Fecal Lipid Content, Serum Lipid Profile, and Intra-Abdominal Fat Accumulation in Normal Rats Supplemented with Rice Bran Oil

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ABSTRACT

This study aimed to investigate the mechanism of rice bran oil on altering lipid absorption and blood lipid level in normal rat. Male Sprague Dawley rat age 3 months old, weighted 250–300 g were grouped into three groups: control (aquabidest 1 ml), orlistat (2.16 mg / 200 g body weight), and rice bran oil (1.04 mg γ-oryzanol / 200 g body weight). The intervention was given through oral gavage, daily for 28 days. Indicators observed were growth performance, total cholesterol and serum triglyceride levels, lipid levels in feces, and accumulation of intra-abdominal fat. The results showed that the treatment did not significantly affect body weight gain. Fecal lipid levels of orlistat, rice bran oil and control group respectively were 0.19g; 0.17g and 0.13 g ($p < 0.05$), while the percentage of indigestible lipids for orlistat, control and rice bran oil group were 26%, 17% and 13% respectively ($p < 0.05$). Total cholesterol and serum triglyceride levels in rice bran oil group were significantly lower than controls. Rice bran oil did not significantly affect the percentage of total intra-abdominal fat and the weight of the heart and kidney ($p > 0.05$). The intervention of rice bran oil was shown to reduce blood cholesterol and triglyceride levels in normal mice and did not accumulate intra-abdominal fat. The results suggest that rice bran oil might have an effect on blood lipid regulation but not by preventing lipid absorption.

Keywords: cholesterol, fecal lipids, normal rat, orlistat, rice bran oil

INTRODUCTION

Lipid accumulation especially in abdominal adipose tissue might disrupt lipid metabolism and is often associated with main risk factors of degenerative diseases such as type 2 diabetes mellitus, hypertension, cardiovascular diseases, and dyslipidemia (Golay & Ybarra 2005; Kotchen 2010; Akil & Ahmad 2011; Klop *et al.* 2013). Rice bran has been acknowledged as one of natural remedies for prevention of degenerative diseases, due to its functional components such as antioxidants, dietary fiber, vitamins, essential minerals, and enzyme inhibitors (Hamada 2000). The antioxidant capacity of rice bran is 28.74 AEAC, 15 times more than tomato juice that has 1.87 AEAC (Damayanthi *et al.* 2010).

Oil can be extracted from rice bran which constitutes up to 18–22% of oil. Rice bran oil has slightly sweet taste due to a high content of 4-monomethylsterols (Cicero & Derosa 2005). It is readily sold in supermarket thus can easily be used to substitute palm oil. Rice bran oil contains γ-oryzanol, a mixture of ferulic acid esters of phytosterols and triterpene alcohols, as bioactive compound which has high antioxidant capacity (Damayanthi *et al.* 2004). Previous studies showed that rice bran oil significantly lowered total cholesterol and serum LDL on rats with hypercholesterolemia (Tong *et al.* 2014; Most *et al.* 2005).

It is hypothesized that the serum lipid lowering effect from rice bran oil is due to its γ-oryzanol content, not from its dietary fiber or fatty acid composition, that inhibit lipid absorption in small intestines and excrete it through feces (Ijiri *et al.* 2015). Study by Bhaskaragoud *et al.* (2016) showed that γ-oryzanol from rice bran oil affect

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lipid absorption by decreasing intestinal lipase activity. Thus, the amount of lipid absorbed also reduced. However, a more detailed mechanism of action of the lipase inhibitor of rice bran oil has not been described.

In this study we examined the effect of rice bran oil on inhibition of lipid absorption compared to orlistat (lipase inhibitor drug). To evaluate lipid absorption of rats fed with standard diet with addition of rice brain oil or orlistat, we examined rat's growth performance, fecal lipid content, serum lipid profile, and the intra-abdominal fat accumulation.

METHODS

Design, location, and time

This study was an experimental study using a Randomized Block Design (RBD). Animal intervention was conducted at IPB Veterinary Teaching Hospital, IPB University. Lipid serum analysis, fecal lipid analysis, and organs weighing were performed at Laboratory of Food Analysis and Chemistry of Department of Community Nutrition of IPB University. The study was conducted from April to November 2018. The experiment obtained ethical clearance from Ethics Commission of IPB Veterinary Teaching Hospital, IPB University number 093/KEH/SKE/VIII/2018.

Materials and tools

Twenty-one male Sprague Dawley rats at 3 month of age were purchased from Tropical Biopharmaca Research Center of IPB University. Rice bran oil (Oryza Grace®) was obtained from supermarket in Bogor. Orlistat (Xenical®) was obtained from pharmacy in Bogor. Control diet was from Indonesia Formula Feed (Indonesia Formula Feed). Blood lipid profile analysis used the Cholesterol Reagent Kit (DiaSys) and Triglyceride Reagent Kit (DiaSys). Fecal lipid analysis used the Soxhlet method with hexane as solvent. Body weight and the weight of intra-abdominal fat, kidney, heart, and liver were measured by digital scale.

Procedure

Animals and treatment. The rats were individually housed in wired-bottomed aluminum cage (170x170x240 mm) and provided with water and control diet ad libitum. The room temperature

was maintained at 25°C with 12 hours dark and 12 hours light period every day. The adaptation period was 7 days, followed by random assignment of the rats into three groups on day 8: 1. control diet (energy 2,750 kcal/100g, 4% fat, 23% crude protein, 17% digestible crude protein, 5% crude fiber) and 1 ml of aquabidest; 2. control diet and orlistat with dose of 10.8 mg/kg BB; 3. control diet and rice bran oil with dose of 57.6 mg γ-oryzanol/day. Orlistat solution was prepared by diluting 64.8 mg of crushed orlistat tablet into 100 ml of 1% Na-CMC to obtain final orlistat content of 0.0648%. Concentration of orlistat solution given to rat was adjusted weekly according to its body weight. Final volume given to rat was not exceeding 5 ml. Treatment was given daily for 28 days. Aquabidest, orlistat, and rice bran oil were given through oral gavage daily at 9 am. Body weight was measured every week. Feed intake was measured every day. Feed efficiency were calculated as proportion of feed intake to body weight gain. All rats were euthanized under anesthesia. Blood was collected through the jugular vein. The liver, heart, adipose tissues (mesenteric, retroperitoneal, and epididymal), and kidney were collected and weighed. Fecal samples were collected during last 4 days of the experiment.

Serum Lipid Profile Analysis. The level of triglycerides and total cholesterol were measured using reagent kit (DiaSys) according to the manufacturer's procedure.

Determination of lipid content of feces. Fat content of feces were measured using Sohxlet method. Feces from the last four days were dried in the oven, then grinded and homogenized. Homogenized fecal sample for analysis were sampled (2 g/sample) using AOAC Official Method 925.08 sampling method. Hexane was used as solvent and heating process was running for 6 hours. Fat content was calculated as percentage of amount of lipid extracted to sample weight.

Data analysis

Data were expressed as means±SEM. Analysis for body weight difference pre and post intervention was performed using paired sample t-test. Feed intake, organ weight, and serum lipid profile were analyzed using ANOVA. All statistics analyses were performed with SPSS version 22.0 for Windows.

RESULTS AND DISCUSSION

Effect of rice bran oil (RBO) on rat growth performance

The effect of RBO on body weight gain, average calorie intake, feed efficiency and lipid absorption are presented in Table 1. After 28 days, total body weight gain of RBO group was not different from the control group. Although the daily calorie intake between groups did not differ significantly, daily lipid intake of RBO group was significantly higher than other groups. This is resulted from the induction of RBO that contributed to additional lipid intake. Both RBO and Orlistat group have higher fecal lipid content.

In this study we observed that in normal physiology state, RBO will not result in significant body weight gain. This finding is in line with previous study which showed that rats intervened with High Fat Diet (HFD) did not lead to excessive body weight gain when given RBO orally along with the diet (Son *et al.* 2009). Moreover, study in normal rats fed either standard diet or rice bran containing diet showed a similar growth performance (Iijiri *et al.* 2015).

It is known that γ-oryzanol might affect lipid absorption which reflected on the number of fecal lipid content. Statistical test results showed that the Orlistat and RBO group had significantly more fecal lipid content than the control group. As it is expected from Orlistat which known to act as inhibitor of the pancreatic lipase enzyme,

the increase in fecal lipid content of RBO group is thought to have different mechanism than Orlistat. The average percentage of absorbed lipid for control, orlistat, and RBO group is 83.49±0.71%, 73.75±1.34%, and 86.96±0.27% respectively. This data show that the amount of lipid absorbed for RBO group was similar to the control group. Thus, unlike orlistat, the lipase enzyme in RBO group is not inhibited. Lipid absorption inhibition is more prominent to rats when fed with whole rice bran oil, so it might be due to the effect of insoluble fiber included in rice bran than γ-oryzanol (Iijiri *et al.* 2015). Possible explanation for higher fecal lipid content might result from higher lipid intake. Therefore, in this study RBO is not effective to prevent lipid absorption in the intestines.

Effect of RBO on serum lipid profile

The effect of RBO on the total cholesterol and triglyceride level are presented in Table 2. RBO has significantly lower total cholesterol and triglycerides level than the control. Moreover, the low level was comparable to Orlistat treatment. This blood lipid lowering effect might be due to γ-oryzanol content in RBO.

It is known that γ-oryzanol is able to inhibit cholesterol and triglycerides absorption in the intestines and excrete it into feces (Rong *et al.* 1997; Wilson *et al.* 2007). However, as mentioned previously, we found that RBO group showed to have higher percentage of

Table 1. Growth performance and lipid absorption

Parameter	Control (n=5)	Orlistat (n=5)	RBO (n=5)
Growth Performance			
Body weight gain (g/28 days)	25.20±8.40[a]	6.40± 9.54[a]	20.00±8.23[a]
Calorie intake (kcal/day)	550.00±81.10[a]	521.30±51.60[a]	491.20±72.40[a]
Feed efficiency	0.04±0.01[a]	0.01±0.02[a]	0.04±0.02[a]
Lipid Absorption			
Lipid intake (g/day)	0.80±0.33[a]	0.76±0.36[a]	1.29±0.28[b]
Fecal lipid content (g)	0.13±0.01[b]	0.19±0.01[a]	0.17±0.01[a]

Data are expressed as the mean± SEM; Different letters (a and b) indicate significant difference at $p<0.05$ in the one-way ANOVA; RBO: Rice Bran Oil

Table 2. Blood lipid profile

Parameter	Control (n=5)	Orlistat (n=5)	RBO (n=5)
Total Cholesterol (mg/dl)	57.99± 0.99[a]	48.15±0.03[b]	46.89±0.20[b]
Triglyceride (mg/dl)	65.30±0.74[a]	50.96±0.26[b]	53.06±0.43[b]

Data are expressed as the mean±SEM; Different letters (a and b) indicate significant difference at $p<0.05$ in the one-way ANOVA; RBO: Rice Bran Oil

lipid absorbed compared to control and orlistat. Therefore, assumption that γ-oryzanol prevent lipid absorption is ruled out. While in this study we cannot confirmed the number of cholesterols absorbed, γ-oryzanol can also target HMG-KoA reductase which suppresses mevalonate synthesis (Chithra *et al.* 2015). Reduction in mevalonate will eventually lower cholesterol level in plasma. We assumed the role of γ-oryzanol is more on regulating lipid metabolism instead of inhibiting lipid absorption.

Unlike saturated fat, total fat intake has proven to have no significant association with total cholesterol and triglyceride level (Joy *et al.* 2007). Rice bran oil composed of mostly unsaturated fatty acids (75%) compared to saturated fatty acids (25%). Thus, even though supplementation of RBO increase the total fat intake, effect on blood lipid profile distortion is low. Along these lines, beneficial effect of RBO on improving plasma lipid level can also be seen in normal physiology state.

Effect of RBO on tissue weight

Rats in the RBO group tend to have lower intra-abdominal adipose tissue (1.11±0.45%bw) compare to control (1.41±0.18%bw) and orlistat (1.23±0.24%bw) group as shown in Table 3. However, these differences are not significant. This finding is in line with previous studies which showed that oryzanol is able to significantly decreased epididymal, mesenteric, and peritoneal fat, but not kidney and heart (Wang *et al.* 2015; Iijiri *et al.* 2015; Charkonpunya 2010; Kobayashi *et al.* 2019).

Mechanism on how RBO able to prevent lipid accumulation is still unknown. However, study by Yang *et al.* (2019) found that rice bran can alleviate adiposity by partially reducing the increase of diglycerides and triglycerides in obese rats. Furthermore, study on molecular level shows that RBO down-regulate lipogenic genes, such as Sod1 and Cat, which reduce hepatic level of triacylglycerol (Ahmed *et al.* 2018). This result suggests that RBO has some roles in regulating

Table 3. Effect on tissue weight

Parameter	Control (n=5)	Orlistat (n=5)	RBO (n=5)
Final Body Weight (g)	305.80±7.70[b]	278.40±15.98[a]	290.30±9.35[a]
Total intra-abdominal fat (%bw)	1.41±0.18[a]	1.23±0.24[a]	1.11±0.45[a]
Mesenteric fat (%bw)	0.34±0.08[a]	0.24±0.05[a]	0.28±0.14[a]
Retroperitoneal fat (%bw)	0.34±0.18[a]	0.31±0.14[a]	0.25±0.24[a]
Epididymal fat (%bw)	0.73±0.09[a]	0.68±0.17[a]	0.58±0.25[a]
Kidney (%bw)	0.69±0.04[a]	0.64±0.04[a]	0.68±0.08[a]
Heart (%bw)	0.32±0.02[a]	0.32±0.04[a]	0.30±0.02[a]

Data are expressed as the mean±SEM; Different letters (a and b) indicate significant difference at $p<0.05$ in the one-way ANOVA; %bw = % body weight; RBO: Rice Bran Oil

adiposity. Further studies are necessary to confirm this mechanism.

CONCLUSION

Beneficial effect of RBO supplementation on blood lipid level in this study does not derived from inhibition of lipid absorption. While the exact mechanism is still unknown, RBO supplementation in normal physiology did not affect both final body weight and tissue weight of intra-abdominal fat, kidney, and heart. This study was performed in rat thus careful interpretation is needed when translating this study result into human subject.

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