

THE INFLUENCE OF GOAT MILK AND SOYBEAN MILK KEFIR ON IL-6 AND CRP LEVELS IN DIABETIC RATS

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Abstract

Background and Aims: Hyperglycemia increases inflammation in type 2 diabetes mellitus (T2DM). Goat and soybean milks have been known to reduce inflammation. The aim of this study is to evaluate the effect of goat milk and soybean milk kefir on IL-6 and CRP levels in diabetic rats. **Materials and Method:** A total of 25 male Wistar rats were divided into the following groups: 1) normal rats; 2) diabetic rats; 3) diabetic rats with intake of goat milk kefir; 4) diabetic rats with intake of soybean milk kefir; and 5) diabetic rats with combination of both types of kefir. Before kefir administration for 4 weeks, plasma glucose was measured. After treatment, plasma glucose, CRP and IL-6 levels were assessed. **Results:** There was a significant decline of plasma glucose in diabetic rats with goat milk kefir ($p=0.021$), and in diabetic rats with the combination of both goat milk and soybean milk kefir ($p<0.001$). After treatment, CRP level in diabetic rats was significantly higher when compared with those that had goat milk kefir ($p=0.043$). All of diabetic rats with kefir had IL-6 values significantly lower than diabetic rats without it ($p=0.021$). **Conclusion:** Both goat milk and soybean milk kefir can reduce IL-6 level, but only goat milk kefir can reduce CRP in diabetic rats.

key words: kefir, goat milk, soybean milk, inflammation, diabetes mellitus.

Background and aims

Hyperglycemia-induced oxidative stress contributes to the development of diabetes complications. Oxidative stress causes insulin resistance, β -cell dysfunction and late diabetic complications [1], and plays a role in the development of chronic inflammation in diabetes [2]. Interleukin-6 (IL-6) and C Reactive Protein [CRP] are sensitive physiological markers of subclinical systemic inflammation associated

with insulin resistance, hyperglycemia, and type 2 diabetes (T2DM) [3]. Many studies reported that elevated levels of IL-6 and CRP can predict the development of type 2 diabetes [3]. IL-6 is produced in a variety of tissues such as endothelial cells and adipocytes [3], while CRP is synthesized by hepatocytes in response to IL-6 during inflammation [4].

Several studies showed that both goat milk and soybean are able to reduce inflammation. Amati et al. [5] reported that the administration

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of goat milk in elderly patients can reduce interleukin 8 (IL-8), interleukin 6 (IL-6), and Tumor necrosis factor alpha (TNF- α). Goat milk contains oligosaccharides which can reduce inflammation in the intestine and colon of the rat [6-8]. Moreover, it was shown that consumption of soybean as much as 30 g/day for short term can reduce plasma concentration of IL-18, TNF- α , and CRP in postmenopausal women with metabolic syndrome [9]. Also, consumption of soybean protein on long term can reduce CRP, plasma glucose, total cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides in type 2 diabetic patients with nephropathy [10].

Many fermented products, especially made of milk and soybean, have beneficial health effects. Administration of fermented milk can reduce CRP and TNF- α in rats with high fat diet and decrease the growth rate of mammary tumors through the reduction of pro-inflammatory cytokines (IL-6) [11,12]. The fermented soybean has an anti-inflammatory activity through the inhibition of pro-inflammatory cytokines production [13] and kefir is one of the fermented products that is made of milk with a complex mixture of bacteria (lactobacilli, lactococci, leuconostocs, aceterobacteria) and yeast (lactose-fermenting and non lactose-fermenting) [14,15]. Kefir has antioxidant, anticancer, anti-inflammatory, antidiabetic [16,17], and anti-hyperglycemic activities [18,19]. In this study we investigated the effects of goat milk kefir, soybean milk kefir, and combination of both on IL-6 and CRP in streptozotocin and nicotinamide-induced diabetes Wistar rats.

Materials and methods

Animals

Twenty five (25) male Wistar rats, (200-300g, 3 months old), were obtained from the Faculty of Veterinary Medicine, Institute

Pertanian Bogor, Indonesia. They were housed in individual cages and acclimatized to the laboratory condition (22-25°C room temperature and 12-hour daylight cycle) for 7 days and had free access to food and water during experimental period. The standard diet was prepared based on a semipurified diet formula for rats by Wotsmann [20] with slight modification (whole wheat flour was substituted by cornstarch). The standard diet in the preliminary experiment consists of casein 24%, DL-Methionie 0.30%, cornstarch 61%, vitamin mix 1%, mineral mix 3.5%, choline chloride 0.2%, alpha cell 5%, and corn oil 5%. (Percentages in diet composition means total % in 100 g diet, e.g. casein 24% mean in 100 g diet contains 24 g casein). This study was approved by the Ethics Committee of Faculty of Medicine, Universitas GadjahMada with the number of ethical clearance KE / FK / 497 / EC.

Induction of diabetes

After a period of adaptation, the rats were randomly divided into five groups: 1) normal rats, 2) diabetic control rats 3) diabetic rats with goat milk kefir intake, 4) diabetic rats with soybean milk kefir intake, and 5) diabetic rats with a combination kefir of goat and soybean milks intake. For induction of diabetes, rats from groups 2, 3, 4, and 5 were injected with nicotinamide (NA) (Sigma-Aldrich, USA) (120 mg/kg body weight - bw, intraperitoneal - i.p) and, after 15 minutes, they were injected with streptozotocin (STZ) (NacalaiTesque, Inc, Japan) (60 mg/kg bw, i.p) [21]. Blood was taken from veins of retroorbital sinus under anesthesia after 5 days of induction, and fasting blood glucose was assayed using a Dyasis glucose kit. In this study, the rats were considered as diabetic rats if they had plasma glucose level higher than 280 mg/dL.

Preparation of soybean milk

Preparation of soybean milk was carried out according to Kasenkas et al. [22] with slight modification. Whole soybean was washed and soaked overnight in distilled water. After the water was discarded, the soybean was mixed with distilled water as much as three times of their weight, then blended and filtered.

Preparation of goat milk kefir, soybean milk kefir, and combination of goat and soybean milk kefir

Goat milk, soybean milk, and combination of both (50% goat milk: 50% soybean milk) were pasteurized at 90°C for 10 minutes, and then cooled at room temperature. Each milk was inoculated by kefir grains as much as 2%. All inoculated milk was incubated at room temperature (25°C) for 18 hours. At the end of the fermentation process, the goat milk kefir, soybean milk kefir, and combination of both were filtered and stored at 4°C until used [22].

Experimental studies

After induction of diabetes, the animals in groups 3, 4 and 5 had a daily administration of goat milk kefir (2 ml/day by gavage), soybean milk kefir (2 ml/day by gavage), and respectively a combination of both milk kefir (2 ml/day by gavage) for 4 weeks. The groups 1 and 2 were given water. The rats daily received 20 g diet and water *ad libitum*. The body weights were recorded weekly. At the end of the experiment, the animals were fasted overnight, and then they were anaesthetized and sacrificed. Blood samples were collected from the aorta to analyze plasma glucose, IL-6, and CRP levels.

Laboratory analyses

Plasma glucose was determined by enzymatic glucose oxidase-peroxidase (GOD-POD) method (DiaSys, Germany). Enzyme Linked Immunosorbant Assay (ELISA) method

was used to measure IL-6 (Boster, Catalog No.EK0412) and CRP (Abcam, ab108827).

Statistical analysis

Body weight, plasma glucose, CRP, and IL-6 were analyzed using one way ANOVA. The limit for statistical significance was set at $p < 0.05$. Results were given as means \pm SD.

Results

Body weight

There was a decrease of body weight in all diabetic rats, but the body weight of normal rats increased. The change in the body weight in all diabetic rats was statistically significant when compared with normal rats (Table 1).

Table 1. The difference of body weight for rats before and after treatment.

Group	Δ Body Weight (g)
Normal rats	46.60 \pm 12.12 ^a
Diabetic control	-12.40 \pm 39.31 ^b
Diabetic + goat milk kefir	-9.00 \pm 23.19 ^b
Diabetic + soybean milk kefir	-23.00 \pm 17.71 ^b
Diabetic + combination of both	-16.80 \pm 21.50 ^b
p	0.001

Values are presented as mean \pm SD.^a and ^b Different notation indicates $p < 0.05$ in One Way ANOVA test followed by Tukey HSD (Honest Significant Difference) test

Glucose level

There was a significant decline of plasma glucose in diabetic rats with goat milk kefir ($P = 0.021$), and in diabetic rats with combination of both goat milk and soybean milk kefir ($P < 0.001$) (Table 2).

CRP and IL-6 levels

The diabetic rats with kefir had significantly lower IL-6 than the diabetic control rats ($P = 0.021$). However, the difference of CRP level was significantly only in diabetic control rats and diabetic rats with goat milk kefir (Table 3).

Table 2. The level of plasma glucose in rats before and after administration of goat milk kefir, soybean milk kefir, and combination of both milk kefir.

Group	Plasma glucose (mg/dL)		95% CI	p
	Before	After		
Normal rats	69.31 ± 8.18	113.40 ± 4.49 ^a	38.56 – 49.6	P<0.001
Diabetic control	283.11 ± 140.02	379.72 ± 83.97 ^b	-235.08 – 41.86	0.125
Diabetic + goat milk kefir	312.21 ± 87.12	126.10 ± 27.97 ^a	46.02 – 326.20	0.021
Diabetic + soybean milk kefir	391.24 ± 146.58	257.38 ± 175.16 ^{a,b}	-80.17 – 347.88	0.157
Diabetic + combination of both	338.41 ± 41.75	112.46 ± 11.11 ^a	180.37 – 271.53	P<0.001
p	0.687	0.002		

Values are presented as mean ± SD. ^a and ^b indicate p < 0.05 in One Way ANOVA test followed by Games-Howell test. ^{a,b} indicate no difference either a nor b. p in row indicate the differences of plasma glucose before and after administration in the same group. p in the last row indicate the differences of plasma glucose between group.

Table 3. The levels of CRP and IL-6 in rats after administration of goat milk kefir, soybean milk kefir, and combination of both milk kefir.

Group	CRP (mg/l)	IL-6 (pg/mg)
Normal rats	0.20 ± 0.02 ^a	564.36 ± 50.83 ^a
Diabetic control	0.25 ± 0.02 ^b	783.78 ± 300.19 ^b
Diabetic + goat milk kefir	0.20 ± 0.03 ^a	546.05 ± 133.60 ^a
Diabetic + soybean milk kefir	0.21 ± 0.03 ^{a,b}	410.12 ± 87.95 ^a
Diabetic + combination of both	0.22 ± 0.02 ^{a,b}	436.19 ± 136.66 ^a
p	0.043	0.021

Values are presented as mean ± SD. ^a and ^b indicate p < 0.05 in One Way ANOVA test followed by Tukey HSD test. ^{a,b} indicate no difference either a nor b. These data were part of the thesis of Kristian SD [23] and Tyas ASA [24]. p in the last row indicate the differences of plasma glucose between group.

Discussion

Hyperglycemia-induced oxidative stress has been suggested to induce the production of inflammatory cytokines [25]. Esposito et al. [26] reported that acute hyperglycemia can increase the levels of IL-6, TNF- α , IL-18 in the circulation and could have a role in impaired glucose tolerance in healthy subjects by an oxidative mechanism. In addition, IL-6 level is independently correlated with CRP in elderly patients with type II diabetes [27].

Our result showed that administration of goat milk kefir or the combination of goat milk and soybean milk kefir can significantly reduce plasma glucose level in streptozotocin and nicotinamide-induced diabetes Wistar rats. The decrease of the glucose level in diabetic rats receiving the combination of goat milk and soybean milk kefir was more important than the one recorded with each of the two kefir. In addition, the diabetic rats with goat milk kefir had a significantly lower CRP level than diabetic control rats. C-reactive protein (CRP) is a major acute-phase protein, expressed and secreted mainly by hepatocytes in response to cytokines such as IL-6 under inflammatory conditions [4,28,29]. Elevated levels of pro-inflammatory cytokines, especially IL-6 and CRP, serve as a common target for intervention in type 2 diabetes [30,31]. Studies in experimental animals showed that renal expression of IL-6 mRNA is increased in streptozotocin-induced diabetic rats compared to normal rats [32]. Systematic review and meta-analysis suggested that abnormal IL-6 and CRP values are significantly related to increased risk of type 2 diabetes [30].

Ojha et al. [33] reported that reducing blood glucose can decline pro-inflammatory cytokines such as IL-6 in streptozotocin-induced rats. In this study, the level of IL-6 in the diabetic rats with kefir was significantly lower than in

diabetic control rats. IL-6 is a member of a family of cytokines which has pleiotropic properties that influences both immunoregulation and non immune events in most of cells and tissues outside the immune system [34,35]. IL-6 is one of proinflammatory cytokines which may be increased due to stimulation of oxidative stress [25].

Goat milk and soybean milk have fairly high leucine content [36]. Leucine is one of the branch-chained amino acids that have a pivotal role in stimulating insulin secretion in pancreatic β cell [37]. Guo et al. [38] reported that supplementation of leucine may improve glucose and insulin sensitivity in multiple rat models of obesity and diabetes.

Several studies showed that goat milk and soybean are able to reduce inflammation. Goat milk contains oligosaccharides which can reduce inflammation in rat's intestine and colon [6-8]. In addition, several studies showed that isoflavones in soybean may decrease blood CRP and proinflammatory cytokines [39,40]. Isoflavone is a protective antioxidant that reduces the formation of reactive oxygen species and free radicals by decomposition of hydrogen peroxide without generating radicals [41]. One study showed that consumption of soybean germ-isoflavone enriched pasta can improve plasma total antioxidant capacity and plasma glutathione in patients with type 2 diabetes [42]. Miraghajani et al. [43] also reported that consumption of soybean milk can reduce CRP

and malondialdehyde in type 2 diabetes patients with nephropathy. According to Azadbakht et al. [10], the long term consumption of soybean protein can reduce CRP, plasma glucose, total cholesterol, LDL, and triglycerides in type 2 diabetic patients with nephropathy. In our study, the lower levels of CRP and IL-6 in diabetic rats receiving kefir may reflect a reduced oxidative stress associated with the consumption of goat milk kefir, soybean milk kefir, or the combination of both. However, the effect of goat milk kefir in reducing CRP level is stronger than soybean one. It may be the inflammatory effect of oligosaccharides in goat milk kefir stronger than isoflavon in soybean milk kefir.

Conclusion

This study showed that goat milk kefir, soybean milk kefir, or the combination of both can significantly reduce IL-6 level, but only goat milk kefir can significantly reduce CRP in streptozotocin and nicotinamide-induced diabetic rats.

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Conflict of interest. We declare that there is no conflict of interest.

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