CHAPTER V

DISCUSSION AND CONCLUSION

Effect of high fat and high sucrose diet on food intake, body weight, blood glucose concentration and systolic blood pressure

The finding of this study indicated that after 20 weeks of high fat and high sucrose (58% and 26%, respectively) diet induction, the male C57BL/6Mlac mice were able to develop obesity, hyperglycemia and an increase visceral fat deposition, while there was no change in the systolic blood pressure. The consumption of foods containing more fat and sucrose in these strain of mice are similar to the happening metabolic syndrome in a person. Especially, the accumulation of excessive abdominal fat plays a role in the beginning state of metabolic syndrome (Grundy, et al., 2005). Consistent with our results, several previous studies also demonstrated that after 16 weeks of high fat and high sucrose diet consumption the mice had been increased in the body weight, the blood glucose and the insulin level (Surwit, et al., 1995), as well as, in this model fed with a high fat diet for 8 weeks developed obesity and hyperglycemia (Rossmeisl, et al., 2003). Recently, it has been reported that increasing consumption of fat (60%) induced obesity in C57BL/6J mice potent to develop characteristic of insulin resistance, non alcoholic fatty liver disease (NAFLD), non alcoholic fatty pancreatic disease (NAFPD), pancreatic and hepatic fat deposition and an enlarge visceral fat accumulation which correlated to the symptom of metabolic syndrome (Fraulob, et al., 2010).

This study indicated that the end of the experiment for inducing with high fat and high sucrose diet, in the HFHS group the body weight did not highly greater than the C group, although it was significant differences. While the body weight during the 1-12 weeks in the HFHS group was less than the C group. From the data it was apparent that might be due to throughout the period of induction with high fat and high sucrose diet, the mice in HFHS group consume less food than the C group (Table 2). The appetite and satiety centers in hypothalamus control the food intake. The previous studies shown that the low glycemic carbohydrates index have digested food slowly

which produced a gradual rise blood glucose, resulting in increased satiety (Ball, et al., 2003) and therefore decreased food intake (Warren, et al., 2003). The reduction of appetite in the HFHS group might related to receiving a lot of fat, resulting in less food intake which is possible that this is a caused of less body weight gain during 1-12 weeks. Nevertheless, although the mice in HFHS group eat less amount of food, even so, when calculating the amount of calories consumption, the HFHS group received more calories than the C group 4 folds. Apart from that at the end of 20 weeks of the induction with high fat and high sucrose (58% and 26%, respectively) diet, the HFHS group increased in abdominal fat accumulation and the body weight gained. In the HFHS group, a possible explanation for long time less amount of food consumption (less appetite) may be related to adipose tissues expansion increasing, leading to the increase leptin secretion from these cells. Leptin hormone had played an important role in the regulation of food intake by it binds to the receptors in the hypothalamus, as a result suppression of appetite, leading to reduction of food intake. Albeit throughout the experiment the mice in HFHS group consumed less food, but when finished of induction found that the body weight of this group was statistically significant higher than the C group. Furthermore, the visceral fat deposition in the HFHS group was significantly increased more than in the C group. Consequently in this study suggested that the C57BL/6Mlac mice fed with high fat and high sucrose diet were able to develop obesity.

Effect of *K. parviflora* extract on visceral fat accumulation and adipocyte morphology

In the present study found that *K. parviflora* extract had shown visceral fat deposition suppressing effect in the HFHS+KPE10 group and decreasing adipocytes diameter in both HFHS treated groups (Figure 20 and 21, respectively) after 8 weeks of treatment. These results are in partly agreement with previous study reported that after 8 weeks of receiving the test feed (mixing between *K. parviflora* powder and Powder Feed MF) *ad libitum*, *K. parviflora* was able to reduce visceral fat accumulation in both normal (Tsumura, Suzuki, Non-obesity; TSNO) and obese (Tsumura, Suzuki, Obese Diabetes; TSOD which a genetic disease animal model in metabolic syndrome) mice, indicating that the *K. parviflora* is effective against obesity

(Akase, et al., 2011). On the other hand, our result demonstrated that *K. parviflora* had no effect to reduce the deposition of abdominal fat in all mice fed normal diet. Therefore, it was concluded that the *K. parviflora* extract is effective in diminish visceral fat accumulation and adipocytes hypertrophy in the mice fed with high fat and high sucrose diet, nevertheless, cannot effect on the mice fed with normal standard chow diet.

Effect of *K. parviflora* extract on fasting blood glucose

This study has demonstrated that after eight weeks of *K. parviflora* extract treatment in the HFHS group, fasting blood glucose concentration decreased but it did not altered in the C group (Figure 15). In this result we are suggesting that the extract of *K. parviflora* had an effect on improving of hyperglycemia. In central obesity people, there were the expansion of adipose tissue, leading to adipocytokine dysregulation, enhance the secretion of proinflammatory and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6), that were the cause of insulin resistance (Coletta and Mandarino, 2011). The previous study has shown that the expression of TNF-α and IL-6 mRNA were inhibited by a flavonoid from *Salvia plebeian*, resulting in insulin sensitivity improving (Wu, et al., 2012). The flavonoids are the main active compounds of *K. parviflora* extract, containing methoxy groups (Sutthanut, et al., 2007). The investigator probably assumes that the secretion of proinflammatory cytokines for instance TNF-α and IL-6 from adipocytes might be suppressed by the *K. parviflora* extract, eventually leading to reduce the blood glucose concentration. Therefore, the *K. parviflora* extract had an anti-hyperglycemia effect.

Effect of *K. parviflora* extract on oral glucose tolerance test

In oral glucose tolerance test, after orally glucose administration in high fat and high sucrose diet-induced mice, the acute and sub-chronic *K. parviflora* extract treatments had no effect on lowering blood glucose concentration. However, the statistical analysis shown that blood glucose levels were not significantly difference between the HFHS treated groups and the HFHS-C group. The results of this study indicated that after 20 weeks for the mice induction with high fat and high sucrose diet

did not develop insulin resistance, it is possible impaired glucose tolerance test only because blood glucose level after 2 hours of glucose loading less than 200 mg/dl (Figure 16 and 18). The previous study indicated that if plasma glucose level is about 140-200 mg/dl after 2 h, that person has impaired glucose tolerance (prediabetes) and if plasma glucose level remains above 200 mg/dl or still high this shows a sign of diabetes (Patel and Macerollo, 2010). In this study, the C57BL/6 Mlac mice fed with high fat and high sucrose diet did not develop insulin resistance, may be due this diet had affected to impair partially the function of pancreas β-cells or some target cells could not respond to insulin only.

Recently, the previous studies have shown that *K. parviflora* power and *K. parviflora* ethyl acetate extract improved insulin resistance in TSOD mice, a spontaneously obese type II diabetes (Akase, et al., 2011; Shimada, et al., 2011). As a consequence, the investigator discovered that *K. parviflora* ethanolic extract had no effect on glucose tolerance alteration, since insulin resistance did not develop in mice fed with high fat and high sucrose diet; it was not as we anticipated.

Effect of *K. parviflora* extract on body weight

This study has demonstrated that eight weeks of *K. parviflora* extract treatment in all HFHS-treated and C-treated groups were unable to change of the body weight (Figure 14). Therefore, this herbal did not effect in the C57BL/6Mlac mice receiving both normal standard and unhealthy diet. In the HFHS group treated with *K. parviflora*, the body weight was continue to high in spite of reduction in visceral fat mass, this could be described as a fat is very light weight compared to the body weight. Thus, the lower of visceral fat content has little effected on body weight. One more possible reason that the body weight of mice did not decreases, probably because we used the dose of *K. parviflora* extract less than the dose used in previous study, which shown *K. parviflora* power suppressed the body weight gain in TSOD mice (Akase, et al., 2011).

Effect of *K. parviflora* extract on serum cholesterol, triglycerides and high-density lipoprotein cholesterol

The results showed that the serum triglyceride concentration in mice fed with high fat and high sucrose diet group was not significantly higher than the control group. In the previous study has shown that the C57BL/6 mice received high fat diet (50% fat, 36% carbohydrate and 14% protein) for 8 weeks, the serum triglyceride level was not higher than the mice fed on normal standard chow diet (Tao, et al., 2009).

Interestingly, our results found that the *K. parviflora* extract administration for 8 weeks (10 mg/kg BW) had a potent effect to suppress serum triglycerides in mice fed with high fat and high sucrose diet. Moreover, it was also revealed that the *K. parviflora* extract (10 and 100 mg/kg BW) was able to lower the cholesterol level and restored a decrease of high-density lipoprotein cholesterol found in mice fed with high fat and high sucrose diet (Table 6). Our result was in agreement with the previous study which demonstrated that 8 weeks of fed with the mixing between *K. parviflora* powder and Powder Feed MF was able to decrease plasma triglyceride and total cholesterol in TSOD mice. Flavonoids in *K. parviflora* powder might inhibit the HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase which is an enzyme in the cholesterol synthesis, resulting in decreased cholesterol synthesis (Akase, et al., 2011). Furthermore, it has been reported that a flavonoid from *K. parviflora* could reduce triglycerides in TSOD mice by inhibiting pancreatic lipase action which is an enzyme in dietary triglycerides absorption (Shimada, et al., 2011), consequential decreased in triglycerides level. Besides that low blood triglyceride concentration results in reduce transferring of triglyceride from very-low-density lipoprotein (VLDL) to HDL-C and subsequently increase HDL-C concentration. On the other hand, this study has demonstrated that the *K. parviflora* extract was unable to restore cholesterol, triglycerides and HDL-C in all mice fed with normal diet. Therefore, *K. parviflora* extract can affect on reduction of cholesterol and triglycerides and increment of HDL-C in mice fed with high fat and high sucrose diet, however, it had no effect on mice receiving a normal diet.

Effect of *K. parviflora* extract on systolic blood pressure

The findings of this study pointed that after 20 weeks induced by high fat and high sucrose diet the mice did not develop to be hypertension (Figure 12). Many studies demonstrated that in the C57BL/6 mouse strains consumption neither high fat diet nor high fat and high sucrose diet, did not develop to hypertension (Surwit, et al., 1995; Parehk, et al., 1998; Angela, et al., 2007; Fraulob, et al., 2010).

Recently, a previous study has shown that the TSOD mice are developed hypertension spontaneous after receiving the *K. parviflora* powder could restore hypertension (Akase, et al., 2011). The investigator discovered that *K. parviflora* ethanolic extract had no any effect to alter systolic blood pressure, since hypertension did not develop in mice receiving an unhealthy diet; it was not as we anticipated.

Conclusion

In summary, the present study demonstrated that 20 weeks high fat and high sucrose diet induction gave rise to obesity, increasing blood glucose concentration, visceral fat deposition, glucose tolerance impairment and dyslipidemia that associated with metabolic syndrome. Eight weeks of the *K. parviflora* extract treatment had a potent effect to suppress symptoms found in mice fed with high fat and high sucrose diet by inhibiting visceral deposition, restoring adipocyte hypertrophy, reducing hyperglycemia, diminishing serum cholesterol and triglycerides concentration. Furthermore, the results of this study showed that the *K. parviflora* extract was capable of improving a decrease in the high-density lipoprotein cholesterol concentration found in the high fat and high sucrose diet groups. In this study found that the *K. parviflora* extract had an effect on mice fed with high fat and high sucrose diet only that mimics the consumption of a Western-style diet (high intakes of fat and sugar). Therefore, the *K. parviflora* is another alternative medical herbal plant as a new potent to prevention of the symptoms from the early stage of the metabolic syndrome.

In the future research, it should further investigate the serum insulin level, the insulin resistance, the tumor necrosis factor-α and interleukin 6 levels in order to uncover more about the biological effect of *K. parviflora* extract on the improvement of insulin resistance and insulin sensitivity in mice receiving unhealthy diet.