Chapter 5

Conclusions and Recommendations

Rice bran particularly rich in dietary fiber, contains significant quantities of essential nutrients and components with antioxidant properties (Shin et al., 1997; Xu and Godber, 1999). The purpose of the study is to find out the benefit of rice bran in preventing metabolic disturbance in metabolic syndrome. The study, therefore, were to find the appropriate method to extract the water soluble components from fresh rice bran, preliminary study the chemical properties of the extract, design the diet that can induce obesity to normal Sprague-Dawley rats and investigate the ability of the rice bran extract RBE's in ameliorating hyperglycemia and hyperlipidemia in those obese rats.

Firstly, the method of water extraction of stabilized rice bran was preliminary verified in 3 conditions. Two of them were the simple warm, 70˚C, and hot, 90˚C, water extracts those mimic the consumption as rice bran tea (Qureshi et al., 2002). The third condition was the enzyme digestion after 70˚C water extraction that mimics the digestion by human gut normal flora (Ardiansyah et al., 2006) which may not be the same as were in rats. It was found that the percentage yields, of water extract at 70˚C, 70˚C plus enzyme treatment and 90˚C extract were 11.98, 12.79 and 13.33 mg/100g fresh rice bran, respectively.

The *in vitro* antioxidative properties in methanolic rice bran extracts as well as the antioxidative effects in animal were reported (Chotimarkorn et al., 2008; Kanaya et al., 2004). Generally, the method with DPPH as a stable free radical to measure radical-scavenging activity was the most simple method and widely used. This method with the molar ratio of samples to DPPH radical indicating the activities of compounds seemed to reflect the activities of samples directly, for values obtained did not vary with the primary concentration of DPPH radical.

Since the simple hot water extract gave a higher yield than the warm one, the antioxidative activity for DPPH radicals was studied in hot water extract only. This was done in comparison with the warm water extract plus enzymes digestion. After the test for anti-oxidation property, the water extract at 70˚C plus enzyme treatment showed the better activity in scavenging the DPPH radical than the hot water extract (EC_{50}=0.155±2.65 mg/mL vs 0.331±16.10 mg/mL). Thus, RBE derived from warm water extraction plus enzyme digestion was used for the studies thereafter. The other study of the anti-oxidation property was done in five varieties of long-grain rice in Thailand. The methanolic rice bran extracts in the aforementioned showed the most effective DPPH radical-scavenging activity of 0.38±0.02 mg/mL EC_{50} (Chotimarkorn et al., 2008). Which was less effective than that of RBE prepared in this study. Thus, it is more likely that any effect of RBE found in this study was derived from the antioxidative property.

Rice bran is a very nutritious product. It contains high content of protein that has higher amino acid score than those found in other plant sources (Wang et al., 1999). The RBE was qualitatively tested for carbohydrate, peptide bond, animo and some functional group. The RBE gave positive reaction to Molisch test, Seliwanoff test and Iodine test, indicating the ketose content in the extract. The positive reaction in Biuret test, xanthoproteic test, Hopkins-Cole test and Sakaguchi test also indicated that the extract contained substance having peptide bonds, aromatic ring and, possibly, iodine as functional group. Therefore, RBE could be a mixture of compounds, and possibly, some complex compounds as well. Unfortunately, there is no data on the chemical composition of RBE yet. Thus, the further quantitative and qualitative studies on the chemical identification are needed.

The environmental factors cause obesity, which is associated with disorders such as glucose intolerance, diabetes, hyperlipidemia, hypertension, and atherosclerosis. Among the environmental factors, intake of high-fat food is well known in contributing to the development of obesity. Obesity induced by high-fat diet is a common model used as a surrogate for the human disease. There are data linking high-fat diet to the development of diabetes in man. (Lichtenstein et al., 2000; Hu et al., 2001). Studies showed that when rat or mouse were fed with high fat diet, they developed obesity and metabolic disorders were manifested (Zuberi A., 2008). Other animal models those gave the same feature are including dogs, rabbits, and hamsters (Hiramatsu & Grill., 2001). Diet-induced obese rat was a fairy good model representing most obesity in human, since it showed many metabolic disorders as were found in human (Bergman et al., 2006).

Claret et al, 2004 demonstrated that a high-fat diet (65% of the energy derived from fat) increased body weight gain and caused impairment of glucose homeostasis and insulin sensitivity in rats. Likewise, the other studies showed that high-fat diet can generate metabolic syndrome with insulin resistance in rodent (Pagliassotti et al., 2000; Buettner et a., 2006).

In this study, the general behaviors of animals were notified according to method described by Chan & Hayes (1989). All of the animals behaved normal. Their respiratory pattern, cardiovascular signs, motor activities, reflexes, and change in skin and fur were normal (data not show). In general, therefore, it can be concluded that RBE had no effect on animal behaviors and general pathological signs.

High-fat (65% of total kcal) diet was able to induce abdominal obesity in normal Sprague-Dawley rats. These rats had high level of fasting blood glucose, impaired glucose tolerance, decreased insulin sensitivity and impaired β-cell function. The increase in weight of abdominal fat pads was shown even if there was no change in the body weight of the rats. RBE at the dose of 2205 mg/kg was able to significantly reduce the increasing body weight gain in comparison with the high-fat diet group (125.98±7.32g vs 160.72±10.03g). The same result was applied to abdominal fat weight of these animals (8.99±0.72g vs 13.95±0.44g). This result was also similar to the other study (Tsutsumi et al.,2000) who found that rice bran may suppress body weight gain and the accumulation of abdominal fat by lipase inhibition and decrease gut absorption of fat. However, they showed no data on fecal fat contents. The explanation could be partly applied to this study, since the body weight gain were decreasing in relation to RBE dosage (Table 4.4). However, there was no sign of oily stool observed in this experiment.

Regarding to glucose homeostasis parameters, FBG were significantly increased when rats were fed with high-fat diet. RBE but metformin co-feeding had no effect on the elevated blood glucose. The data, at least, emphasized that high-fat diet can cause hyperglycemia as was shown by others (Storlien et al., 1993). AUC-G were significantly reduced in high-fat feeding rats when they were also received RBE and metformin. The decrement tended to be dose-dependent and RBE gave a better effect than metformin. The results supported other studies (Kahlon et al., 1992; Seetharamaiah & Chandrasekhara, 1989). The effective dose of 220.5 mg RBE /kg rat

BW, can be calculated back to dose of 1.47 g RBE/kg human BW. The resulted was similarity with Qureshi et al. (2002) reported that rice bran water soluble were fed in both types of diabetes had decreased levels of glycosylated hemoglobin and fasting glucose (15%, 33%), respectively. Serum insulin levels were increased (4%) and serum total cholesterol, LDL-cholesterol, apolipoprotein-B, and triglycerides levels were decreased (7%) with rice bran water solubles in both types of diabetes. The reduction of glycosylated hemoglobin and a slight increase in insulin levels indicate that consumption of rice bran water solubles can control blood glucose levels in human diabetes. However, the study of Qureshi et al. (2002) in both type diabetes patient, the study design of the effects of rice bran water solubles in conjunction with American Heart Assosiation (AHA) Step-1 diet. In this study, the animal model was characteristics of prediabetic which inconsistent with Qureshi et al. (2002) who study in diabetes patient.

RBE showed a trend to restore the % homeostasis model assessment of β-cell function (HOMA-β), though there was no statistic significance, but not the homeostasis model assessment of insulin resistance (HOMA-IR). Since RBE was able to ameliorate glucose clearance of the whole body system, its effectiveness could be derived from being an effective secretagogue of β-cell. This is especially true because the clearance is virtually depend on the proper amount and action of insulin secreted by β-cell as well. Indeed, the study did showed that RBE reduced AUC-G but had no effect on FBG. Therefore, the chemical properties of the content in RBE are worth evaluated in the near future. The second line of explanation is that the increased glucose clearance rate effect could be secondary to its effect on reducing abdominal fat mass. The evidence and more detailed will be discussed in the next paragraph.

Metabolism as was intervened by high-fat diet and RBE treatment gave a high variation in blood parameters rendering a chance to look at the correlations among the parameters. Although, it is a virtue that HOMA-β was significant correlated with either FBG or HOMA-IR while serum insulin was significantly correlated with either HOMA-IR or HOMA-β, since the homeostasis was assessed using both FBG and serum insulin. However, it is interesting that HOMA-β and FBG were statistically significant correlated with abdominal fat weight which positively correlated with serum triglyceride and LDL-C as well. This is, therefore, hinted the close link between glucose homeostasis and blood lipid, at least, in diet-induced metabolic disease. Serum triglyceride is not a direct result of glucose homeostasis imbalance or vise versa, but, it is unless via high abdominal fat weight or abdominal obesity.

The further important point to concern regarding to the issue is that how and what kind of fat molecules derived from abdominal fat influence the glucose homeostasis, especially the β-cell function. The most feasible one would be the massive flow of fatty acid derived from visceral fat lipolysis as was previously shown in dog (Bergman et al., 2006). Indeed, high level of free fatty acid, not triglyceride, was shown to insult β-cell viability (Zhou & Grill, 1995; Kharroubi et al., 2004).

Interestingly, the high dose of cholesterol ingestion did not increase total blood cholesterol level in high-fat feeding rats. Though there was an increase in blood levels of LDL-C and decrease HDL-C in these high-fat feeding rats, but there was no statistic significance. The amount of cholesterol intake, 47.4 mg./kg. rat weight /day, is equal to 2,844 mg/ 60 kg man/day. The calculation is based on the amount of 250 mg cholesterol per a hen egg plus the amount of 7.8 % from pork liver and using the formula proposed by Regan-Shaw, Nihal and Ahmed (2007). If the calculation is correct the result of the study would indicate the daily intake of approximately 10 egg yolks per day do not raise blood total cholesterol in man, even thought in the present of 65.26 % of total calories derived from fat.

In conclusion, the amount of 65% of total calories from fat was able to induce abdominal obesity, leading to a state of metabolic syndrome. The abnormal glucose homeostasis was clearly shown by hyperglycemia, elevated AUC-G and decreased HOMA-β. RBE was able to ameliorate the glucose clearance in high-fat induced obese rats. It is the first study ever that the weighing of abdominal fat mass that cannot be done in human was brought to relate with the prediabetes parameters in human. This gave the opportunity to gain further knowledge in the pathoetiology of diabetes.

Finally, two questions are worth further investigated. How and what kind of molecules in RBE that effect the glucose clearance rats? Would RBE has an effect on lipolysis of abdominal fat mass?