Effect of Goat Milk Yogurt Fortified with Red Rice Bran Flour on SGPT Levels of Rats (*Rattus norvegicus*) Model Diabetes Mellitus Induced Streptozotocin

I R Putri¹, A E P Haskito²*, and D A O A Permana³

¹,²Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, East Java 65151, Indonesia
³Veterinary Anatomy and Histology Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, East Java 65151, Indonesia

*Corresponding Author: drherika1989@gmail.com

Abstract. Diabetes mellitus is a metabolic disease characterized by an increase in high blood sugar levels caused by a lack of insulin secretion or impaired insulin work or both. Goat milk yogurt with fortified red rice bran flour can be used as a therapy for diabetes mellitus because it contains high fiber and antioxidants. This study was aimed to determine the effect of giving goat milk yogurt fortified with red rice bran flour on the levels of SGPT in rats diabetes mellitus induced by streptozotocin. This study used RAL with 20 male Wistar rats divided into 5 groups, namely K1 (negative control), K2 (positive control), P1, P2, and P3. Streptozotocin induction of 45 mg/kgBW at K2, P1, P2, and P3. In therapeutic group used volume of 1 ml/kgBW, 2 ml/kgBW, and 3 ml/kgBW. The data of this study were analyzed quantitatively using the one-way ANOVA statistical test followed by Tukey test α = 0.05. The results showed that the administration of goat milk yogurt fortified with red rice bran flour could reduce SGPT levels in rats. The conclusion that the volume of administration of 2 ml/kgBW is an effective volume of administration to reduce SGPT levels by 50.50 ± 7.59.

1. Introduction

Diabetes mellitus is a multifactoral disease characterized by chronic hyperglycemia syndrome and impaired carbohydrate, fat, and protein metabolism, caused by insufficiency of insulin secretion or endogenous insulin activity or both. Chronic Diabetes mellitus can cause long-term organ damage, especially the eyes, kidneys, nerves, liver, and blood vessels. Hyperglycemia in diabetes mellitus causes glucose autooxidation, protein glycation, and activation of the polyol metabolic pathway and then accelerates the formation of Reactive Oxygen Species (ROS) or formation of oxidative stress. Reactive Oxygen Species (ROS) causes an increase of free radicals in the body. These free radicals damage various body tissues, such as the liver cells. The liver is an organ that functions as a neutralizer of toxic substances that enter the body, and subjected to the increase of free radical concentration [1]. Damage of the hepatic cells membrane lead to elevated levels of Serum Glutamate Pyruvate Transaminase (SGPT) in the blood circulation.

Goat milk contains nutrients, such as protein, fat, carbohydrates, vitamins, minerals and enzymes that are easily utilized by the body because they have smaller molecular sizes. In addition, goat milk contains many Monounsaturated Fatty Acids (MUFAs), Polyunsaturated Fatty Acids (PUFAs), and...
Medium Chain Triglycerides (MCTs) which are bioactive components. The bioactive components of the peptide function as antimicrobial, antihypertensive, and antioxidative agent. Goat milk could prevent lactose intolerance because of its very small fat globules [2].

Yogurt is a milk product fermented with Lactic Acid Bacteria (LAB). In the fermentation process of milk into yogurt, occurs hydrolysis process by LAB results in bioactive peptides. Goat milk yogurt contains bioactive peptides consisting of 16 amino acids, as antioxidants [3]. Bioactive peptide of antioxidants contained in milk include proline, histidine, tyrosine or tryptophan [4].

Rice bran is a by-product of rice milling obtained from the outer layer of rice karyopsis. Red rice bran contains fiber and has a higher antioxidant activity than black rice bran and white rice bran. The main antioxidants contained in red rice bran are vitamin E (tocopherol and tocotrienol), γ-oryzanol at 88.07%, and vitamin B15 [5].

This research was conducted to see the effect of 4% red rice bran fortified goat milk yogurt fortification on SGPT levels in rats (Rattus norvegicus) with diabetes mellitus model, so it is expected to be used as an alternative treatment for diabetes mellitus in pets.

2. Material and Methods
2.1 Animal model of Diabetes Mellitus
The experimental animals used in this study were 20 male rats (Rattus norvegicus), Wistar strain aged 8-12-week with an average body weight 130-200 grams in healthy condition. The use of experimental animals has received ethical approval with No: 1115-KEP-UB. The rats (Rattus norvegicus) were divided into 5 treatment groups and acclimatized for 7 days by feeding 20g/head/day and drinking ad-libitum.

2.2 Making Goat Milk Yogurt Fortified with Red Rice Bran Flour
Yogurt making is done by preparing the mother culture by pasteurizing goat milk using the High Temp Short Time (HTST) technique until milk 72°C for 15 seconds. Then allowed to stand until the temperature reaches 45°C and then inoculated the starter 0.5% and 4% red rice bran. Incubated for 2-3 hours until the pH reaches 4-4.5. The therapy used the red rice bran fortified goat milk yogurt given in rats using gastric sonde with a volume of administration 1 mL/kgBW, 2 mL/kgBW, and 3 mL/kgBW for 14 days [6].

2.3 Streptozotocin Induction
Streptozotocin inducted by intraperitoneal administration at 45 mg/kg BW dose, before the induction rats were fasted for 12 hours and after induction rats were incubated for 2 days, then the determination of the condition of diabetes mellitus was measured with a glucometer to ensure an increase in blood glucose and diabetes mellitus in the rats (Rattus norvegicus).

2.4 Administration Goat Milk Yogurt Fortified with Red Rice Bran Flour
The therapy used the goat milk yogurt fortified with red rice bran flour given in rats using gastric gavage with a volume of administration 1 mL/kgBW, 2 mL/kgBW, and 3 mL/kgBW for 14 days [6].

2.5 Measurement of Blood Glucose Levels
Blood glucose measurements were carried out before the treatment of rats (Rattus norvegicus) and performed before and after the induction of streptozotocin. Measurement of blood glucose performed before induction streptozotocin aims to determine blood glucose levels before the animals exposed to diabetes mellitus. Re-measurement of blood glucose levels after induction of streptozotocin. If the blood glucose level of the rats (Rattus norvegicus) has reached 200 mg / dl, the rat is already in a condition of diabetes mellitus. Measurement of glucose levels conducted after 14 days which had been treated using goat milk yogurt with a photograph of red rice bran.
2.6 Dissection and blood sampling in Rats
Dissection and blood sampling in Rats performed on the 25 day after the therapy using goat milk yogurt with red rice bran flour fortification was completed. The first step is euthanasia of experimental animals was done by cervical dislocation, then surgery was performed. Rats are placed in a dorsal recumbency, surgery is done by making an incision in the abdomen. The abdomen is opened and expanded towards the lateral, so the organs in the abdominal cavity are visible. Blood sampling taken from the ventricle of the heart using disposable syringe ±3 ml. Blood is collected in the venoject tube without anticoagulation, closed and tilted. The serum is taken using a micropipette and transferred to the microtube for centrifugation at 3000 rpm for 10 minutes. Serum is stored at -20°C until it will be used again. Serum obtained from centrifugation results is used as a sample for the measurement of SGPT enzyme levels.

2.7 Measurement of SGPT levels of blood serum
The principle of determining SGPT blood serum is carried out using the kinetic method principles established by the International Federation of Chemical Chemistry (IFCC) using the UV-Vis spectrophotometer. Rat’s blood serum (0,1 mL) obtained on day 25 then mixed with 1 mL SGPT reagents. Afterwards, put in a cuvette, left for 5 minutes to react and then measured its absorption using a UV-Vis spectrophotometer at a wavelength of 365 nm. Measurements were perfomed four times at 60-second intervals. The results of the calculation of SGPT levels are expressed in units of units/liter (U/L) which is the number of enzymes in 1 liter of serum that can produce NAD+ at the same time unit.

2.8 Data analysis
This research data is quantitative data and statistical analysis was performed with the One Way ANOVA Test using Statistical Package for The Science (SPSS) and followed by Tukey test α = 0.05.

3. Results and Discussion
3.1 SGPT Levels in Blood Serum in Rats (Rattus norvegicus)
The results of blood glucose levels in rats (Rattus norvegicus) induced by streptozotocin and after receiving therapy using goat milk yogurt with fortified red rice bran flour fortification are presented in Table 1

| Treatment Group | Average SGPT levels SGPT (U/L) ± SD | Increased levels of SGPT (%) on negative controls | Decrease levels of SGPT (%) on positive controls |
|-----------------|-------------------------------------|--------------------------------------------------|-----------------------------------------------|
| K1  (Negative Controls) | 27.25 ± 1.71a | - | - |
| K2  (Positive Controls) | 105.75 ± 43.75b | 288.07 | - |
| P1 (1 ml/kg BB) | 58.25 ± 6.84a | - | 44.91 |
| P2 (2 ml/kg BB) | 50.50 ± 7.59a | - | 52.24 |
| P3 (3 ml/kg BB) | 57.75 ± 4.24a | - | 45.39 |

Note: Notations a and b show significant differences (p<0.05) between treatment groups.

The levels of SGPT on the negative control group (K1) with an average value of 27.25±1.71 U/L represents the average standard of normal SGPT levels in rats (Rattus norvegicus) of Wistar strains. Statistical test results showed a significant difference (p<0.05) between the negative control group (K1) and the positive control (K2). According to Yusuf et al.[7], normal SGPT rate of rats (Rattus norvegicus) strain Wistar, ie 17.5-30.2 U/L. The rats in the negative control group (K1) has normal SGPT levels because were only given feed in the form of rations and ad libitum drinking water and without any other treatment. SGPT levels in the positive control group (K2) had an average value of 105.75±43.75 U/L.
The SGPT levels in the positive control group (K2) had an average value of 105.75±43.75 U/L. The levels in the positive control group (K2) increased by 288.07% against negative control (K1). Rats (Rattus norvegicus) in the positive control group (K2) were given streptozotocin at a dose of 45 mg/kg body weight. According to Hasanah [8], streptozotocin is a diabetogenic agent that can damage pancreatic β cells. Diabetogenic mechanism of streptozotocin through DNA alkalization in the nitroseurea group results in damage to pancreatic β cells by forming free radicals, such as superoxide (O$_2^-$), hydrogen peroxidase (H$_2$O$_2$), and hydroxyl radicals (OH$^-$) so that they are reactive in the body. Administration of therapy using goat milk yogurt with a fortification of red rice bran flour is able to lower the level of blood serum SGPT. Statistical test results showed the three treatment groups (P1, P2, and P3) were significantly different (p<0.05) to positive control (K2). Treatment group 1 (P1) with a volume of 1 ml/kg body weight obtained an average value of SGPT level 58.25 ± 6.84 U/L which decreased by 44.91%. Treatment group 2 (P2) with a volume of administration of 2 ml/kgBB obtained an average value of SGPT levels 50.50 ± 7.59 U/L which decreased by 52.24%. Treatment group 3 (P3) with a volume of administration of 3 ml/kgBB obtained an average value of SGPT levels of 57.75 ± 4.24 U/L which decreased by 45.39%. Low SGPT levels are caused by the ability of bioactive peptides found in goat milk yogurt serves as an antioxidant. According to Zakaria et al. [9], bioactive peptides can inhibit the enzyme α-glucosidase which can decrease hyperglycemic. This will reduce triglyceride lipolysis in adipose tissue. which reduce the release of free fatty acids and there is no fatty liver (steatosis) so that SGPT levels decrease in blood serum.

Red rice bran contains phenolic acid, flavonoids, anthocyanins, proanthocyanin, tocopherol, Tocotrienol, γ-orizanol, and filic acid [10]. Tocopherol and tocotrienol are non-enzymatic antioxidants which are part of vitamin E. These antioxidants can protect cells from free radical damage by donating one free electron to free radicals or receiving an unstable electron so that it becomes more stable [11].

4. Conclusion
Administering goat milk yogurt with red rice bran flour fortification with 2 ml/kg BW dosage of administration is an effective dose that can lower the levels of SGPT and SGOT in rats (Rattus norvegicus) which is given streptozotocin dose 45 mg/kgBB.

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