spermatozoa produced will increase, thus heightening the expectation to help overcome male infertility and reduce the prevalence in the world population of infertility (Amdekar & Singh, 2016; Liu et al., 2005).

5 CONCLUSIONS

The administration of jicama juice (Pachyrhizus erosus) and kefir grains as a synbiotic drink can decrease malondialdehyde levels and increase testicular superoxide dismutase activity in rats with hyperlipidemia.

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