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# Anti-Hypercholesterolemia Effect of Black Rice Bran in Male Winstar Rat

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**Abstract**

This study was designed to evaluate the efficacy of Black Rice Bran (BRB) to mitigate the onset of hypercholesterolemia in Winstar rats fed with atherogenic diets. The rat was fed with the experimental diets during a 12-h period for 16 weeks. The body weights were measured every week. At the end of week-16, total of cholesterol, HDL, LDL and triacylglycerol (TAG) in their blood were measured immediately. The rats were sacrificed to remove the heart and liver, then analyzed the total cholesterol (TC) content in those organs. The rats fed with diet contained 0.5% Cholesterol and Cholic Acid exhibited more severe hypercholesterolemia than others fed with diet contained only 0.5% cholesterol. The inclusion of the BRB in the diets significantly ($p < 0.05$) decrease the level of TC, LDL and TAG of rats plasma fed control diets that either contained or were absent in bile salt ($p < 0.05$). There were no differences in HDL-level for all treatments. Conclusion: supplementation of atherogenic experimental diets with BRB decreased lipid levels for hypercholesterolemic rats, grace of bioactive components present in BRB, ex: anthocyanin, oryzanol, and fiber

*Keywords:* Black Rice Bran; Anti-Hypercholesterolemia; Winstar Rat

## Introduction

Rice is Indonesian staple food and rice bran is a by-product of rice milling. White rice brand and rice bran oil are reported to have beneficial effect for human health (Cara *et al*., 1992; Kahlon *et al*., 1992; Ausman *et al*., 2005). Ling *et al*. (2001) and Xia *et al*. (2003) showed that the outer layer of black rice has functional effect. This layer was source of fibre, oil, flavonoids, polyphenols and anthocyanidins. Pigmented rice contained two main of anthocyanin, those are Cyanidin-3-Glucoside and Peonidin-3-Glucoside (Hu *et al*., 2003; Abdel-Aal *et al*., 2006).

Anthocyanin is natural phenolic that colouring a lot of fruits and vegetables (Murota *et al*., 2002; Kerckhoffs *et al*., 2002; Lee *et al*., 2006; Wu *et al*., 2006). Anthocyanin reduced the risk of coronary heart and atherosclerosis disease risk through its antioxidant, anti-platelet and anti-inflammation activities (Hu *et al*, 2003; Xia *et al*., 2003); then anthocyanin rich-diets are good for our health (Ling *et al*., 2001; Xia *et al*., 2003; Galvano *et al*., 2004).

Some researchers reported that black rice and or the outer layer of this rice significantly reduced lipid level in the blood (Ling *et al*., 2002), plaque of atherosclerotic in hypercholesterolemia rabbit, and Apo-*E* in the rat (Ling *et al*., 2001; Xia *et al*., 2003). Lichtenstei *et al*. (1994), Suh *et al*. (2005), Juliano *et al*. (2005) and Ausman *et al*. (2005) showed the positive effect of rice brand oil or ɣ -Oryzanol on human health. The oil contained some fatty acids like Oleic, Linoleic, and Palmitic, and also Sterol and Oryzanol (Lichtenstein *et al*, 1994; Chen & Cheng, 2006). ɣ-Oryzanol had antioxidant activity too (Juliano *et al*., 2005; Suh *et al*., 2005). Based on such research, the aim of this study is to investigate the effect of black rice bran on cholesterol level of rats.

## Materials and Methods

*Materials*

The rat used was male white rat Winstar (*Rattus norvegicus*) age of 5 weeks with weight in average of 120-150 g and in good health. The rats were obtained from Laboratory of PUSVETMA Surabaya. Research sample is dry simplicial from black rice bran (*Oryza sativa* L. *indica*), that bought from farmer in Kepanjen area, Malang, East Java, Indonesia. Test kit was used for measurement of the total cholesterol, HDL, LDL and triacylglycerol (TAG).

*Method*

This research used Block Random Design with Feed Diets Varieties as experimental factor; those are No cholesterol (NK) as negative control; Cholesterol 0,05% (P) as positive control, Cholesterol + BRB (P+BR), Cholesterol+Cholic Acid (C), Cholesterol+Cholic Acid+BRB (C+BR). The experiment was repeated 5 times respectively.

The rats was handled based on Manual "Perawatan dan Penggunaan Hewan Coba" Vol. 1 from Dewan Perawatan Hewan (1993). The rats were settled in stainless steel cage and maintained under controlled condition: in temperature at 25$^{o}$C, under light (14:10: dark cycle). The rats were acclimatization for a week. The rat divided into 5 groups of experiments based on the feed diets (NK, P, P+BR, C, C+BR). The feed composition for each group of experiment was prepared as described in Table 1.

Table 1. Feed Diet Composition for Each Experiment

| Feed Diet Composition (g/100g) | Group Experiments* | | | | |
|---|---|---|---|---|---|
| | NK | P | P + BR | C | C+BR |
| Casein | 25,5 | 25,5 | 25,5 | 25,5 | 25,5 |
| Corn starch | 47,0 | 46,50 | 43,50 | 46,00 | 43,25 |
| Sucrose | 3,00 | 3,00 | 3,00 | 3,00 | 3,00 |
| Cephacellulose | 5,00 | 5,00 | 5,00 | 5,00 | 5,00 |
| Vitamin mix | 3,00 | 3,00 | 3,00 | 3,00 | 3,00 |
| Choline chloride | 0,20 | 0,20 | 0,20 | 0,20 | 0,20 |
| Methionine | 0,30 | 0,30 | 0,30 | 0,30 | 0,30 |
| Mineral mix | 3,00 | 3,00 | 3,00 | 3,00 | 3,00 |
| Canola oil | 10,0 | 7,00 | 7,00 | 7,00 | 7,00 |
| Butter | - | 3,00 | 3,00 | 3,00 | 3,00 |
| Cholesterol | - | 0,50 | 0,50 | 0,50 | 0,50 |
| Black Rice Bran | - | - | 3,00 | - | 3,00 |
| Cholesterol | - | 0,50 | 0,50 | 0,50 | 0,50 |
| Cholic Acid | - | - | - | 0,05 | 0,05 |

* The experiments: No cholesterol (NK); Cholesterol 0,05% (P), Cholesterol + BRB (P+BR), Cholesterol+Cholic Acid (C), Cholesterol+Cholic Acid+BRB (C+BR)

Period of experiment was 16 weeks. The rat was feed daily based on the group of experiments. The body weight was measured once in a weeks. At the end of experiments (weeks-16), the rats were sacrificed and the bloods were collected in heparinized tubes. Meanwhile heart and liver were removed. The tubes were centrifuged on 4$^{o}$C at 1000 rpm for 5 minutes. The hearts and livers were rinsed in 0,9% NaCl at 4$^{o}$C, air-dried, and then weighed. Total Cholesterol (TG), HDL, LDL and TAG of blood plasma were measured using test kit (Sigma, St. Louis, MO, USA). Cholesterol content in the hearts and livers were analysed

based on Folch *et al*. (1957).

**Statistical analysis**

Data was analysed statistically using ANOVA at α = 0,05%, then analysed further using BNT. All the analysis was performed using SPSS 20.

**Result and discussion**

*The Effect on Weight of the Winstar Rat Body, Heart, and Liver*

There was no significant difference between group of experiment for body weight, heart weight, liver weight, and feed efficiency ratio (FER) (Table 2). It means the addition of black rice bran at 3% in feed did not give a significant effect. Xia *et al*. (2003) and Ling *et al*. (2002) researches showed that the addition of black rice bran on experiment feed, for rat and or rabbit, did not increased the body weight of the animals.

Table 2. The Experiment Effect on Weight of Winstar Rat Body, Heart, and Liver

| Experiments | Body weight (g) | FER* | Heart weight (g) | Liver weight (g) |
|---|---|---|---|---|
| NK | 297,6 | 0,483 | 1,07 ± 0,04 | 12,62 ± 0,33 |
| P | 308,3 | 0,467 | 1,03 ± 0,02 | 19,39 ± 0,85 |
| P + BR | 306,4 | 0,470 | 1,10 ± 0,05 | 18,60 ± 0,22 |
| C | 246,8 | 0,491 | 1,13 ± 0,05 | 19,99 ± 0,35 |
| C + BR | 258,9 | 0,462 | 1,06 ± 0,02 | 15,30 ± 0,91 |

* FER= Feed Efficiency Ratio

The C+BR-Rats had lower liver weight compared to P+BR and C-Rats. Black rice bran contained soluble dietary fibre, unsaturated fatty acid, and sterol. The dietary fibre bound fatty acids, cholesterol, and bile salt in the intestine, thus decreased fat absorption (Cara *et al*., 1992; Vissers *et al*., 2000; Vaskonen *et al*., 2002; Xia *et al*., 2003). Those will decrease fat content in the liver, especially for BRB-Rats.

*The Effect on Total Cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Triacylglycerol (TAC) of The Winstar Rat*

The addition of BRB significantly decreased Total Cholesterol (TC), LDL-C and TAG content in the rats. Data in Table 3 showed that TC of P+BR-rats and C+BR-rats were significantly lower ($p < 0,05$) than TC of P-rats and C-rats respectively. It also happened for LDL-C and TAG of P-BR-rats and C+BR-rats, if compared to TC of P-rats and C-rats respectively. There was no significant effect of BRB addition on HDL-C content of the rats.

Table 3. Experiment Effect on Total Cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), and Triacylglycerol (TAG) of Winstar Rat

| Experiments | TC (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | TAG (mmol/L) |
|---|---|---|---|---|
| NK | 1,79 ± 0,10 | 1,11 ± 0,09 | 0,68 ± 0,09 | 0,51 ± 0,04 |
| P | 2,51 ± 0,16 | 0,91 ± 0,06 | 1,65 ± 0,08 | 0,78 ± 0,08 |
| P + BR | 2,18 ± 0,11 | 0,92 ± 0,08 | 1,27 ± 0,05 | 0,49 ± 0,05 |
| C | 4,20 ± 0,12 | 0,73 ± 0,12 | 3,47 ± 0,12 | 0,55 ± 0,03 |
| C + BR | 2,52 ± 0,13 | 0,81 ± 0,14 | 1,71 ± 0,14 | 0,43 ± 0,02 |

The decrease of Total Cholesterol Content in the rats may be affected by oryzanol, anthocyanin and dietary fibre in BRB. The soluble dietary fibre can increase hypocholesterolemic effect (Fernandez *et al*, 1997; Mekki *et al*., 1997; Behall *et al*., 2004). Bifidobacterium in the intestine will ferment the fibre and Short Chain Fatty Acid (SCFA), then decreases cholesterol in the blood and other tissues (Hara *et al*., 1999).

The oryzanol decreases the cholesterol absorption (Lichtenstein *et al*.., 1994; Cicero & Gaddi, 2001; Berger *et al*., 2005; Suh *et al*., 2005). Oryzanol significantly decreased Total Cholesterol, LDL, and VLDL content in the hypercholesterolemia rats (Suh *et al*., 2005) and in hypercholesterolemia man (Berger *et al*., 2005).

Frank *et al*. (2002) reported that Cyanidin-3-O-Glucoside did not influenced fat profile of hypercholesterolemia rats, but Nielsen *et al*. (2005) proved that anthocyanin extracted from blackcurrant significantly decreased total cholesterol, LDL, VLDL, and TAG in the blood of hyperlipidaemia-inherited rabbits.

*The Effect on Cholesterol Level in The Heart and Liver of Winstar Rat*

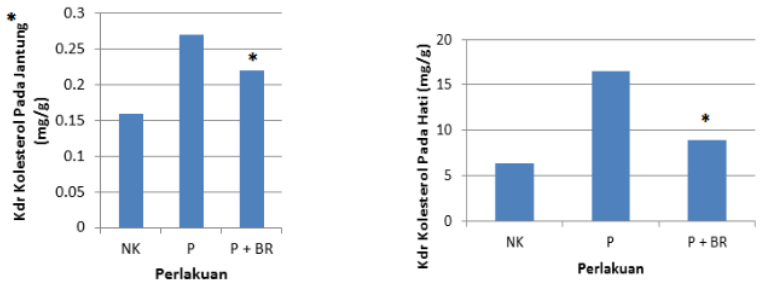


Figure 1. Cholesterol Content in The Heart and Liver of Winstar Rat Feed with Diet Contained Cholesterol and or BRB comparing to No Cholesterol Diet.

* Significantly different at α< 0,05, ** Significantly different at α< 0,01

*NK: No Cholesterol, P: cholesterol 0,05%, P+BR = Cholesterol+BRB*

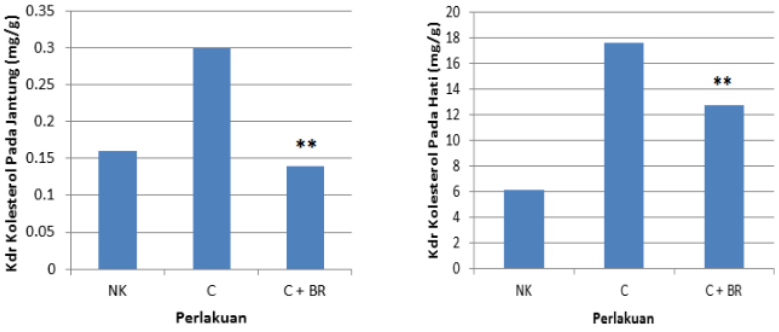


Figure 2. Cholesterol Content in The Heart and Liver of Winstar Rat Feed with Diet Contained Cholesterol and Cholic Acid and or BRB comparing to No Cholesterol Diet.

* Significantly different at α< 0,05, ** Significantly different at α< 0,01

*NK: No Cholesterol, C: cholesterol 0,05%+Cholic Acid, C+BR = Cholesterol+Cholic Acid+BRB*

Cholesterol accumulation in the liver and heart of the rats treated no BRB feed (P and C) were significantly lower than the ones in the rats treated with BRB feed (P+BR and C+BR) (Figure 1 and 2). The cholesterol in those P and C-rats were significantly higher than control (NK). It may be influenced by the anthocyanin, oryzanol and soluble dietary fibre in the BRB, which play important role in the decrease of cholesterol absorption (Lichtenstein *et al*., 1994; Cicero & Gaddi, 2001; Berger *et al*., 2005; Suh *et al*., 2005) in the blood and other tissues (Hara *et al*., 1999). It was supported by Xia *et al*. (2003) that shown the importance of BRB in the decrease of LDL and TAG in the hypercholesterolemia rats (Table 2). The dietary fibre in the BRB can bind the bile salt then inhibit fatty acid absorption (Ausman *et al*., 2005).

## Conclusion

Supplementation of atherogenic experimental diets with Black Rice Bran (BRB) decreased lipid levels in plasma, heart, and liver of the rat; and also LDL, total cholesterol, and triglyceride in plasma of the Winstar rat. It needs to study further, the effect of BRB on human.

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