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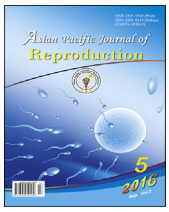

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Effect of black soybean natto extract (*Glycine soja*) on reproduction system of hypercholesterolemia male mice

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ABSTRACT

Objective: To analyze the effect of black soybean natto treatment on the recovery of the reproduction system in hypercholesterolemic male mice model.
Methods: Thirty male mice were divided into five groups, they were normal mice (K–), a hypercholesterolemic mice model (K+), a hypercholesterolemic mice model with 200 mg/kg BW of natto extract (P1), a hypercholesterolemic mice model treated with 400 mg/kg BB of natto extract (P2), and a hypercholesterolemic mice model treated with 800 mg/kg BB of natto extract (P3). Natto extract was given orally for 30 d.
Results: Black soybean natto extract influenced on reproduction system of male hypercholesterolemic mice by increasing the testosterone level and increasing the density and motility of sperm.
Conclusion: Black soybean natto extract is promising as hypercholesterolemic therapy for treating the male reproduction system.

1. Introduction

Hypercholesterolemia is a condition in cases where the level of total cholesterol is ≥ 240 mg/dL, low-density lipoprotein is ≥ 160 mg/dL, and triglycerides are ≥ 150 mg/dL in the human body [1,2]. Hypercholesterolemia is related to male reproduction system dysfunction, including a decreased in the quality of produced semen that leads to infertility [3].

Lifestyle changes, a high-fat and high-cholesterol diet, and low physical activity cause the increases of the fatty substance and cholesterol level in blood. A high cholesterol diet can enhance radical oxygen production and lipid peroxidation in some tissues. Lipid peroxidation is an important factor that can induce morphological changes in spermatozoa [4]. A high-fat diet causes the reproduction problems by increasing the cholesterol level in the testes, leading to gonad cell degeneration and hypothalamus-pituitary gland dysfunction which are related to short penis figuration and spermatogenesis interferences. A low high-density lipoprotein level will lead to an increasing in total cholesterol which causes erection dysfunction [5].

To balance a high-fat diet in cases of treatment and prevention of reproduction system dysfunction, a high-oxidant diet is urgently needed. The oxidant can repress the harmful effect of fat. Soybean has high oxidant content and is widely available. However, fermented soybean has more oxidant content than not-fermented ones. Among of fermented soybean food, natto is well-known as fermented product of soybean. Natto has fibrinolytic activity because it contains nattokinase enzyme [6]. Natto consumption could decrease the total cholesterol level in a hypercholesterolemia rat model serum [7].

Commonly natto is made with yellow soybean (*Glycine max* L.). According to Dajanta *et al.* [8] black soybean natto has higher antioxidant activity than yellow soybean natto. The amount of aglycone daidzein and genistein isoflavones are higher in the black soybean natto than in unfermented black soybean [9]. Daidzein isoflavones have a chemically estrogen-like structure that leads to them having high estrogen activity [10]. The function of daidzein in male reproduction system is still questionable. Weber *et al.* [11] found that the testosterone plasma level and androstenedione in the male rat decreased after treatment with a diet containing 600 μg/g of isoflavones for 5 weeks. A high isoflavones diet using soybean is related to low-quality of sperm production [12]. Zhang *et al.* [10] found that daidzein had a direct effect

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on Leydig cells and was ability to increase testosterone production *in vitro*.

Studies about the effect of isoflavones on human or hypercholesterolemic animal model are still very limited. This study analyzed black soybean natto extract as an antioxidant-rich diet in relation to a disturbance of the reproduction system in a male hypercholesterolemia mice model. The results of this study were expected to provide information about the treating of the male reproduction system recovery through spermatogenesis improvement.

2. Material and methods

2.1. Animal models

This study used a 7-weeks-old male mice, and they were divided into 3 treatment groups randomly. Each group was treated with a high-fat diet and black soybean natto extract orally at various concentrations: 200 mg/mL, 400 mg/mL, 800 mg/mL. Positive control was given with a high-fat diet without natto extract. A volume 0.5 mL/20 g mice weight was given every day for 4 weeks through *ad libitum*. An adrenaline injection at a dose 0.000 84 mg/20 g was administered intravenously on the first day of treatment, except in the negative control group [13]. A high-fat diet was given and natto extract extraction performed at the same time for 4 weeks. The high-fat diet was consisted 56.7% of Hi-Gro Medicated 551 food +20% of coconut oil +23.3% quail egg yolk [14].

2.2. Extract preparation

Black soybean was washed and soaked for 12 h then boiled for 6 h to soften it. Then the boiled black soybean was supplemented with 10% (b/b) of natto (ready to use). The black soybean mixed with natto was tightly wrapped then fermented for 3 d on 40 °C in 80% air humidity. The fermented black soybean was then stored in a refrigerator [15]. This was then extracted using distilled water and processed in a blender. Homogenated natto was centrifuged at 2000 r/pm for 10 min and filtrated. The supernatants were diluted with distilled water into some concentrations then stored in freezer at −4 °C [6].

2.3. Density and motility of epididymis sperm

Mice were dissected on the 31st d after treatment then the caudal epididymides were taken. Two caudal epididymides were sunken into 0.9% of NaCl then the fatty tissue was removed. A number of spermatozoa were measured with a hemocytometer under a binocular microscope (M = 400×) then analyzed using following equation (1):

$$\text{Spermatozoa concentration} = \text{N}/\text{conversion factor} \times 10^{16} \quad (1)$$

Information: N = number of spermatozoa in Neubauer column; Conversion factor = 5.

Spermatozoa motility was categorized as (a), (b), (c), or (d) following these criteria: (a) moving fast toward, (b) moving slowly or in randomly directions, (c) not moving forward, (d) not motility [16]. Based on the WHO [16] standard, normal spermatozoa has categories (a) and (b) $\geq$ 50%. We used that standard, a number of spermatozoa from the (a) and (b) categories were analyzed based on the percentage.

2.4. Testicular assessment

Testes weights were measured for testicular index calculation according to Yuan *et al.* [17] equation (2):

$$\text{Testis index} = \text{testis weight}/\text{body weight} \times 100 \quad (2)$$

Testes were stored in formalin 10% then it was cross-sectioned and stained with Hematoxylin and Eosin. Seminiferous tubule were observed with a microscope (M = 100×) and zoomed in on using Dino-lite software.

2.5. Hormone measurement

Testosterone hormone was measured through mice blood. Blood was taken from heart tissue then stored at room temperature for 30 min. Then blood was centrifuged at 3 000 r/min for 25 min to obtain blood serum. Blood serum was then stored in the freezer at −20 °C until it was ready to be observed. Testosterone level was measured using ELISA at a wavelength at 450 mn.

2.6. Statistical analysis

Data were analyzed using one-way ANOVA (α = 0.05). Statistical analysis was carried out using SPSS 22.0 for Windows software. Data were shown as mean ± standard error.

3. Results

This study found that there is significantly ($P < 0.05$) physiological recovery of male reproduction in hypercholesterolemia mice model after treatment of black soybean natto extract (Table 1).

3.1. Testis weight

Testis weight of male hypercholesterolemia mice fed with black soybean natto extract showed a significantly decreased ($P < 0.05$) after 30 d of treatment compared to positive control. Testes Index is significantly different ($P < 0.05$) between the positive control group and the treated groups.

3.2. Density and motility of sperm

The density and motility of sperm of the male hypercholesterolemic mice models were significantly decreased ($P < 0.05$) compared to negative control. Black soybean natto extract treatment for 30 d has proven that it can increase the motility and density of sperm of male hypercholesterolemic mice (Figure 1).

3.3. Testosterone level

Black soybean natto extract can significantly increase the testosterone hormone level of male mice ($P < 0.05$). Furthermore, the testosterone level was lower in the control groups than in the treatment groups (Table 1).

Table 1
High-fat diet effect on serum testosterone, seminiferous tubule diameter, testes weight, and testes index.

Parameter	Treatment				
	K–	K+	P1	P2	P3
Testosterone level (ng/mL)	$23.44^{a} \pm 0.83$	$21.8^{a} \pm 0.42$	$30.22^{b} \pm 0.91$	$30.42^{b} \pm 1.74$	$29.77^{b} \pm 2.96$
T. Seminiferous diameter (μm)	$217.26^{c} \pm 3.06$	$162.06^{a} \pm 1.27$	$185.43^{b} \pm 5.56$	$238.76^{d} \pm 3.29$	$197.3^{bc} \pm 14.13$
Testes weight (g)	$0.52^{a} \pm 0.03$	$0.72^{c} \pm 0.05$	$0.61^{abc} \pm 0.03$	$0.63^{ab} \pm 0.04$	$0.68^{bc} \pm 0.04$
Testicular index	$2.12^{b} \pm 0.13$	$1.68^{a} \pm 0.1$	$1.57^{a} \pm 0.08$	$1.65^{a} \pm 0.13$	$1.79^{a} \pm 0.08$

Different letters indicate $P < 0.05$ in comparison. (K–) = Negative control; (K+) = Positive control (High Ffat diet); (P1) = High fat diet + 200 mg/mL of natto extract; (P2) = High fat diet + 400 mg/mL of natto extract; (P3) = High fat diet + 800 mg/mL of natto extract.

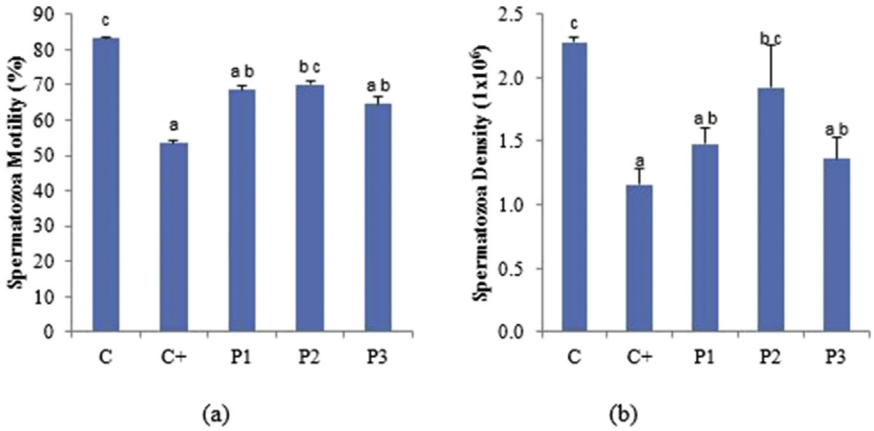

Figure 1. Sperm motility (1a) and spermatozoa density (1b) of hypercholesterolemic male mice treated with black soybean natto extract.

4. Discussion

A high-cholesterol diet is the main factor that caused hyperlipidemia, atherosclerosis, and another lipid metabolism disturbances that lead to abnormality of the male reproduction system [5]. This study found that a decrease in the testosterone level occurred in high-fat-fed mice group. In contrast, it increased in mice group fed with black soybean natto extract. This is probably due to the isoflavones content in soybean, which was expected to be a facilitator of testosterone production. Furthermore, isoflavones stimulated spermatogenesis, sperm maturation, and testes growth [17].

Soybean isoflavones have estrogen-like activity in the male reproduction system that is bound to ERα and ERβ. The daidzein isoflavone stimulated testosterone synthesis through non-genomic mechanisms. Daidzein can modulate the endocrine system by altering the steroidogenic gene expression related to enzyme activity by increasing the human chorionic gonadotropin. Furthermore, the increasing level of human chorionic gonadotropin will be followed by an increasing in testosterone production [10].

Hypercholesterolemia causes a disturbance in hypothalamus-pituitary axis activity. The increase of oxidative stress and decrease of endogenous antioxidant activity lead to spermatogenesis disturbance [5]. This study found that black soybean natto extract can increase the density and motility of sperm compared to a high-fat fed mice group. The antioxidant activity of black soybean natto was expected to maintain the quality of sperm by protecting it from free radicals. Black soybean has higher antioxidant activity than yellow soybean. This founding is in accordance with Jedlinska *et al.* [18] who found that antioxidant supplementation can protect DNA inside the sperm from free radicals and increase testes barrier stability.

Moreover, soybeans are rich in arginine, an amino acid substance, which has an important role in spermatogenesis. It blocks and restraints glycolysis inhibitors in sperm cells, and provides energy for sperm cells, assists spermatogenesis recovery, and increases spermatozoa density. Another mechanism that can improve sperm quality is L-arginine which increased nitric oxide production, which can protect sperm from membrane disruption due to lipid peroxidase [14]. This study also found that black soybean natto extract can increase testosterone level, and protected the quality of sperm and testes in mice fed with a high-fat diet.

Furthermore, it can be concluded that black soybean natto extract affects the reproduction system of hypercholesterolemia male mice by increasing the testosterone level and the density and motility of sperm. However, the further study aimed revealing the role of isoflavone in male reproduction physiology is necessary.

Conflict of interest statement

The authors declare that they have no competing interest.

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The two authors contributed equally to the article.

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