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Effect of Goat Milk Kefir Fortified with Vitamin D₃ on Blood Glucose and Insulin in Rats

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ABSTRACT

Background: Insulin resistance can trigger the accident of type 2 diabetes mellitus (T2DM). Insulin resistance will increase blood glucose levels and resulting hyperglycemia. Vitamin D₃ has important role to maintain glucose tolerance through insulin secretion and sensitivity. Goat milk has higher vitamin D₃ content than cow's milk. Kefir known has good effect for diabetes. Goat milk kefir with vitamin D₃ potentially lowers blood glucose and insulin levels in diabetes person.

Aim: To investigate the effect of goat milk kefir fortified with vitamin D₃ on blood glucose and insulin.

Methods: This research was designed with randomized pre-post control group design. A total of 20 male Wistar rats were divided randomly into 4 groups: normal rats (K- group); diabetic rats (K+ group); diabetic rats with goat milk kefir treatment (P1 group); diabetic rats with goat milk kefir with vitamin D₃ treatment (P2 group). Treatment was conducted for 28 days. Statistical analysis used Paired t-test on normal data and Wilcoxon test on abnormal data. Different test between groups using ANOVA for normal data and Kruskal Wallis for abnormal data.

Results: Significant difference on blood glucose levels between the treatment groups was found (p=0.049). Goat milk kefir with vitamin D₃ did not decrease insulin levels and blood glucose levels significantly. However, there was found a decrease on insulin and blood glucose levels in diabetic Wistar rats.

Conclusion: There was an effect of goat milk kefir with vitamin D₃ fortification on decreased insulin and blood glucose levels in diabetic Wistar rats but it was not statistically significant.

Keywords: Goat milk kefir, Fortification, Vitamin D₃, Blood glucose, Insulin

INTRODUCTION

Insulin and blood glucose have correlation with diabetes. Blood glucose is a major precursor of diabetes. It can be seen from the amount of blood glucose levels in the body. One of the signs that a person has diabetes mellitus is value of actual blood glucose levels >200 mg/dl and fasting blood glucose levels >126 mg/dl.¹ Some conditions that can trigger the increasing of blood glucose levels is age, weight, physical activity, and sex.² Insulin is a regulates hormone of blood glucose. In the condition of diabetes mellitus, insulin cannot work properly. There is some malfunction of signaling between insulin and blood glucose. Insulin resistance initiates the incidence of diabetes mellitus type 2. In the condition of insulin resistance, blood glucose and insulin levels will increase.³

Insulin resistance is a metabolic syndrome that influenced by various factors, one of which is free radicals. Kefir is milk fermentation product. Kefir made by inoculating the kefir seeds. Kefir seeds have potential effect as probiotics and antioxidants.⁴ Potential effect of antioxidant in kefir has a good benefits for insulin resistance because insulin resistance triggered by oxidative stress.⁵ Research shows that consumption of kefir can reduce fasting blood glucose in patients with diabetes mellitus type 2.⁶ Kefir is dairy products that have potential effects to reduce hyperglycemia by increasing insulin.⁷ Goat milk contains high levels of protein, vitamin A, vitamin D, thiamin, riboflavin, niacin, pantothenic acid, calcium and phosphorus than cow's milk.⁸ Goat's milk has distinctive aroma and flavor that's made goat milk consumption not as much as cow's milk.⁹ Fermentation is one way to improve undesirable sensory characteristics in goat's milk. In the fermentation process, organic components will be broken

down by enzymes into smaller forms, making them more digestible, stable and can increase the taste of food.¹⁰ Goat milk has higher vitamin D content than cow milk. However, vitamin D content of both milk still low to sufficient daily intake. Vitamin D₃ (cholecalciferol / calciol) is a type of vitamin D obtained from animal foods and also can be formed in the skin through UV exposure.¹¹ Vitamin D plays an important role to maintain glucose tolerance through insulin secretion and sensitivity.¹² Risk of diabetes increase on Vitamin D deficiency condition.³ Vitamin D deficiency causes dysregulated glucose metabolism by disrupting glucose-stimulated secretion in the hyperglycemic phase.¹³ Therefore, This study aimed to investigate the effect of goat milk kefir fortified with vitamin D₃ on blood glucose and insulin.

METHODS

This was a true experimental study with randomized pre-post control group design in mice with kefir vitamin D₃ fortified. In vivo research was held in Animal Laboratory of Medical Faculty Diponegoro University.

Subjects: The sample in this study was 24 male Wistar rats aged 2 months with 150-300 grams weight. Mice were obtained from UD. Tiput Abadi Jaya, Yogyakarta, Indonesia.

Kefir Production: Kefir was made from goat milk. Milk was pasteurized at 72°C for 1 minute then, cooled to 26°C. Then, 2% of kefir seeds were inoculated and incubated for 18 hours at 25°C. When fortification of vitamin D₃ was done, kefir stored in the refrigerator at 4°C. Kefir was produced in Diponegoro University Integrated Laboratory.

Treatments: Rats were placed in individual cage and acclimatized for 7 days with free feeding and drinking. After

acclimatization, the rats were divided into 4 groups: normal rats (K-); diabetic rats (K+); diabetic rats with goat milk kefir treatment (P1); and diabetic rats with goat milk kefir with vitamin D₃ treatment (P2). Diabetic induction via intraperitoneal using nicotinamide (NA) 120 mg/kgW. After 15 minutes, they were induced with streptozotocin (STZ) 60 mg/kgW. STZ is well known to cause pancreatic β -cell damage, whereas NA is administered to rats to partially protect insulin-secreting cells against STZ.¹⁴ 5 days after injections, blood samples were taken for observe fasting blood glucose levels via plexus retro orbital. Ketamine 60-100 mg/kgW and xylazine 4-10 mg/kgW were used as anesthesia. Interventions with doses of 2ml/day orally were done for 28 days. All subjects were fed 20 gr/day and ad libitum drinking. In the end of study, rats were fasted for 8-10 hours before taking blood. Blood glucose levels were measured using GOD-PAP method (glucose oxidase-peroxidases) while insulin measurement used ELISA method (enzyme linked immunosorbent assay).

Data Collection and Analysis: Data from blood glucose and insulin levels were analyzed using statistical data processing program. Normality of the data was tested with the Saphiro-Wilk test. Differences between blood glucose and insulin levels before and after treatment were analyzed using Paired t-test while the abnormal data were tested using Wilcoxon test. Differences of blood glucose and insulin levels between the sample groups were tested using ANOVA and then followed by Post-Hock statistic test. The

abnormal data were tested using Kruskal Wallis test and continued with Mann-Whitney test. The limit for statistical significance was set at $p < 0.05$ (95% degree of trust). Result were reported as Mean \pm SD

Ethical Clearance: Ethical approval for this study was obtained from ethical research committee at Department of Nutritional Science Faculty of Medicine, Diponegoro University and Kariadi Central General Hospital (ethical clearance number is No.73/EC/H/FK-RSDK/IX/2017).

RESULTS

Diabetic induction by STZ and Na showed the increasing of insulin levels even though there was no statistically significant difference (Table 1). A tendency of decreased insulin levels was found in the intervention group. Insulin levels in the group with goat milk kefir intervention showed higher decrease than the group with goat milk kefir fortified with vitamin D. The decrease of insulin levels in goat milk kefir group was -219.31 pg/ml, while the decrease of insulin levels in goat milk kefir fortified with vitamin D group was -85.32 pg/ml.

Blood glucose levels test showed significant differences in each treatment group (Table 2). Intervention with goat milk kefir showed statistically significant difference on blood glucose levels between pre and post intervention. The blood glucose in the group of normal rats and diabetic rats tent to increase.

Table 1. Insulin Difference Between Treatment Groups

| Group | Insulin (pg/ml) | | | P |
|---|---------------------|---------------------|--------------------|--------------------|
| | Pre | Post | Δ Pre-Post | |
| Normal rats (K-) | 431.56 \pm 80.34 | 435.55 \pm 354.44 | 4.00 | 0.984 ¹ |
| Diabetic rats (K+) | 391.09 \pm 74.82 | 409.83 \pm 135.23 | 18.80 | 0.776 ¹ |
| Diabetic rats with goat milk kefir (P1) | 511.53 \pm 233.94 | 292.22 \pm 150.39 | -219.31 | 0.080 ^x |
| Diabetic rats with goat milk kefir with vit D ₃ (P2) | 538.43 \pm 252.06 | 453.11 \pm 202.14 | -85.32 | |
| P | 0.582 ^y | 0.446 ^z | 0.399 ^z | |

Annotation: ¹ Paired t; ^x Wilcoxon; ^y One Way ANOVA; ^z Kruskal Wallis

Table 2. Blood Glucose Difference Between Treatment Groups

| Group | Blood Glucose (mg/dL) | | | p ^t |
|---|----------------------------------|-----------------------------------|---------------------|----------------|
| | Pre | Post | Δ Pre-Post | |
| Normal rats (K-) | 157.44 \pm 17.82 ^a | 235.78 \pm 35.40 ^a | 78.34 ^b | 0.007* |
| Diabetic rats (K+) | 349.94 \pm 92.44 ^b | 489.90 \pm 96.86 ^{ab} | 139.96 ^b | 0.082 |
| Diabetic rats with goat milk kefir (P1) | 416.42 \pm 101.92 ^b | 346.16 \pm 100.19 ^{ab} | -70.26 ^b | 0.158 |
| Diabetic rats with goat milk kefir with vit D ₃ (P2) | 459.28 \pm 80.83 ^b | 410.50 \pm 164.98 ^b | -48.78 ^b | 0.647 |
| p ^y | 0.001* | 0.014* | 0.049* | |

Annotation: * Significant; ^t Paired t; ^y One Way ANOVA

DISCUSSION

Kefir is a fermented milk product that has potential effect as a probiotic and antioxidant. Kefir contained Exopolysaccharide called kefiran. The bioactive component of Exopolysaccharide activates the hormone glucagon-like peptide 1 (GLP-1), gastric inhibitory peptide (GIP) and adenilic cyclase via adenosine monophosphate cycle (cAMP), Ca²⁺ ion sensitivity and activation of protein kinase A. This reaction increases insulin release of β -pancreatic cells. Exopolysaccharide is also a biopolymer that lowering blood glucose by inhibits blood glucose absorption. It coats the intestinal microvilli thus inhibiting glucose uptake.¹⁵ Results showed the decrease of insulin and blood glucose levels in the group of diabetic rats which was given goat

milk kefir intervention (P1 group). The insulin levels in the diabetic rats with kefir intervention decreased because the insulin sensitivity worked physiologically resulting in the decline in the blood glucose level.

Antioxidant effects on kefir help to cope inflammatory events. Probiotics are known to ward off insulin resistance by reducing the inflammatory response which is one of the causes of insulin resistance.¹⁶ Lactic acid bacteria in kefir can trigger the immune system to produce anti-inflammatory cytokines such as IL-10 and TGF- β . IL-10 acts as an immune-stimulant by increasing the life-span of cells by increasing the production of anti-apoptotic B-cell lymphoma 2 (Bcl-2). The anti-inflammatory effect of IL-10 is due to a decrease in the production of pro-inflammatory

cytokines. TGF- β may regulate the immune system by inhibiting the proliferation of T cells directly by decreasing the production of pro-inflammatory cytokines.^{17,18} *Lactobacillus helveticus* as lactic acid bacteria may decrease NF- κ B activation by decreasing expression of pro-inflammatory cytokines such as IL-1 β , TNF - α , IL-6, cyclooxygenase-2, nitric oxide induced synthase and increased expression of IL-10.¹⁹ *Lactobacilli kefiranofaciens* in kefir can suppress the production of pro-inflammatory cytokines and promote the production of anti-inflammatory cytokines.²⁰ Yeast in kefir such as *Saccharomyces cerevisiae* acts as inhibiting the activation of nuclear factor kappa B (NF- κ B) and MAPK thereby decreasing the expression of inflammatory cytokines such as IL-8, IL-6, and TNF- α .^{21,22}

Kefir helps to cope with the incidence of diabetes mellitus by increasing blood glucose uptake in muscle tissue, improving the signaling of insulin and reducing inflammation through the antioxidant effects.²³ Kefiran has a role to activate PI 3-kinase, thus helping insulin signaling. With good insulin signaling, insulin sensitivity works well and the incidence of insulin resistance can be prevented. In other way, probiotic bacteria of kefir produce insulin lipotropic polypeptide and glucagon-like-peptide-1 which play a role in increasing the induction of blood glucose uptake by muscle.²³

Intervention of goat milk kefir fortified with vitamin D₃ showed decreased levels of insulin and blood glucose in the group of hyperglycemic rats (P2 group). Vitamin D has protective effect in the incidence of inflammation that occurs in pancreatic beta cells. Vitamin D can reduce the formation of inflammatory mediators by modulating the formation of cytokines. In the modulation mechanism, NF- κ B (which is a transcription factor that plays a role in stress stimulation) has been inactivated. In addition, NF- κ B regulates the immune response in the incidence of infection. With inactive of NF- κ B, the incidence of inflammation occurring in pancreatic beta cells can be suppressed.²⁴ Besides to having a protective effect against pancreatic beta cells from inflammatory, vitamin D also plays a role in secretion of insulin. Vitamin D in the form of 1.25 OH₂D enters to the pancreatic beta cells. In the pancreatic beta cells, 1.25 OH₂D will bind to the VDR-RXR (vitamin D receptor retinoic acid receptors) and form VDRE (vitamin D response element) which plays a role in increasing the activation of insulin gene transcription process thus increase insulin synthesis.²⁵

Insulin sensitivity is a key to the incidence of insulin resistance. It is also associated with vitamin D. Vitamin D can directly increase insulin sensitivity by stimulating the expression of INS-R (insulin receptor) by activating PPAR (peroxisome proliferator-activated receptor) in which PPAR is a factor transcription associated with regulation of fatty acid metabolism in muscle tissue and adipose tissue. With the increased regulation of metabolism, it will also increase the regulation of blood glucose. This is because macronutrient metabolism is interconnected.^{26,27} Vitamin D is also likely to have an indirect effect on increasing insulin sensitivity. Vitamin D plays a role in the regulation of extracellular calcium, the rate of calcium in cells and intracellular calcium.^{28,29}

The results showed that pure kefir can lower insulin levels and blood glucose more effective than kefir with fortified vitamin D₃. A possibility that may cause the condition to occur is insulinopenic. The insulinopenic condition causes various malfunctions in the body. One of the effects is calcium imbalance that triggers increased parathyroid hormone (PTH). In sub-optimal vitamin D levels, high amount of PTH will disturb the sensitivity of insulin.^{30,31} Increased levels of PTH are associated with abnormal glucose metabolism. PTH may decrease signaling stimulation between insulin and blood glucose by inhibiting GLUT-4 phosphorylation. In other way, PTH also inhibits the stimulation of insulin signaling by reducing GLUT-1.³²

CONCLUSION

The intervention of goat milk kefir and vitamin D₃ fortified goat milk kefir decreased insulin and blood glucose levels in diabetic rats even though it was not significantly difference. For further research, fortification levels of vitamin D₃ need to increase and intervention time should be reviewed for optimal effect.

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