

**EFFECTS OF THE CRUDE EXTRACT FROM THE
FRUIT RIND OF RAMBUTAN (*Nephelium lappaceum* L.)
ON OBESITY IN MALE WISTAR RATS**

Aree Thinkratok



**A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Environmental Biology**

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ผลของสารสกัดจากเปลือกเงาะที่มีต่อโรคอ้วนในหนูขาวเพศผู้พันธุ์วิสตาร์



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ปีการศึกษา 2554

**EFFECTS OF THE CRUDE EXTRACT FROM THE FRUIT RIND
OF RAMBUTAN (*Nephelium lappaceum* L.) ON OBESITY IN
MALE WISTAR RATS**

Suranaree University of Technology has approved this thesis submitted
in partial fulfillment of the requirements for a Master's Degree.

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โรคอ้วนเป็นปัญหาสำคัญด้านสาธารณสุขซึ่งสามารถทำให้เกิดโรคต่างๆ ที่เกี่ยวข้องกับโรคอ้วนได้ การใช้ยาลดความอ้วน เช่น ยาอริสแตทและยาไซบูทรามิน ส่งผลให้เกิดอาการข้างเคียงที่ไม่พึงประสงค์ ดังนั้นการใช้สารสกัดจากพืชน่าจะเป็ทางเลือกที่ดีที่จะใช้ทดแทนยาลดความอ้วนได้ เปลือกเงาะประกอบไปด้วยสารประกอบฟีนอลิกซึ่งสามารถป้องกันโรคอ้วนได้ ดังนั้นการทดลองในครั้งนี้มีจุดประสงค์เพื่อศึกษาผลของสารสกัดจากเปลือกเงาะต่อโรคอ้วนในหลอดทดลอง และในสัตว์ทดลอง โดยประกอบด้วย 3 การทดลอง ดังนี้

การทดลองที่ 1 ศึกษาความเป็นพิษเฉียบพลันและความเป็นพิษกึ่งเรื้อรังของสารสกัดจากเปลือกเงาะในหนูขาวเพศผู้พันธุ์วิสตาร์ จากการศึกษาความเป็นพิษเฉียบพลัน พบว่าค่าความเข้มข้นสูงสุดของสารสกัดจากเปลือกเงาะที่ไม่ทำให้เกิดผลข้างเคียงใดๆ ที่ให้ทางปากมีค่าเท่ากับ 3000 mg/kg และค่าความเข้มข้นต่ำสุดที่ก่อให้เกิดผลข้างเคียงอย่างใดอย่างหนึ่งของสารสกัดจากเปลือกเงาะมีค่าเท่ากับ 4000 mg/kg ดังนั้นปริมาณของสารสกัดจากเปลือกเงาะที่ทำให้หนูขาวตายร้อยละ 50 (LD₅₀) มีค่ามากกว่า 5000 mg/kg ส่วนการศึกษาความเป็นพิษกึ่งเรื้อรัง พบว่าค่าความเข้มข้นสูงสุดของสารสกัดจากเปลือกเงาะที่ไม่ทำให้เกิดผลข้างเคียงใดๆ ที่ให้ทางปากมีค่าเท่ากับ 1000 mg/kg และค่าความเข้มข้นต่ำสุดที่ก่อให้เกิดผลข้างเคียงอย่างใดอย่างหนึ่งของสารสกัดจากเปลือกเงาะมีค่าเท่ากับ 2000 mg/kg

การทดลองที่ 2 ศึกษาผลของสารสกัดจากเปลือกเงาะต่อเอนไซม์ไลเปสจากตับอ่อน เอนไซม์อัลฟาอะไมเลส และเอนไซม์อัลฟาไกลูโคซิเดสในหลอดทดลอง และค่าทางชีวเคมีต่างๆ ของพลาสมาในหนูขาวที่ถูกชักนำให้เกิดภาวะเลือดมีสารไขมันมากด้วย lipid emulsion ผลการทดลองพบว่า เปลือกเงาะที่สกัดด้วยเอทานอล (85%) ให้ผลผลิตเท่ากับ 18.05% มีสารประกอบฟีนอลิกทั้งหมดเท่ากับ 416.18 ± 0.01 mg gallic acid/g dry extract และมีปริมาณแอนโทไซยานินทั้งหมดเท่ากับ 14.90 ± 0.8 μ g/g dry extract และค่าความสามารถของปริมาณสารสกัดที่ทำการยับยั้งอนุมูลอิสระ 50% (IC₅₀) เท่ากับ 0.288 ± 0.04 mg/ml dry extract การศึกษาในหลอดทดลองพบว่าสารสกัดจากเปลือกเงาะ แทนนิน และยาอริสแตทมีผลยับยั้งต่อการทำงานของเอนไซม์ไลเปสจากตับอ่อน เอนไซม์อัลฟาอะไมเลส และเอนไซม์อัลฟาไกลูโคซิเดส ส่วนการศึกษาในสัตว์ทดลอง พบว่าสารสกัดจากเปลือกเงาะ แทนนิน และยาอริสแตท ที่ให้ทางปากสามารถยับยั้ง

การเพิ่มขึ้นของระดับไตรกลีเซอไรด์ในพลาสมาเนื่องจากการได้รับ lipid emulsion ได้อย่างมีนัยสำคัญทางสถิติ

การทดลองที่ 3 ศึกษาผลของสารสกัดจากเปลือกเงาะต่อการแสดงออกของโปรตีนฟอสบริเวนสมองส่วนไฮโปทาลามัสในหนูขาว พบว่าสารสกัดจากเปลือกเงาะชักนำให้เกิดการแสดงออกของโปรตีนฟอสเฉพาะในส่วนของ arcuate nucleus เท่านั้น พบการเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติของจำนวน Fos-positive neuron ที่บริเวณของ arcuate nucleus ตั้งแต่ -3.60 ถึง -3.96 มิลลิเมตร จาก bregma ในกลุ่มที่ได้รับสารสกัดจากเปลือกเงาะเมื่อเปรียบเทียบกับกลุ่มควบคุม

โดยสรุป การศึกษาในครั้งนี้ชี้ให้เห็นว่าสารสกัดจากเปลือกเงาะมีประโยชน์ในการป้องกันและรักษาโรคอ้วนได้



สาขาวิชาชีววิทยา

ปีการศึกษา 2554

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AREE THINKRATOK : EFFECTS OF THE CRUDE EXTRACT FROM
THE FRUIT RIND OF RAMBUTAN (*Nephelium lappaceum* L.) ON
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RAMBUTAN/OBESITY/LIPASE ENZYME/ALPHA-AMYLASE ENZYME/
ALPHA-GLUCOSIDASE ENZYME/BIOCHEMICAL PARAMETERS/ACUTE
TOXICITY/SUB-CHRONIC TOXICITY/FOS/ARCUATE NUCLEUS

Obesity has become an important public health problem that can cause a number of obesity-related diseases. The use of commercial anti-obesity drugs, such as orlistat and sibutramine, can cause unpleasant side effects. To overcome these problems, the use of plant extracts is probably a better way to replace these anti-obesity drugs. The fruit rind of rambutan (*Nephelium lappaceum* L.) contains phenolic compounds that can help to prevent obesity. Therefore, this experiment aimed to study effects of the rambutan rind extract on obesity *in vitro* and *in vivo*. This study consisted of 3 main experiments:

Experiments 1 investigated acute and sub-chronic toxicities of the rambutan rind extract in male Wistar rats. The acute toxicity data indicated that the no-observed-adverse-effect level (NOAEL) for the oral dose of the rambutan rind extract was 3000 mg/kg and the lowest-observed-adverse-effect level (LOAEL) was 4000 mg/kg. The evaluated oral LD₅₀ of the rambutan rind extract was then greater than 5000 mg/kg. The sub-chronic toxicity indicated that the NOAEL of the rambutan rind extract was 1000 mg/kg and the LOAEL was 2000 mg/kg.

Experiment 2 investigated effects of the rambutan rind extract on pancreatic lipase, alpha-amylase, and alpha-glucosidase activities *in vitro* and plasma biochemical parameters in lipid emulsion-induced hyperlipidemia rats. The results showed that the rind of rambutan extracted by 85% ethanol had a yield of 18.05%. The rambutan rind extract contained total phenolic content (416.18 ± 0.01 mg gallic acid/g dry extract), total anthocyanin content (14.90 ± 0.8 $\mu\text{g/g}$ dry extract), with IC_{50} value of 0.288 ± 0.04 mg/ml extract. *In vitro* study, the rambutan rind extract, tannin, and orlistat showed potent inhibitory effects on pancreatic lipase, alpha-amylase, and alpha-glucosidase activities. *In vivo* study, the rambutan rind extract, tannin, and orlistat significantly suppressed the increased plasma level of triglyceride (TG) caused by lipid emulsion administration.

Experiments 3 investigated effect of the rambutan rind extract on the rat hypothalamic expression of the neuronal activation marker Fos. The rambutan rind extract induced Fos expression only in the arcuate nuclei (ARC). A significant increase in the number of Fos-positive neurons was shown in the area of the ARC ranging from -3.60 to -3.96 mm from bregma following the rambutan rind extract compared to the control.

In conclusion, the present findings suggested that the rambutan rind extract displayed beneficial effects in prevention and treatment of obesity.

School of Biology

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CURRICULUM VITAE

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CHAPTER I

INTRODUCTION

1.1 Rational of the study

Nowadays, obesity has become a major health problem worldwide that has serious economic and social consequences. Obesity is a result of changes in lifestyles and eating habits. Obesity and overweight are the fifth leading risk for global deaths and at least 2.8 million adults die each year as a result of being overweight or obese (World Health Organization, 2011). In 2010, the World Health Organization (WHO) surveyed the prevalence of obesity in male and female aged 15 years old or over (Figure 1.1). The prevalence of obese males were 2.33%, 1.69%, 2.58%, 8.96%, 12.38%, 22.94%, 23.68%, 28.41%, 30.06%, and 44.22%, whereas the prevalence of obese females were 1.13%, 11.02%, 11.05%, 7.58%, 24.52%, 22.11%, 26.27%, 29.14%, 41.03%, and 48.28% in Japan, Malaysia, Thailand, France, Brazil, Germany, United Kingdom, Australia, Mexico, and United States, respectively. In 2010, approximately 43 million children under the age of 5 years were overweight and approximately 2.3 billion adults were expected to be overweight and approximately 700 million adults were expected to be obese in 2015 (WHO, 2011). Therefore, obesity prevention and treatment are a high public health priority.

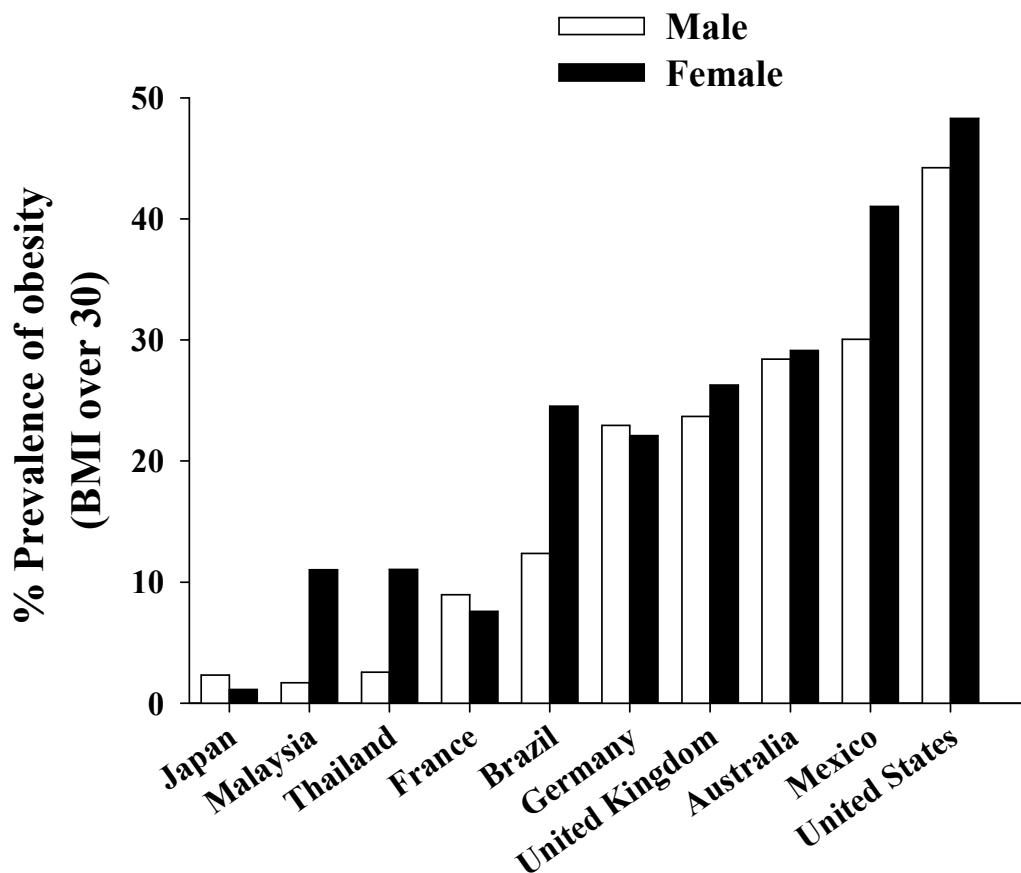


Figure 1.1 Prevalence of obese males and females (aged 15 years or over) in countries selected in 2010 (WHO, 2011).

Several literatures have presented well-documented links between obesity and increased mortality, accelerated aging and morbidity due to hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, congestive heart failure, stroke, osteoarthritis, pulmonary dysfunction (obstructive sleep apnea and hypoventilation syndrome), certain types of cancer (breast, endometrium, colon, and prostate), gastrointestinal diseases (fatty liver, cirrhosis, gastroesophageal reflux, and gallstones), menstrual abnormalities, increase in surgical risk, impaired fertility,

increased pregnancy risks, brain disease (Alzheimer's disease), anxiety, depression, and suicide (Devlin et al., 2000; Kopelman, 2000; Roth et al., 2004; Wickelgren, 1998). In addition, 44% of the diabetes burden, 23% of the ischaemic heart disease burden, and 41% of certain cancer burdens are attributable to obesity and overweight (WHO, 2011).

Successful obesity treatment plans incorporate diet, exercise and behavior modification with or without pharmacologic therapy and/or surgery. Many attempts have been made to correct the metabolic disparity of the obesity condition, producing a number of reagents including sibutramine (serotonin and noradrenaline uptake inhibitor), orlistat (gastrointestinal lipid uptake inhibitor), rimonabant (cannabinoid (CB1) receptor antagonists), and bupropion (dopamine-noradrenaline-reuptake inhibitor) (Adan et al., 2008; Davidson et al., 1999; Gadde et al., 2001; James et al., 2000; Padwal and Majumdar, 2007; Van Gaal et al., 2005). However, administration of these drugs is known to often cause undesirable side effects such as dry mouth, anorexia, constipation, insomnia, dizziness, nausea, diarrhea, tachycardia, hypertension, depressive symptoms, anxiety, palpitations, and memory impairment (Adan et al., 2008; Bray et al., 2001; Davidson et al., 1999; Gadde et al., 2001; James et al., 2000; Van Gaal et al., 2005). Therefore, the use of plant extracts is probably a better way to replace these anti-obesity drugs.

Fruits and vegetables are particularly rich sources of antioxidant components, including phenolic compounds (Bagchi and Preuss, 2007; Kubola and Siriamornpun, 2008). Anti-obesity effects of phenolic compounds have been demonstrated by many studies. Phenolic compounds found in apple and tea reduced the weight of visceral adipose tissues and the triglyceride content of blood and liver in rats fed high-fat diet

(Ohta et al., 2006). Phenolic compounds found in the roots of *Salacia reticulata* (SRHW) may be involved in the anti-obesity effects through inhibiting the activity of fat metabolizing enzymes (pancreatic lipase, lipoprotein lipase, and glycerophosphate dehydrogenase) and enhancing lipolysis (Yoshikawa et al., 2002). Anti-obesity action of peanut (*Arachis hypogaea* L.) shell extracts (PSE) containing luteolin and flavonoid may be attributed to the inhibition of fat absorption in the digestive tract as PSE could inhibit the activity of a number of lipases (pancreatic lipase, lipoprotein lipase, and possibly hormone sensitive lipase). PSE significantly increased in fecal lipid excretion, and significantly lowered body weight, body weight gain, liver size, triacylglycerol content in the liver, as well as the serum glucose and insulin in high-fat diet fed rats (Moreno et al., 2006). *Nelumbo nucifera* leaves extract (NNE) containing several flavonoids and alkaloids could impair digestion and inhibit absorption of lipids and carbohydrates *in vitro*, accelerate lipid metabolism and up-regulate energy expenditure in high-fat diet fed mice (Ono et al., 2006). *Hibiscus sabdariffa* calyces aqueous extract containing anthocyanins significantly reduced body weight gain in monosodium glutamate (MSG)-induced obese mice, and significantly increased liquid intake in both healthy and MSG-induced obese mice (Alarcon-Aguilar et al., 2007). Nomame Herba containing primarily proanthocyanidin (condensed tannin) could inhibit lipase activity *in vitro*, reduce body weight, liver weight, and parametrial fat, reduce plasma triglyceride, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) elevation in rats fed a high-fat diet (Yamamoto et al., 2000). *Garcinia cambogia* seeds extract (GCSE) containing several flavonoids significantly decreased body weight, plasma very-low-density lipoprotein levels and high-density lipoprotein levels, and triacylglycerol pool in adipose tissue and the liver. GCSE

significantly increased plasma low-density lipoprotein and chylomicrons levels, and triacylglycerol pool in the gastrointestinal system in male rats (Oluyemi et al., 2007). *Ilex paraguariensis* extract (IPE) containing phenolic compounds (flavonoids and phenolic acids) significantly decreased weight gain, adiposity, epididymal fatpad weight, the serum levels of cholesterol, triglycerides, low-density lipoprotein, and glucose, and levels of gene and protein expression in high fat diet-induced obese mice (Arçari et al., 2009). *Glycyrrhiza chalcones* roots extract (GCE) containing flavonoids could inhibit the activity of pancreatic lipase *in vitro*. GCE significantly decreased body weight gain, plasma total triglycerides, and total cholesterol levels in the high fat diet-induced obese rats (Birari et al., 2011). *Kaempferia parviflora* rhizome extract (KPE) containing flavonoids significantly increased low-density lipoprotein, and significantly decreased body weight, visceral fat, subcutaneous fat, plasma total cholesterol, triglycerides, high-density lipoprotein and insulin levels in Tsumura, Suzuki, Obese Diabetes (TSOD) mice (Akase et al., 2011). Thus, consumption of fruits and vegetables containing high amount of phenolic compounds may contribute to the prevention of obesity.

The fruit rind of rambutan was reported to contain a large variety of substances possessing antioxidant activity such as ascorbic acid and phenolic compounds (anthocyanins, flavonoids, tannins, ellagic acid, corilagin, and geraniin) (Khonkarn et al., 2010; Palanisamy et al., 2008; Palanisamy et al., 2011a; Palanisamy et al., 2011b; Thitilertdecha, Teerawutgulrag, and Rakariyatham, 2008; Thitilertdecha et al., 2010; Wall, 2006; Worngsiri, Chavadej, and Disyaborn, 1993). Apart from its high antioxidant activity, antibacterial (Palanisamy et al., 2008; Thitilertdecha et al., 2008), anti-herpes simplex virus type 1 (Nawawi et al., 1999), antiproliferative (Khonkarn

et al., 2010), and anti-hyperglycemic (Palanisamy et al., 2011b) activities of the rambutan rind extract have been reported. It has been recently reported an *in vitro* anti-hyperglycemic activity of the geraniin-enriched rambutan rind extract (Palanisamy et al., 2011a). Flavonoids and oleanane-type triterpene oligoglycosides from the rambutan rind extract showed inhibitory effects on fatty acid synthase activity *in vitro* (Zhao et al., 2011). It is hoped that natural antioxidants found in the fruit rind of rambutan could be candidate for treatment of obesity. Therefore, the effect of the rambutan rind extract on obesity was investigated.

1.2 Research objectives

The experiments were designed to clarify the following:

1. To study acute and sub-chronic toxicities of the rambutan rind extract in adult rats.
2. To study the effects of the rambutan rind extract on pancreatic lipase, alpha-amylase and alpha-glucosidase activities *in vitro*, and plasma biochemical parameters levels in postprandial hypertriglyceridemic rats.
3. To study the acute effect of the rambutan rind extract on Fos expression in the hypothalamus and the nucleus of the solitary tract in rats.

1.3 Research hypothesis

The rambutan rind extract have anti-obesity effects in rats through the inhibition of fat metabolizing enzyme (pancreatic lipase), carbohydrate hydrolyzing enzymes (alpha-amylase and alpha-glucosidase), and the neuronal activation in the brain areas involved in the regulation of food intake and body energy balance.

1.4 Expected results

1. The findings will provide the first evidence of the rambutan rind extract on acute and sub-chronic toxicities in rats.

2. The findings will provide the new evidence of the beneficial effects of the rambutan rind extract on anti-obesity as it may inhibit pancreatic lipase, alpha-amylase and alpha-glucosidase activities, decrease triglyceride levels, stimulate neuronal activity in the paraventricular nucleus of the hypothalamus and stimulate neuronal activity in the nucleus of the solitary tract, inhibit neuronal activity in the lateral hypothalamic area and the perifornical area of the hypothalamus, and inhibit or stimulate neuronal activities in the arcuate nucleus of the hypothalamus.

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CHAPTER II

LITERATURE REVIEW

2.1 Rambutan (*Nephelium lappaceum* Linn.)

Rambutan (*Nephelium lappaceum* Linn.) is a tropical fruit of the Sapindaceae family. It is native to Southeast Asia. This fruit is an important commercial crop in Asia, where it is consumed fresh, canned, or processed, and appreciated for its refreshing flavour and exotic appearance (Palanisamy et al., 2008). The rambutan fruit is ovoid, or ellipsoid, pinkish-red, bright-or deep-red, orange-red, maroon on dark-purple, yellowish-red, or all yellow or orange-yellow; 3.4-8 cm long. Its thin, leathery rind is covered with tubercles from each of which extends a soft, fleshy, red, pinkish, or yellow spine 0.5-2 cm long. Pulp is white, translucent, juicy, acid or sweet flesh, 0.4-0.8 cm thick, adhering more or less to the ovoid or oblong, somewhat flattened seed, which is 2.5-3.4 cm long and 1-1.5 cm wide (Morton, 1987).

The rambutan fruits are used for treating severe dysentery, diarrhea, fevers, and stomachic and used as astringent and vermifuge. The leaves are used poultice on the temples to alleviate headache. The roots decoction is used for treating fevers and the bark is used as astringent (Palanisamy et al., 2008; Ragasa et al., 2005). In Malaysia, the dried fruit rind is sold in drugstores and employed in local medicine (Morton, 1987; Palanisamy et al., 2008). The fruit rind of rambutan contained a large variety of substances possessing antioxidant activity such as ascorbic acid and

phenolic compounds (Tables 2.1 and 2.2). Apart from high antioxidant activity (Tables 2.3 and 2.4), antibacterial (Palanisamy et al., 2008; Thitilertdecha et al., 2008), anti-herpes simplex virus type 1 (Nawawi et al., 1999), antiproliferative (Khonkarn et al., 2010), and anti-hyperglycemic activities (Palanisamy et al., 2011b) of the rambutan rind extract has been reported. It has been recently reported an anti-hyperglycemic activity of the geraniin-enriched rambutan rind extract (Palanisamy et al., 2011a). Geraniin, an ellagitannin, isolated from the rambutan rind extract has been shown to be the key compound that responsible for anti-hyperglycemic activities of the rambutan rind extract since geraniin could inhibit alpha-glucosidase and alpha-amylase activities, *in vitro* (Palanisamy et al., 2011a). Flavonoids and olean-type triterpene oligoglycosides, isolated from the rambutan rind extract, potently inhibited fatty acid synthase activity *in vitro* (Zhao et al., 2011).

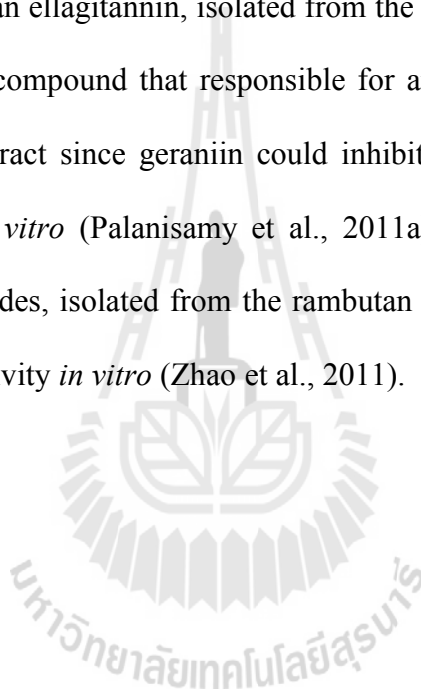


Table 2.1 Yields, total phenolic, total anthocyanin, and tannins contents in the rambutan rind extract.

Extraction method	Solvent	% Yield (% w/w)	Total phenolic content	Total anthocyanin content (mg/100 g of fresh extract)	Tannins (% extraction)	References
	Butanol	7.4	1.73 ± 0.03 ^a	-	-	
	Ethanol	10.7	0.72 ± 0.05 ^a	-	-	
Macerate	Ethyl acetate	0.3	2.28 ± 0.02 ^a	-	-	Khonkarn et al. (2010)
	Hexane	0.6	0.29 ± 0.01 ^a	-	-	
	Methanol	25.5	2.05 ± 0.11 ^a	-	-	
Macerate	Ethanol	17.8 ± 0.2	762 ^b	-	-	Palanisamy et al. (2008)
	Water	13.2 ± 0.2	300 ^b	-	-	Palanisamy et al. (2011a)
Macerate	Ethanol	30.58	-	-	-	Palanisamy et al. (2011b)
	Ethanol	33.2 ± 0.1	975 ± 34 ^b	-	-	
Macerate	Water	23.4 ± 0.3	428 ± 21 ^b	-	-	
	Aqueous	2.06 g/100 g	393.2 ± 7.4 ^c	-	-	
Macerate	Ether	2.84 g/100 g	293.3 ± 0.5 ^c	-	-	Thitilertdecha et al. (2008)
	Methanol	25.1 g/100 g	542.2 ± 0.0 ^c	-	-	
	Ethanol:					
Macerate	Water:Acetic acid (60:39:1)	-	-	181.3	-	Sun et al. (2011)
Macerate	Water	-	-	-	21.18	Wongsiri et al. (1993)

a = mg gallic acid/ml; b = mg gallic acid/g dry weight extract; c = mg catechin/g dry extract

Table 2.2 Geraniin, ellagic acid, corilagin, and ascorbic acid contents in the rambutan rind extract.

Extraction method	Solvent	Geraniin	Ellagic acid (mg/g dry extract)	Corilagin (mg/g dry extract)	Ascorbic acid (mg/100 g fresh weight)	References
Macerate	Ethanol	37.9 ± 8.3 ^d	-	-	-	Palanisamy et al. (2011b)
	Water	15.2 ± 2.3 ^d	-	-	-	
Macerate	Ethanol	3.79 % ^e	-	-	-	Palanisamy et al. (2011a)
Macerate	Methanol	568 ^d	53.5	71.9	-	Thitilertdecha et al. (2010)
-	-	-	-	-	36.4	Wall, 2006

d = mg/g extract; e = % extract

Table 2.3 Antioxidant activities of the rambutan rind extract in the free radical scavenging activities determined by DPPH, galvinoxyl, and ABTS methods.

Extraction method	Solvent	Antioxidant activities			References
		DPPH-scavenging activity	Galvinoxyl-scavenging activity	ABTS-scavenging activity	
Macerate	Butanol	-	-	TEAC values = 16 mM/mg	Khonkarn et al. (2010)
	Ethanol	-	-	TEAC values = 15 mM/mg	
	Ethyl-acetate	-	-	TEAC values = 23 mM/mg	
	Hexane	-	-	TEAC values = 1 mM/mg	
	Methanol	-	-	TEAC values = 22 mM/mg	
Macerate	Ethanol	IC ₅₀ = 3.7 µg/ml	IC ₅₀ = 1.7 µg/ml	IC ₅₀ = 1.7 µg/ml	Palanisamy et al. (2008)
	Water	IC ₅₀ = 18 µg/ml	IC ₅₀ = 19.4 µg/ml	IC ₅₀ = 16.5 µg/ml	
	Aqueous	IC ₅₀ = 9.67 µg/ml	-	-	
Macerate	Ether	IC ₅₀ = 17.3 µg/ml	-	-	Thitilertdecha et al. (2008)
	Methanol	IC ₅₀ = 4.94 µg/ml	-	-	

DPPH = 1, 1-diphenyl-2-picrylhydrazyl; ABTS = 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)

IC₅₀ = 50 % Inhibition concentration; TEAC values = Trolox equivalent antioxidant capacity values

Table 2.4 Antioxidant activities of the rambutan rind extract in the superoxide-scavenging activity, β -carotene bleaching, lipid peroxidation, and FRAP methods.

Extraction method	Solvent	Antioxidant activities			References
		Superoxide -scavenging activity	β -carotene bleaching	Lipid peroxidation FRAP activity	
Macerate	Butanol	-	-	EC values = 15 mM/mg	Khonkarn et al. (2010)
	Ethanol	-	-	EC values = 11 mM/mg	
	Ethyl-acetate	-	-	EC values = 19 mM/mg	
	Hexane	-	-	EC values = 1 mM/mg	
	Methanol	-	-	EC values = 20 mM/mg	
Macerate	Ethanol	41.4%	-	-	Palanisamy et al. (2008)
	Water	47.5%	-	-	
Macerate	Aqueous	-	< 10 μ g/ml	IC ₅₀ = 0.55 μ g/ml	Thitilertdecha et al. (2008)
	Ether	-	< 10 μ g/ml	IC ₅₀ = 0.81 μ g/ml	

FRAP = Ferric reducing antioxidant power; IC₅₀ = 50 % Inhibition concentration; EC values = Equivalent capacity values

2.2 Phenolic compounds and obesity

Phenolic compounds, one of the most widely occurring groups of phytochemicals, are secondary metabolites that are derivatives of the pentose phosphate and phenylpropanoid pathways in plants. These compounds are of considerable physiological and morphological importance in plants (Balasundram et al., 2006). Plant phenolics comprise a great diversity of compounds, such as tannins, flavonoids (anthocyanins, flavanols, flavonols, flavones, and among other) and several classes of non-flavonoids (phenolic acids, lignins, and stilbenes) (Chirinos et al., 2008). Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, anti-obesity, cardioprotective, and vasodilatory effects (Aberoumand and Deokule, 2008; Balasundram et al., 2006). These beneficial effects have been attributed to the antioxidant activity of phenolic compounds. Anti-obesity of action of phenolic compounds has been demonstrated in many studies. Phenolic compounds found in apple and tea could reduce the weight of visceral adipose tissues and the triglyceride content of blood and liver in rats fed high-fat diet (Ohta et al., 2006). Phenolic compounds found in the roots of *Salacia reticulata* could inhibit activities of fat metabolizing enzymes (pancreatic lipase, lipoprotein lipase and glycerophosphate dehydrogenase) and enhance lipolysis (Yoshikawa et al., 2002). *Corchorus olitorius* leaves extracts containing phenolic compounds significantly increased high-density lipoprotein-cholesterol, and decreased body weight gain, liver weight, liver

triglyceride levels, epididymal adipose tissue weight, plasma levels of total cholesterol, triglyceride, glucose, insulin, and free fatty acid and down-regulated gene expression of liver tissue in mice fed high-fat diet (Wang et al., 2011).

Flavonoids are a group of phenolic compounds with strong antioxidant activity that have been identified in fruits and vegetables (Mojzis et al., 2008). Naturally occurring flavonoids are generally classified into six classes according to their chemical structures, such as flavanones, flavones, isoflavonoids, flavanols (flavans), flavonols, and anthocyanins (Li and Jiang, 2007). Numerous studies have indicated that flavonoids possess anti-carcinogenic, anti-viral, anti-inflammatory, anti-estrogenic or estrogenic, and anti-angiogenic activities (Havsteen, 2002; Middleton et al., 2000; Mojzis et al., 2008). Recent interests in these substances have been stimulated by the potential health benefits arising from the antioxidant activities of these phenolic compounds. As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capacity in both *in vivo* and *in vitro* systems. The functionality of flavonoids in human health is supported by the ability of the flavonoids to induce human protective enzyme systems, and by a number of epidemiological studies suggesting protective effects against cardiovascular diseases, cancer, obesity, osteoporosis and other age-related diseases (Aoki et al., 2007; Morris and Zhang, 2006). Anti-obesity of properties of flavonoids have been demonstrated in many studies. Anti-obesity action of peanut (*Arachis hypogaea* L.) shell extracts (PSE) containing luteolin and flavonoid may be attributed to the inhibition of fat absorption in the digestive tract as PSE could inhibit a number of lipases, including pancreatic lipase, lipoprotein lipase and, possibly, hormone sensitive lipase. PSE significantly increased fecal lipid excretion and lowered body

weight, body weight gain, liver size, triacylglycerol content in the liver, as well as the serum glucose and insulin in high-fat diet fed rats (Moreno et al., 2006). *Nelumbo nucifera* leaves extract containing several flavonoids and alkaloids could impair digestion and inhibit absorption of lipids and carbohydrates *in vitro*, accelerate lipid metabolism and up-regulate energy expenditure in high fat diet fed mice (Ono et al., 2006). *Garcinia cambogia* seeds extract (GCSE) containing several flavonoids decreased body weight, plasma very-low-density lipoprotein levels and high-density lipoprotein-cholesterol levels, and triacylglycerol pool in adipose tissue and the liver. GCSE significantly increased plasma low-density lipoprotein-cholesterol and chylomicrons levels, and triacylglycerol pool in the gastrointestinal system in male rats (Oluyemi et al., 2007). *Ilex paraguariensis* extract containing flavonoids and phenolic acids significantly decreased weight gain, adiposity, epididymal fatpad weight, the serum levels of cholesterol, triglycerides, low-density lipoprotein-cholesterol, and glucose in high fat diet-induced obese mice (Arçari et al., 2009). *Glycyrrhiza chalcones* roots extract (GCE) containing flavonoids possessed pancreatic lipase inhibitory effects *in vitro*. GCE significantly decreased body weight gain, plasma levels of total triglycerides, and total cholesterol in the high fat diet-induced obese rats (Birari et al., 2011). *Kaempferia parviflora* rhizomes extract containing flavonoids significantly increased low-density lipoprotein-cholesterol, and significantly decreased body weight, visceral fat, subcutaneous fat, plasma total cholesterol, triglycerides, high-density lipoprotein-cholesterol, and insulin levels in Tsumura, Suzuki, Obese Diabetes (TSOD) mice (Akase et al., 2011). *Fraxinus excelsior* seed extract containing phenolic compounds significantly decreased body weight gain, omental and retroperitoneal fat, average liver weight, fatty liver, blood

glucose levels, and plasma insulin levels in the high fat diet-induced obese mice (Ibarra et al., 2011). Lotus leaves extract containing flavonoids, alkaloids, triterpenoids, polyphenols, and steroids significantly increased high-density lipoprotein-cholesterol, and significantly decreased body weight gain, relative weights of epididymal and retroperitoneal adipose tissues, the serum of total cholesterol, triglyceride, and low-density lipoprotein-cholesterol levels in the high fat diet-induced obese rats (Du et al., 2009).

Anthocyanins are natural pigments belonging to the flavonoid phenolic group of compounds. Anthocyanins are widely distributed in plants that are consumed in the human diet such as crops, beans, vegetables, and fruits (Lee et al., 2008). Chemically, they are polyhydroxylated or polymethoxylated glycosides or acylglycosides of anthocyanidins which are oxygenated derivatives of 2-phenylbenzopyrylium or flavylium salts. Anthocyanins are responsible for most of the red, blue, and purple colors of fruits, vegetables, flowers and other plant tissues or products. They are particularly abundant in berries and other fruits with red, blue or purple color and in red wines (Mazza and Miniati, 1993). The six anthocyanidins (pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin) commonly found in plants are classified according to the number and position of hydroxyl and methoxyl groups on the flavan nucleus. The most commonly occurring anthocyanidin in nature is cyanidin (Mazza, 2007). In particular, these anthocyanins are associated with a wide range of biological activities including antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic and α -glucosidase inhibitory activities, improvement of vision, induction of apoptosis, and neuroprotective effects (Lee et al., 2008; Mazza, 2007; Talavéra et al., 2004). In addition, these pigments may reduce the risk of coronary heart disease through

modulation of arterial protection inhibition of platelet aggregation or endothelial protection (Lila, 2004; Mazza, 2007). Anthocyanins are found to protect against obesity, enhance memory function, prevent free radicals generation, decrease lipid peroxidation, reduce pancreatic swelling and decrease blood sugar concentrations in urine and blood serum (Alarcon-Aguilar et al., 2007; Jayaprakasam et al., 2006; Lee et al., 2008; Lila, 2004; Mazza, 2007; Talavéra et al., 2004). *Hibiscus sabdariffa* calyces aqueous extract containing anthocyanins significantly reduced body weight gain in monosodium glutamate (MSG)-induced obese mice and increased liquid intake in healthy and MSG-induced obese mice (Alarcon-Aguilar et al., 2007). Black soybean (*Glycine max* L.) seed coats extract containing anthocyanins significantly increased the high-density lipoprotein-cholesterol levels, and lowered weight gain, weights of epididymal and perirenal fat pads, and also reduced the levels of serum triglyceride and cholesterol in rats fed a high fat diet (Kwon et al., 2007).

Tannins are polyphenolic secondary metabolites of higher plants that can be classically divided into hydrolysable tannins and proanthocyanidins (condensed tannin). Hydrolysable tannins are esters of phenolic acids and a polyol, usually glucose. The phenolic acids are either gallic acid in gallotannins or other phenolic acids derived from the oxidation of galloyl residues in ellagitannins. Proanthocyanidins are far more common in our diet. They are polymers made of elementary flavan-3-ol units. (Khanbaee and Van Ree, 2001; Santos-Buelga and Scalbert, 2000). Tannins are the active principles of plant-based medicines, especially in Asian (Japanese and Chinese), the tannin-containing plant extracts are astringents and diuretics, and used against diarrhea, stomachache, and duodenal tumours. Tannins can be used as antidotes for heavy metals and alkaloids poisoning because tannins can

precipitate these substances (Khanbabaee and Ree, 2001). Anti-obesity action of tannins has been demonstrated in many studies. The study of rats fed with high cholesterol diets supplemented with 1000 mg/kg tannic acid (tannin) demonstrated that, tannin significantly increased in the excretion of neutral, acidic, and total fecal sterols, and lowered plasma levels of triglyceride and cholesterol, and also reduced cholesterol in liver. Tannin significantly lowered the hepatic HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA) and ACAT (acyl CoA cholesterol acyltransferase) activities, the levels of plasma and hepatic TBARS (thiobarbituric acid-reactive substances), hepatic antioxidant enzyme activities of GSH-Px (glutathione peroxidase) and SOD (superoxide dismutase) (Park et al., 2002). Nomame Herba containing primarily proanthocyanidin (condensed tannin) could inhibit lipase activity *in vitro*, reduced body and liver weight, parametrial fat, reduce plasma triglyceride, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) elevation in rats fed a high-fat diet (Yamamoto et al., 2000). *Murraya koenigii* leaves extract containing tannins, flavonoids, carbazole alkaloids, furanocoumarins, and terpenoids significantly reduced body weight gain, plasma total cholesterol, and triglyceride levels in the high fat diet-induced obese rats (Birari et al., 2010). Longan flowers extract (LFE) containing condensed tannins, proanthocyanidins, flavonoids, and ascorbic acid significantly increased high-density lipoprotein-cholesterol, fecal triglyceride, and cholesterol excretions. LFE significantly decreased body weight, epididymal and perirenal fat, serum triglyceride levels, lipids and cholesterol levels in the liver, and down-regulated pancreatic lipase activity, sterol regulatory element binding protein-1c (SREBP-1c), and fatty acid synthase (FAS) gene expressions, as well as up-regulated low-density lipoprotein receptor (LDLR) and peroxisome proliferator-activated-

receptor-alpha (PPAR- α) gene expressions in liver tissue of rat fed hypercaloric-diet (Yang et al., 2010). Grape seeds extract (*Vitis vinifera* Linn.) containing proanthocyanidins significantly increased high-density lipoprotein-cholesterol and suppressed the increase of body weight, food intake, accumulated fat pads, and serum levels of total cholesterol and triglycerides in the high fat diet-induced obese rats (Arora, Ansari, and Nazish, 2011). *Cassia fistula* and *Senna alata* leaves extract containing condensed tannins and hydrolysable tannins significantly lower body weight and weight of parametrial fat in high fat diet-induced lipidemia in mice (Chichioco-Hernandez and Leonido, 2011).

Ascorbic acid (vitamin C) is an antioxidant and a cofactor for various biochemical reactions, and acts as an electron donor for different enzymes (Kaidar-Person et al., 2008). Other possible functions of ascorbic acid include a role in the regulation of endogenous cholesterol synthesis, cardiovascular disease and protective role against common cold (Kaidar-Person et al., 2008). Many health benefits have been attributed to ascorbic acid such as antioxidant, anti-atherogenic and anti-carcinogenic activities (Naidu, 2003).

2.3 Anti-obesity drugs

The anti-obesity drugs are pharmaceutical substances with different putative actions like reducing food intake, altering food metabolism, or increasing energy expenditure (Chaput et al., 2007).

Sibutramine (Meridia[®], Reductil[®]) is an appetite suppressants of the phenylamine family that selectively inhibits the reuptake of neurochemicals such as monoamines, noradrenaline, serotonin, and dopamine. It reduces food intake,

increases thermogenesis, and fatty acid catabolism (Connoley et al., 1999). There is a dose-dependent reduction of body-weight with a mean decrease of 4.5 kg in patients receiving sibutramine when compared to placebo group (Anderson et al., 2002; Li et al., 2005). While sibutramine can certainly induce weight loss, wide ranges of negative side effects are accompanied. Side effects of sibutramine include insomnia, increases in blood pressure and pulse rate, dry mouth, dizziness, drowsiness, flushing, nausea, constipation, anorexia, and headache (Kim et al., 2003).

Bupropion (Wellbutrin[®], Zyban[®]) is approved for the treatment of depression and smoking, it also acts as appetite suppressant. Bupropion is chemical derivatives of substituted amphetamine that selectively inhibits the reuptake of neurochemicals such as norepinephrine and dopamine. Bupropion caused weight loss in a dose-dependent manner. Side effects of bupropion include dry mouth, constipation, headache, anxiety, dizziness, nausea, paresthesia, and insomnia (Anderson et al., 2002).

Rimonabant (Acomplia[®]) selectively blocks the cannabinoid-1 (CB1) receptors in the endocannabinoid system. Activation of these receptors by endogenous cannabinoid, such as anandamide, increased appetite (Wadden et al., 2005). The CB1 receptor is a member of the seven transmembrane G-protein-coupled receptor superfamily. These receptors couple to a variety of signaling pathways, including inhibition of adenylate cyclase, inhibition of calcium channels and activation of potassium channels (Jonsson et al., 2006). CB1-receptor blockade with rimonabant was found to reduce body weight and waist circumference, improve plasma glucose-insulin homeostasis, and increase in plasma high-density lipoprotein-cholesterol levels. Side effects of rimonabant include insomnia, dizziness, nausea, anxiety,

depression, diarrhea, and suicidal (Christensen et al., 2007; Padwal and Majumdar, 2007; Samat et al., 2008).

Orlistat (Xenical[®]) is a weight-loss agent for treatment of obesity. The compound is a partly hydrated derivative of an endogenous lipstatin produced by bacterium *Streptomyces toxytricini*. Orlistat is a pancreatic lipase inhibitor that reduces dietary fat absorption by around 30% (Bray, 2000; Bray and Tartaglia, 2000). Orlistat can improve lipid blood levels (Mittendorfer et al., 2000; Sjöström et al., 1998), improve glucose metabolism in obese patients with and without diabetes (Heymsfield et al., 2000; Kelley, 2002; Lindgarde, 2000), and reduce high blood pressure (Rossner et al., 2000). The use of orlistat frequently results in adverse events including diarrhea, fecal incontinence, oily stools, fecal urgency, deficiencies of fat-soluble vitamins, flatulence, and vomiting (Ioannides-Demos et al., 2006; Rucker et al., 2007; Padwal, Li, and Lau, 2004; Padwal and Majumdar, 2007).

2.4 Definition of obesity

Obesity is defined as a condition characterized by excess body fat that is quantified by the elevation in body weight of patients (Seidell and Flegal, 1997). In general, it is accepted that obesity results from positive energy balance in which energy intake exceeds expenditure (Wood et al., 1998). It is characterized by enlarged fat mass and elevated lipid concentration in blood (Devlin et al., 2000; Fujioka et al., 2002). The amount of fat mass is increased when the number and/or size of adipocytes are multiplied by proliferation and differentiation. Differentiated adipocyte stores fatty acids (FAs) in the form of triglycerides (TGs) in their cytoplasm, with an involvement of various enzymes such as stearoyl-CoA desaturase-1 (SCD-1) and fatty acid

synthase (FAS). This overall lipid synthetic process is called lipogenesis (Weissman, 1999). Adiposity has been shown to have a strong linear correlation with elevated plasma levels of fatty acids. If blood fatty acids levels are elevated for prolonged periods by excessive energy intake, triglyceride can be accumulated in non-adipose tissues including liver and muscle, which can lead to pathological consequences such as the development of fatty liver or ketosis (Herdt, 2000).

The widely accepted means of assessing obesity is the body mass index (BMI). A very good correlation has been found between BMI and the percentage of body fat in a population. Table 2.5 provides the World Health Organization (WHO) classification of adults according to the BMI (Bagchi and Preuss, 2007):

$$\text{BMI} = \frac{\text{Kg}}{\text{m}^2} = \frac{\text{Body weight in Kg}}{\text{Height in m}^2}$$

Table 2.5 Classification of adults according to BMI (Bagchi and Preuss, 2007).

Classification	BMI	Risk of co-morbidities
Underweight	< 18.50 (but risk of other clinical problems increased)	Low
Normal range	18.50 - 24.99	Average
Overweight	≥ 25.00	
Pre-obese	25.00 - 29.99	Increased
Obese class I	30.00 - 34.99	Moderate
Obese class II	35.00 - 39.99	Severe
Obese class III	≥ 40.00	Very severe

From Table 2.5, the current value settings are as follows: a BMI lower than 18.50 suggests the person is underweight and may indicate malnutrition, eating

disorder, or other health problems, a BMI of 18.50 to 25.00 may indicate optimal weight while a number above 25.00 may indicate the person is overweight, a number above 30.00 suggests the person is obese (over 40.00, morbidly obese).

2.5 The physiology of obesity

The amount of fat in the body (adiposity) is not, as was once thought, a passive result of bad habits or over-indulgence. Rather, it is precisely regulated as part of the process of energy balance, a process whereby energy intake (food intake) is matched to energy expenditure (metabolism and exercise) and the size of the body's energy stores (the fat mass). The major organ regulating this system is the brain, although multiple organ systems participate in the process (Woods and Seeley, 2002). The hypothalamus has been focused on as the center of the brain that controls appetite, energy expenditure, and body weight (Lawrence, Turnbull, and Rothwell, 1999; Schwartz, 2000). Many hypothalamic nuclei are important in the regulation of food intake and energy homeostasis, including the arcuate nucleus (ARC), paraventricular (PVN), ventromedial (VMH), lateral hypothalamic (LHA), and perifornical (PFA) nuclei (Lawrence, Turnbull, and Rothwell, 1999; Schwartz, 2000; Valassi et al., 2008; Williams et al., 2001). The nucleus of the solitary tract (NTS) in the brainstem receives much of the information pertinent to satiety, including vagal afferent information and gustation, and it integrates and relays this information to the hypothalamus (Berthoud and Neuhuber, 2000; Broberger and Hökfelt, 2001). Figure 2.1 shows how circulating signals related to the size of the fat mass (adiposity signals) are integrated with signals from the gastrointestinal system (satiety signals) to control

energy balance (Schwartz et al., 2000). Main brain pathways involved in eating behavior regulation are shown in Figure 2.2 (Valassi et al., 2008).

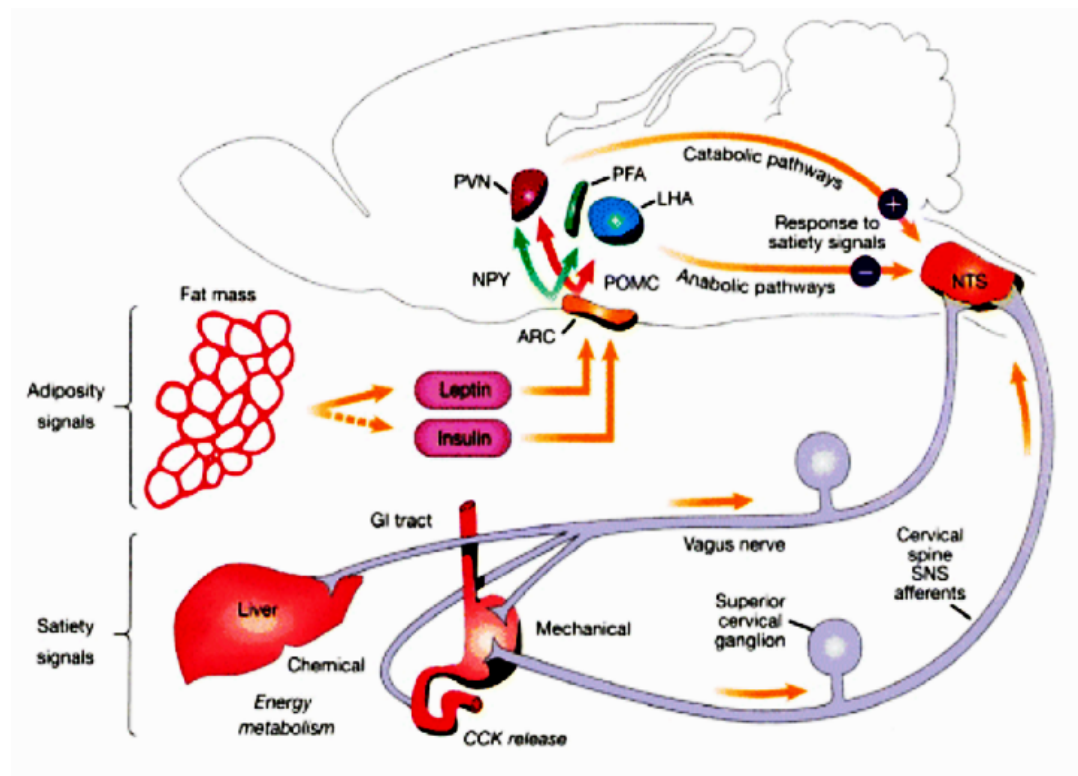


Figure 2.1 Pathways by which signals related to the fat mass are integrated with signals from the gastrointestinal system to control all aspects of energy balance. Adiposity signals are connected through central autonomic pathways to centers that process satiety signals. Reduced input from adiposity signals (e.g. after weight loss) increases meal size by reducing brain response to satiety signals (Schwartz et al., 2000).

Adiposity and satiety signals enter the brain at different levels (Figures 2.1 and 2.2). Adiposity signals enter the brain at the level of the hypothalamus. Neural signals from the gastrointestinal (GI) system and the liver provide information about the food

is being eaten, for example, the taste of the food, how much the stomach is distended, and the chemical content of the food. These satiety signals are sent to the brainstem. The brain responds to the hormone signals *via* integrated neuropeptide pathways, leading to a number of outputs that are directly related to energy homeostasis. These outputs include neuroendocrine activation from the pituitary gland, motor behaviour (eating, exercise, etc.) and autonomic activity. It has become apparent that the autonomic nervous system has a much greater impact than was once thought upon many fundamental processes of metabolism, including lipolysis, the secretion of insulin and glucagons from the pancreas, and glucose synthesis and secretion from the liver. It is important to note that, while energy expenditure tends to decrease with ageing, mainly because of the absence of occupational activity and extreme physical exertion, energy intake does not tend to decrease to the same extent, for a number of reasons, including lifetime habits. Thus, there is a tendency over time for the body weight to increase (Woods and Seeley, 2002).

Cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) have been reported to act as satiety signals to reduce food intake (Figure 2.2) (Lee et al., 1997; Valassi et al., 2008). Satiety signals are generated in the GI tract during a meal and regulate food intake on meal-to-meal basis, inducing a sense of fullness. After entering the GI lumen, nutrients trigger the secretion of several peptides which, in addition to other actions, activate vagal and sympathetic pathways afferent to the NTS in the caudal brainstem where they provide information on the chemical and mechanical properties of the nutrients (Wood, 2004). The NTS expresses both pro-opiomelanocortin (POMC) and leptin receptors, which suggests

that this brain area, like the ARC, is able to integrate peripheral satiety and adiposity signals with hypothalamic and suprahypothalamic information (Schwartz et al., 2000).

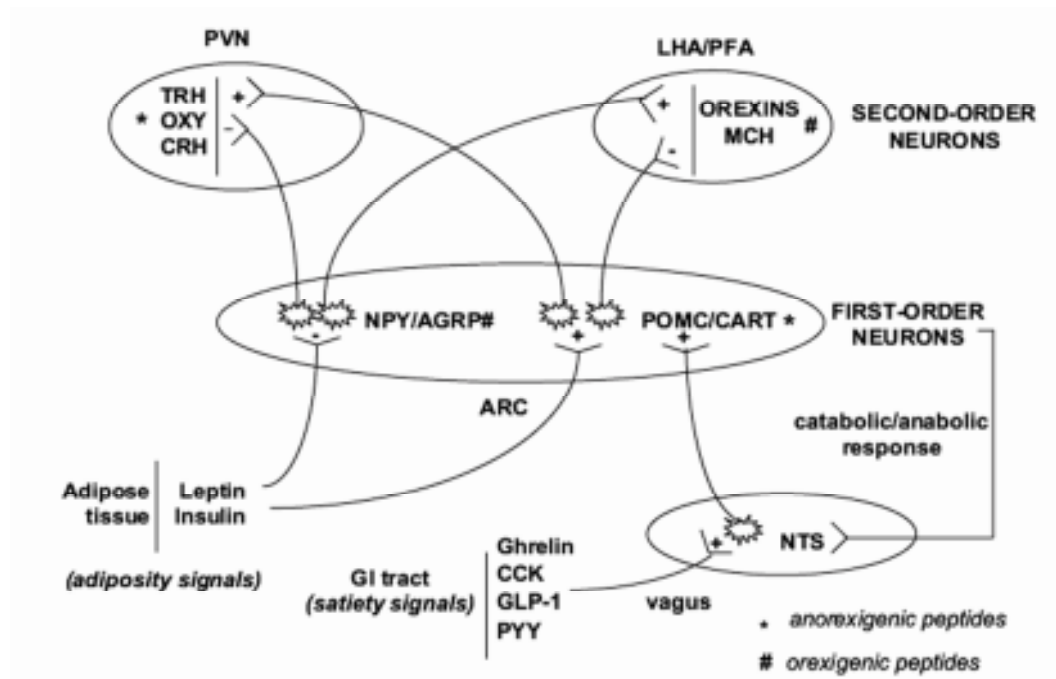


Figure 2.2 Schematic representation of the chief brain pathways involved in the regulation of eating behavior. (ARC: arcuate nucleus, NTS: nucleus of the solitary tract, CCK: cholecystokinin, GLP-1: glucagon-like peptide 1, PYY: peptide YY, PVN: paraventricular nucleus, LHA: lateral hypothalamic area, PFA: perifornical area, NPY: neuropeptide Y, AGRP: Agouti-related peptide, POMC: pro-opiomelanocortin, CART: cocaine- and amphetamine-regulated transcript, CRH: corticotropin-releasing hormone, TRH: thyrotropin-releasing hormone, OX: oxytocin, MCH: melanin concentrating hormone) (Valassi et al., 2008).

CCK is mainly produced by neuroendocrine secretory cells lining the intestinal lumen. CCK peptides exert their action on 2 distinct receptor subtypes: CCK-A (alimentary), now called the CCK-1R, which is mostly expressed peripherally; and CCK-B (brain), renamed the CCK-2R, which is primarily present in the brain (Kissileff et al., 2003). When nutrients enter the lumen and binds to specific receptors (CCK-1R) located on vagal sensory terminals delivering to NTS a sense of fullness (Valassi et al., 2008). CCK reduces food intake *via* the parasympathetic nervous system during food ingestion (Kissileff et al., 2003) and increases absorption by retarding stomach emptiness (Helm et al., 2003).

GLP-1 is secreted by the endocrine L cells in the ileum (Drucker, 1998) in response to the entry of nutrients into the small intestine and coreleased with PYY (Kim et al., 2005). In addition to stimulating insulin release, GLP-1 also inhibits glucagon, delays gastric emptying (Naslund et al., 1999), inhibits food intake (Flint et al., 1998) and aids in the metabolism and absorption of specific macronutrients (Verdich et al., 2001). GLP-1 inhibits food intake by binding to GLP-1 receptors on afferent nerves in the liver and/or GI tract and activating vagal afferent nerves to the central nerve system and the NTS (Havel, 2001). Intraventricular administration of GLP-1 inhibits food intake (Turton et al., 1996; Tang-Christensen et al., 1996), and central administration of GLP-1 antagonists increases feeding in rodents (Turton et al., 1996; Meeran et al., 1999)

PYY is released from distal intestinal endocrine cells (L cells) following detection of fatty acids, fibre and bile acid in the gut (Onaga et al., 2002). The release of PYY appears to be confined to the very end of the meal or the period immediately thereafter in humans and it causes a decrease in gastric emptying and consequently a

reduction in food intake (Batterham et al., 2002). Moreover, when co-released with GLP-1, an additive effect on energy intake is observed (Neary et al., 2005). In addition to its effects on food intake, PYY may regulate energy expenditure (Sloth et al., 2007).

Ghrelin is produced by the stomach and may activate the growth hormone secretagogue receptor (Kojima et al., 1999). Circulating ghrelin concentrations were increased during fasting and decreased when nutrients presented in the stomach (Tschöp, Smiley, and Heiman, 2000), and lowered in obese and lean human subjects (Tschöp, Smiley, and Heiman, 2000). In contrast to the anorexigenic effects of other GI peptide hormones, peripheral or central administration of ghrelin increases food intake in rodents, whereas administration of antagonists against ghrelin inhibit feeding (Shintani et al., 2001; Tschöp, Smiley, and Heiman, 2000; Wren et al., 2000).

Adiposity signals composed of leptin and insulin are shown in Figures 2.1 and 2.2. Leptin, the *ob* gene product, is produced mainly in the adipose tissue and enters the brain in proportion to its plasma levels. Leptin maintains long term control on adiposity and regulates adaptive metabolic changes in response to nutritional modifications (Ahima and Osei, 2004). Leptin is also able to regulate short-term energy intake, modulate meal size according to changes in energy balance (with negative energy balance). Low leptin signaling activates anabolic and inhibits catabolic circuits, enhancing neuropeptide Y (NPY) and agouti-related peptide (AGRP) release and blocking the activity of POMC and cocaine- and amphetamine-regulated transcript (CART) neurons, results in an increase in meal size and a decrease in energy expenditure (Schwartz et al., 2000). Insulin, when body weight augments, insulin resistance occurs with attendant increase in insulin secretion. The

hormone enters the brain in proportion to its circulating levels, contributing to reduce energy intake through the activation of catabolic pathways (Schwartz et al., 2000). Central administration of insulin significantly reduces feeding and body weight in animal models (Vettor et al., 2002). Insulin and leptin both activate POMC neurons, but they seem to differentially regulate AGRP, with leptin inhibiting and insulin stimulating its synthesis (Valassi et al., 2008 quoted in Wanting et al., 2005).

The ARC, adjacent to the third ventricle, is the chief hypothalamic area involved in the control of food intake and contains two interconnected groups of “first-order” neurons releasing NPY and AGRP, which enhance food intake, and the anorexigenic substances POMC and CART (Valassi et al., 2008). The axons of these neurons project to “second-order” neurons, located in part in the PVN, where the anorexigenic substances thyrotropin-releasing hormone (TRH), corticotropin-releasing hormone (CRH) and oxytocin are secreted, and in part in the LHA and PFA, where the orexin molecules melanin-concentrating hormone (MCH) and orexins are produced. When adiposity signals reach the ARC, anorexigenic peptides are released which activate a catabolic circuit. In contrast, the activation of anabolic pathway leads to the release of orexigenic peptides and occurs when adiposity signal concentrations in the brain are low (Valassi et al., 2008). Long term high fat diet for 14 days significantly decreased the number of neurons carrying α -MSH and CART peptide in the ARC of the rat hypothalamus (Tian et al., 2004).

NPY is a 36-amino acid hypothalamic orexigenic neuropeptide secreted in the ARC (Angelopoulos et al., 2005 quoted in Demont et al., 1992), from which NPY neurons project to second-order neurons located in the PVN, LHA, PFA, VMH and dorsomedial (DMN) nuclei, and to other brain regions, setting in motion the anabolic

pathway (Ramos et al., 2004). Furthermore, 90% of NPY neurons are found to be co-expressed with AGRP (Schwartz et al., 2000). Central administration of NPY inhibits thermogenesis, enhances food intake and promotes adipogenesis in rats (Williams et al., 2004). AGRP is another potent orexigenic peptide. The release of AGRP from the ARC is inhibited by leptin infusion. AGRP influences food intake mainly through the competitive antagonism of central melanocortin receptors (Ollmann et al., 1997).

POMC, the precursor of several molecules including alpha-melanocyte-stimulating hormone (α -MSH), is the main regulator of food intake and body weight (Valassi et al., 2008; Lawrence et al., 1999). In the brain, POMC is located primarily in the ARC. Two of the five melanocortin receptors (MC-Rs), MC3-R and MC4-R, are expressed in the hypothalamic regions associated with feeding behaviour (e.g. the PVN and VMN) (Lawrence et al., 1999). CART, a neuropeptide involved in feeding, is widely distributed in the brain, gut, pituitary gland, adrenals, and pancreas (Gautvik et al., 1996). Ninety percent of CART neurons are co-localized with POMC neurons in the ARC and project to second-order neurons likely mediating the anorexigenic effect of leptin (Aja et al., 2001).

2.6 Fos protein

Fos, the translational product of immediate early gene *c-fos*, exerts influence on cellular functions by regulating the induction of its downstream target genes as a transcription factor. Fos is induced rapidly and transiently in neurons after applying a variety of stimuli (Herrera and Robertson, 1996; Hughes and Dragunow, 1995). After translation, Fos/Jun heterodimer nucleoprotein complex binds with high affinity to a DNA-specific sequence identified as activating protein-1 (AP-1) site (Hughes and

Dragunow, 1995; Sassone-Corsi et al., 1988). AP-1 is a collective term referring to dimeric transcription factors composed of Jun, Fos or activating transcription factor (ATF) subunits that bind to a common DNA site (Karin et al., 1997). Several *cis* elements mediate *c-fos* induction. Proximal to the *c-fos* TATA box (TGACGTCA) is a cAMP-responsive element (CRE) or ATF proteins, which all mediate *c-fos* induction *via* cAMP- and Ca²⁺-dependent signaling pathways in response to neurotransmitters and polypeptide hormones (Sheng et al., 1991). Expression of *c-fos* and its protein product Fos has been extensively used to map stimulus-evoked functional activity in the brain (Nikolaev et al., 2002). Fos can be identified by immunohistochemistry to be in the nuclei of neurons (Bullitt, 2004).

The effect of high fat diets (saturated), n-3 or n-6 polyunsaturated fatty acids, or a low fat diet over periods of 1, 7, and 11 weeks on Fos expression in the hypothalamus have been demonstrated in mice (Wang et al., 1999). Fos immunoreactive neurons in the dorsal part of lateral hypothalamic (dLH) area were significantly increased after saturated fat feeding for 1 week whereas the VMH activity was decreased when compared to the low fat diet group. Fos immunoreactive neurons in the PVN area were significantly increased after saturated fat feeding for 7 and 11 weeks when compared to the low fat diet group. A significant increase in the number of Fos immunoreactive neurons in the VMH was shown after n-3 or n-6 polyunsaturated fatty acids feeding for 1 week compared to the high fat diet group (Wang et al., 1999). The effect of lipid emulsion on Fos expression in the hypothalamus and the NTS have been demonstrated in rats (Lo et al., 2007). Significant increases in the number of Fos-positive neurons were demonstrated in the PVN, VMH, and ARC of the hypothalamus and the NTS in brainstem at 4 h after lipid

emulsion administration when compared to the control group (normal saline). Fos-positive neurons induced by lipid emulsion were abolished by CCK-1 receptor antagonist, Lorglumide. Thus, the neuronal activation induced by lipid emulsion arises within the gastrointestinal tracts which are relayed to the brain *via* a CCK-1 receptor dependent pathway (Lo et al., 2007). The effect of CCK on Fos expression in the NTS have been demonstrated in rats (Zittel et al., 1999). CCK dose-dependently increased the number of Fos-positive neurons were demonstrated in the medial and intermediate subnucleus of the NTS when compared to the control. CCK doses of 4 mg/kg or higher can inhibit food intake independent of the vagus nerve. Food intake induced Fos expression levels in the NTS similar to those induced by CCK. Thus, CCK and the NTS are interacting parts of food regulating mechanisms that are involved in the short-term regulation of food intake and the neural control of feeding by the caudal brainstem (Zittel et al., 1999).

2.7 Adipose tissue

Adipose tissue is specialized connective tissue (Albright and Stern, 1998). Adipose tissue functions can be classified into three aspects. First, it is related to lipid metabolism including TGs storage and FAs release. Second, it catabolizes TGs in order to release glycerol and FAs that participate in glucose metabolism in liver and other tissues. Finally, adipocytes secrete adipokines, which include hormones, cytokines and other proteins with specific biological functions (Morrison and Farmer, 2000). Adipose tissue has an important influence on physiological processes such as development and growth of the adipocyte and energy homeostasis (Bays et al., 2008). There are two types of adipose tissue depending on its cell structure, location, color,

vascularization and function: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is the primary site of energy storage in a lipid droplet of the adipocytes in the form of TGs, whereas BAT contains multilocular adipocytes or cells with various lipid droplets. This cell has a large number of mitochondria and is specialized in heat production and, therefore, energy expenditure. Nevertheless, in humans, BAT is present only in newborns for regulating thermogenic process (Gesta et al., 2007).

2.7.1 Lipogenesis

Lipogenesis is the synthesis of esterified FAs, which from TGs from carbohydrates or other energy sources acquired in the diet (Figure 2.3). In rats, lipogenesis occurs in liver and WAT, whereas in humans, lipogenesis contributes mildly to the fat balance (McDevitt et al., 2001). It occurs predominantly in liver and to a lesser extent in adipose tissue, even with high-carbohydrate diets. In rodents, nutritional status and small changes in insulin levels are factors that influence lipogenesis rate (Vázquez-Vela et al., 2008 quoted in Huber et al., 1965). Lipid synthesis is augmented during postprandial state and after carbohydrate consumption and is inhibited under fasting conditions (Sebokova et al., 1997). Lipid accumulation in adipose tissue depends on circulating FA uptake (Zechner et al., 2000). FAs are provided by the enzymatic hydrolysis of TG contained in the chylomicrons by the lipoprotein lipase. After FAs enter the adipocyte, reesterification is necessary for lipid storage in TG form (Hirata et al., 1999). Several enzymes involved in adipose tissue lipogenesis are induced by insulin. These are fatty acid synthase (FAS), acetyl CoA carboxylase (ACC) and malic enzyme (ME). Newly synthesized FAs are used as substrates in TG synthesis (Sul and Wang, 1998).

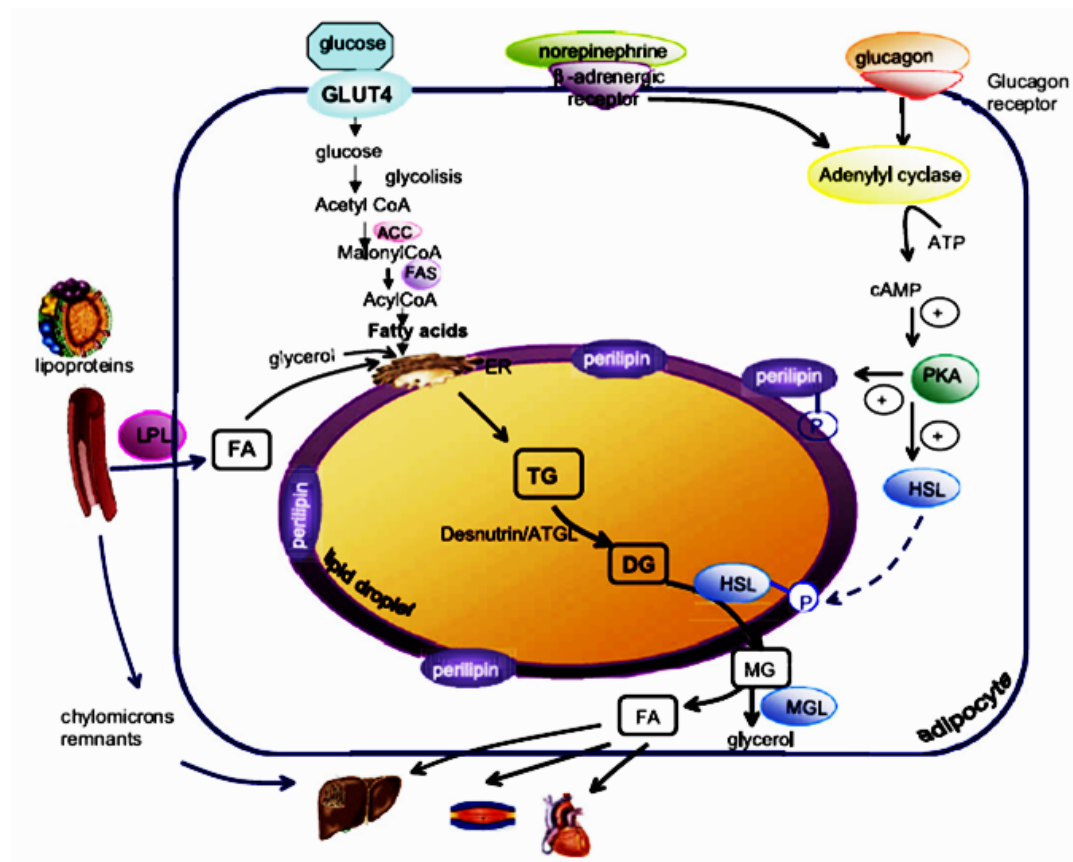


Figure 2.3 Lipogenesis and lipolysis (Vázquez-Vela et al., 2008). TG: triglyceride, DG: diglyceride, FA: fatty acid, MG: monoglyceride, MGL: monoglyceride lipase, PKA: protein kinase A, ACC: acetyl CoA carboxylase, HSL: hormone-sensitive lipase, LPL: lipoprotein lipase, FAS: fatty acid synthase.

2.7.2 Lipolysis

TG stored in the lipid droplets are first hydrolyzed by the enzyme adipose triglyceride lipase (ATGL), also known as desnutrin, releasing a diacylglycerol moiety and FA (Villena et al., 2004). After hydrolysis by ATGL, diacylglycerols are then hydrolyzed sequentially by the hormone-sensitive lipase

(HSL) and monoglyceride lipase (MGL), producing FFAs and glycerol (Fredrikson, Tornqvist, and Belfrage, 1986; Holm, 2003). Different lipases gain access to the lipid droplet when proteins that coat the vesicle (perilipins) are phosphorylated. Perilipin normally prevents lipolysis of TG by its localization surrounding the lipid droplet leading to prevent the access of lipases (Brasaemle et al., 2000). Beta-adrenergic stimulation of adipocytes and the subsequent protein kinase A-dependent phosphorylation of HSL and perilipin trigger the translocation of HSL from the cytoplasm to the lipid droplet and induce neutral lipid hydrolysis (Egan et al., 1992). During fasting, glucagon and catecholamines stimulate lipolysis in the adipocytes by activating *via* PKA several lipases, resulting in a mobilization of FFA from the adipocyte to the circulation, which are then bound to albumin and transported to muscle, liver, heart and other tissues for its oxidation or reesterification (Lafontan et al., 2000).

2.7.3 Digestive enzymes activities

Pancreatic lipase (PL) enzyme

Lipases are enzymes that digest fats, including triacylglycerol and phospholipids. The human lipases include the pre-duodenal (lingual and gastric) and the extra-duodenal (pancreatic, hepatic, lipoprotein and the endothelial) lipases (Mukherjee, 2003). PL (triacylglycerol acyl hydrolase), the principal lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient digestion of triglycerides. It removes fatty acids from the α and α' position of dietary triglycerides, yielding β -monoglycerides and long chain saturated and polyunsaturated fatty acids as the lipolytic products (Mukherjee, 2003; Shi and Burn, 2004; Thomson et al., 1997).

PL is responsible for the hydrolysis of 50-70% of total dietary fats (Figure 2.4). Thus, PL inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural products as anti-obesity agents (Birari and Bhutani, 2007).

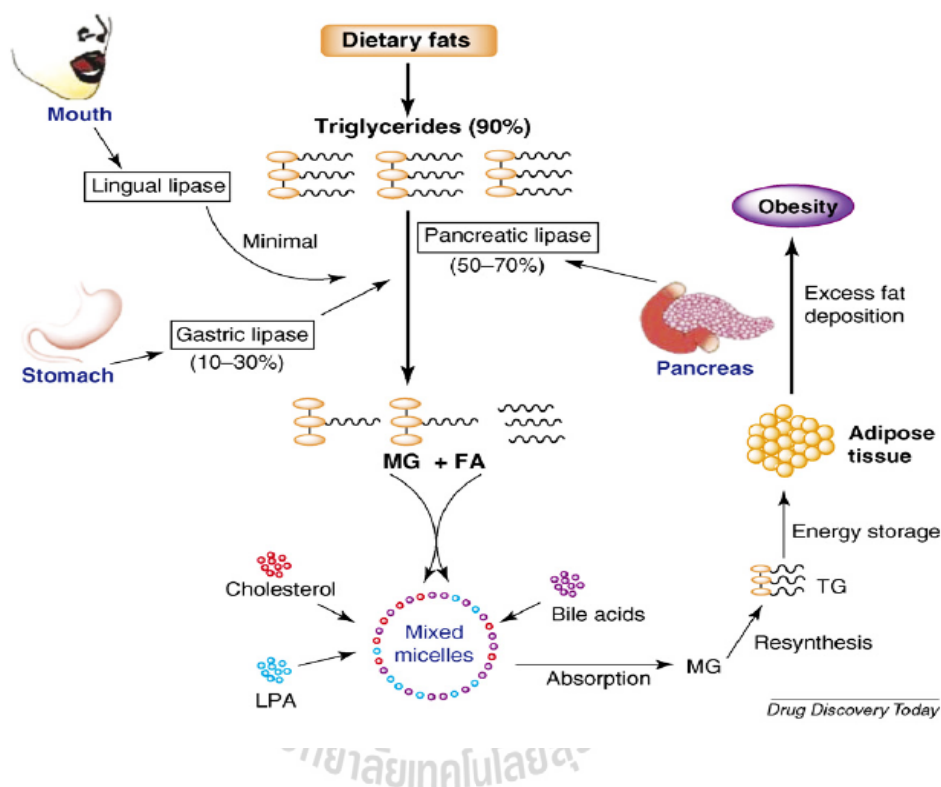


Figure 2.4 Physiological role of pancreatic lipase in lipid absorption (Birari and Bhutani, 2007). (LPA: lysophosphatidic acid, TG: triglyceride, FA: fatty acids, and MG: monoglycerides).

Alpha-amylase and alpha-glucosidase enzymes

Alpha-amylase and alpha-glucosidase are the key enzymes involved in the metabolism of carbohydrates. Alpha-amylase catalyzes the hydrolysis of α -1,4-glucan bonds in starch, maltodextrins, and maltooligosaccharides. This enzyme is

present in animals, plants, bacteria, and fungi. The digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of the polymeric substrate into shorter oligomers. Later on in the gut there are further hydrolyzed by pancreatic alpha-amylase into disaccharides, oligosaccharides, and polysaccharides (Ferey-Roux et al., 1998). Thereafter, alpha-glucosidase enzyme, located in the brush border surface membrane of intestinal cells, further catalyzes the final step in the digestive process of carbohydrates (Gao et al., 2007). Alpha-glucosidase catalyzes the hydrolysis of α -1,4-glucosidic bond in disaccharides, oligosaccharides, and polysaccharides, which are ultimately converted into glucose (Nashiru et al., 2001). Liberated glucose is then absorbed by the intestinal and results in postprandial hyperglycemia. Therefore, inhibition of these enzymes can decrease the postprandial hyperglycemia after a mixed carbohydrate diet by delaying the process of carbohydrate hydrolysis and absorption.

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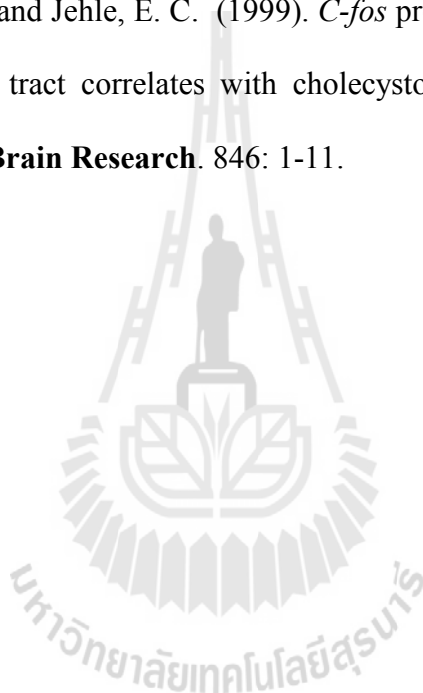
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CHAPTER III

ACUTE AND SUB-CHRONIC TOXICITY STUDIES OF THE RAMBUTAN RIND EXTRACT IN MALE WISTAR RATS

3.1 Abstract

Rambutan (*Nephelium lappaceum* L.) has various uses in medicine and the fruit rind of rambutan can be considered as an easily accessible source of natural antioxidant, antibacterial, anti-herpes simplex virus type 1, antiproliferative, and anti-hyperglycemic activities. Since toxicological data of the rambutan rind extract has not been reported, this study aimed to investigate acute and sub-chronic toxicities of the rambutan rind extract in male Wistar rats. In the acute toxicity study, rats (n=48) were orally administered a single dose of 1000, 2000, 3000, 4000, or 5000 mg/kg of rambutan rind extract. In the sub-chronic toxicity study, rats (n=32) were daily orally administered of 500, 1000 or 2000 mg/kg of the rambutan rind extract for 30 days. All rats were observed for mortality, signs of toxicity, food consumption, and body weight for 14 days after dosing in acute toxicity study and for 30 days in sub-chronic toxicity study. Relative organ weight (ROW), serum and plasma biochemical parameters, histopathological examination, and hematological parameters (only in sub-chronic) were performed at the end of each observation period. In the acute

toxicity study, the lethal dose (LD₅₀) was found to be greater than 5000 mg/kg (p.o.) of the rambutan rind extract. No mortality was found after oral administration of 500, 1000, 2000, 3000, and 4000 mg/kg of the rambutan rind extract. The 5000 mg/kg of the rambutan rind extract resulted in 12.5% mortality (one death out of eight rats). Some signs of toxicity; hypo-activity was observed at 4000 and 5000 mg/kg, convulsion was observed in rats that died later. Moreover, significant decreases in body weight gain, food consumption, serum glucose levels and ROW of liver and heart were observed. In the sub-chronic toxicity study, no mortality and sign of toxicity was found at the dose of 500 and 1000 mg/kg of the rambutan rind extract. The mortality rate at the dose of 2000 mg/kg of the rambutan rind extract was 12.5% (one death out of eight rats) and the observed sign of toxicity was hypo-activity. In addition, significant decreases in body weight gain, food consumption, plasma triglyceride and BUN levels, mean corpuscular hemoglobin concentrations (MCHC) and lymphocyte, while significant increases in ROW of testes and heart, platelet count, neutrophil and eosinophil were observed. Therefore, the acute toxicity data indicated that the no-observed-adverse-effect level (NOAEL) for the oral dose of the rambutan rind extract was 3000 mg/kg and the lowest-observed-adverse-effect level (LOAEL) was 4000 mg/kg. The evaluated oral LD₅₀ of the rambutan rind extract was then greater than 5000 mg/kg in male Wistar rats. In the sub-chronic toxicity, the NOAEL of the rambutan rind extract was 1000 mg/kg and the LOAEL was 2000 mg/kg. This is the first report of acute and sub-chronic toxicities studies of the rambutan rind extract. The present findings of acute and sub-chronic toxicities in rats will help for future clinical studies of the medicinal safety and *in vivo* experimental studies of the pharmacological potential of the medicine plant.

3.2 Introduction

Rambutan (*Nephelium lappaceum* L.) belongs to the Sapindaceae family which is native to Southeast Asia. The dried fruit rind of rambutan has been employed in traditional medicine for the treatment of astringent, stomachic, acts as a febrifuge and relieves diarrhea and dysentery (Morton, 1987; Palanisamy et al., 2008). The fruit rind of rambutan contained a large variety of substances possessing antioxidant activity such as ascorbic acid and phenolic compounds (anthocyanins, flavonoids, tannins, ellagic acid, corilagin, and geraniin) (Palanisamy et al., 2008; Thitilertdecha et al., 2008, 2010; Wall, 2006). Apart from its high antioxidant activity, antibacterial (Palanisamy et al., 2008; Thitilertdecha et al., 2008), anti-herpes simplex virus type 1 (Nawawi et al., 1999), antiproliferative (Khonkarn et al., 2010), and anti-hyperglycemic activities (Palanisamy et al., 2011b) of the rambutan rind extract have been reported. It has been recently reported an anti-hyperglycemic activity of the geraniin-enriched rambutan rind extract (Palanisamy et al., 2011a). Geraniin, an ellagitannin, isolated from the rambutan rind extract has been shown to be the key compound that responsible for anti-hyperglycemic activities of the rambutan rind extract since geraniin could inhibit alpha-glucosidase and alpha-amylase activities, *in vitro* (Palanisamy et al., 2011a). Flavonoids and olean-type triterpene oligoglycosides from the rambutan rind extract were shown to inhibit fatty acid synthase activity *in vitro* (Zhao et al., 2011). Although, the rambutan rind extract has long been used in traditional medicine and various purposes, the study on the toxicity of the fruit rind of rambutan has never been reported. Therefore, this study aimed to investigate the safety of the hydroethanolic extract of the rambutan rind by

determining its behavioural and pharmaco-toxicological effects after acute and sub-chronic oral administration in male Wistar rats.

3.3 Materials and methods

3.3.1 Plant material

Rambutan (*Nephelium lappaceum* L.) fruits were obtained from local market in Nakhon Ratchasima province during June-August 2008.

3.3.2 Preparation of plant extract

The fruit rind of rambutan was washed with copious amounts of water and allowed to air dry at room temperature for 2-3 h. The fruit rind was then cut into small thin pieces and dried at room temperature for 2-4 days. The dried rind was powdered using an electric mill with a 1 mm mesh. The dried powder was extracted by maceration method with 85% aqueous ethanol (100 g dried powder/ 500 ml of 85% aqueous ethanol) for 7 days in the dark at room temperature. The obtained suspension was filtered through No.1 Whatman filter paper (Whatman International Ltd., Maidstone, England). The filtrate was collected, concentrated using a rotary evaporator (Rotavapor[®] model R-205, Buchi, Switzerland) and then converted into crude extract by freeze dryer (Labconco Corporation Ltd., Missouri, USA). The obtained crude extract was stored at -20 °C until further used and the weight percentage yield was determined. This stock extract was used in all experiments performed in this chapter.

3.3.3 Animals

Male Wistar rats were obtained from Institutional Animal Care, Suranaree University of Technology (SUT). They were maintained under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 20 ± 1 °C) with free access to food and water. All studies were conducted under permit of the SUT Animal Care and Use Committee.

3.3.4 Materials

0.9% Normal saline solution

0.9% Normal saline solution was prepared by adding 9 g of sodium chloride (NaCl; Sigma Chemical Co., St. Louis, MO, USA) to 900 ml of deionized distilled water (DI). This solution was adjusted volume to 1000 ml with DI water in a volumetric flask.

Heparinsed saline solution

Heparinsed saline (5 IU/ml) solution was prepared by adding 1 ml of a 5000 units/ml heparin solution (LEO Phamaceutical product, Ballerup, Denmark) to 999 ml of 0.9% normal saline solution.

0.1 M Phosphate buffer solution (pH 7.4)

0.1 M Phosphate buffer solution (pH 7.4) was prepared by adding 1.065 g of di-sodium hydrogen phosphate anhydrous (Na_2HPO_4 ; BDH Ltd., UK) and 0.397 g of sodium dihydrogen orthophosphate 1-hydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; BDH) to 90 ml of DI water. This solution was stirred on a hot plate magnetic stirrer (VELP Scientifica, Europe) at 50-60 °C for 1 h, left to be cool down to room temperature and

then adjusted to pH 7.4 with 1 M HCl or 2 M NaOH. This solution was adjusted volume to 100 ml with DI water in volumetric flask.

10% Buffered formalin solution

10% Buffered formalin solution was prepared by adding 10 ml of formalin (CH₂O, 40%; Carlo Erba Reagents, Italy) to 90 ml of phosphate buffer solution (0.1 M, pH 7.4) and kept in amber bottle.

Eosin working solution

Eosin working solution contained 39.8% v/v of eosin Y 1% aqueous solution (Bio-Optica; Italy), 47.8% v/v 95% alcohol (Commercial grade; Carlo Erba), and 0.4% v/v acetic acid glacial (CH₃COOH, analytical grade; Carlo Erba). This solution was adjusted volume to 100 ml with DI water in volumetric flask.

3.3.5 Acute toxicity study

Acute toxicity test was performed according to the Organization of Economic Co-operation and Development (OECD) guideline 423 for testing of chemical (OECD, 2001). Forty eight rats (282.12 ± 5.37 g) were selected by stratified randomization and then divided into six groups, with eight rats in each group. Rats were fasted overnight and then given single oral doses of 1000, 2000, 3000, 4000, and 5000 mg/kg of the rambutan rind extract dissolved in DDD water at a dosing volume of 10 ml/kg BW while the control group received DDD water (10 ml/kg). All rats were observed for mortality and signs of toxicity (behaviors: tremors, convulsions, sedation, salivation, increase activity, hypo-activity, irritability, itching the nose and mouth on the cage floor, diarrhea, lethargy, sleep, and coma) for the first, second,

third, and fourth hours and thereafter once daily for 14 days. Daily food consumption and weekly body weight changes for each rat were measured throughout the study.

At the end of the observation period, all rats were fasted overnight and then anesthetized with pentobarbital sodium (Nembutal, Ceva Sante Animale, Libourne, France) at dose of 60 mg/kg (i.p.). Blood samples (3 ml from each rat) were collected *via* cardiac puncture into non-heparinized tubes and centrifuged at 5,000 g for 10 minutes. The serum was separated and frozen at -20 °C until further biochemical analysis. The serum biochemical parameters [triglyceride (TG), total cholesterol (TC), glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] were measured by automated analyzer (Hitachi 911, Japan).

3.3.6 Sub-chronic toxicity study

Sub-chronic toxicity test was performed by the modified method of Maphosa, Masika, and Moyo (2010) and OECD guideline 407 (OECD, 1995). Thirty two rats (262.19 ± 13.39 g) were selected by stratified randomization and then divided into four groups, with eight rats in each group. Rats were daily orally administered of 500, 1000, and 2000 mg/kg of the rambutan rind extract dissolved in DDD water at a dosing volume of 2 ml/kg while the control group daily received DDD water (2 ml/kg) for 30 days. The doses of the rambutan rind extract were selected based on the results of the acute toxicity study. All rats were observed for mortality and signs of toxicity as mentioned in acute toxicity study for the first, second, third, and fourth hours and thereafter once daily for 30 days. Rats were maintained on an ad libitum diet and tap water throughout the study. Daily food

consumption and weekly body weight changes for each rat were measured throughout the study.

On the 31st day, all rats were fasted overnight and then anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg BW, i.p.). Blood samples (3-4 ml from each rat) were collected *via* cardiac puncture into EDTA (Ethylenediamine tetraacetic acid) tubes and heparinized tubes. Blood with EDTA was used immediately for the determination of hematological parameters while heparinized blood was centrifuged at 2000 rcf for 5 minutes, plasma obtained was stored at -20 °C until further biochemical analysis. Hematological parameters [red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC), red blood cell distribution width (RDW), platelet count, white blood cells (WBC), neutrophil, lymphocyte, monocyte, eosinophil, and basophil] were determined by Coulter[®] HmX Hematology Analyzer (Beckman Coulter Inc., Ireland). The plasma biochemical parameters [TG, TC, glucose, AST, ALT, creatinine, and blood urea nitrogen (BUN)] were determined by A15 Analyzer Automatic Clinical Chemistry (BioSystems S.A., Spain).

After blood collection, the rats from both acute and sub-chronic toxicity studies were perfused through the left ventricle of the heart with 200-250 ml of heparinized saline at a flow rate of 40 ml/min by using peristaltic pump (model SP 311, VELP Scientifica, Europe). Immediately after starting the pump, the right atrium was cut to allow an escape route for the blood and perfusion fluid. After the atrium effluent was clear, the organs (heart, liver, kidneys, spleen, and testes) were quickly dissected, removed, carefully examined, and weighted individually. Each organ to

body weight ratio (relative organ weight: ROW) was calculated as (weight of organ (g) ÷ body weight of rat (g) on the day of sacrifice) × 100% (Abdullab et al., 2009; Aniagu et al., 2005). After that, kidneys and liver were fixed in 10% buffered formalin solution for 7 days and processed for histopathological examination.

3.3.7 Tissue paraffin embedding process

Briefly, the tissues were fixed in 10% buffered formalin solution for 1 h, dehydrated two times with serial diluted alcohol (70%, 80%, 95%, and 100%) for 1 h each. After that, the tissues were cleared two times with xylene (Carlo Erba) for 1 h each and then infiltrated two times with paraffin at 56-58 °C for 1 h each. The infiltrated tissues were then embedded into paraffin block using Histocentre 2 tissue embedding system (Shandon, England) and sectioned at 5 µm using rotary microtome (Microm HM 355S, Microm GmbH., Germany). The sections were floated with DI water containing gelatin in tissue floating bath (Medex Nagel GmbH., Germany) at 45 °C and then mounted onto slides. After that, the sections were dried by using hotplate (Medex Nagel) at 45 °C for 24 h. Sections were finally stained with haematoxylin and eosin for microscopic examination.

3.3.8 Histological staining with haematoxylin and eosin

After the sections were dried, the sections were deparaffinized with xylene three times with xylene (Carlo Erba) for 5 min each, hydrated with serial diluted alcohol at concentration 100% (3 times), 95% (2 times), and 70% for 5 min each, respectively. The sections were washed in running tap water for 5 min, washed with DI water for 7 min. After that, the sections were stained nucleus with

haematoxylin (Bio-Optica) for 15 min, washed in running tap water for 5 min, washed with 95% alcohol for 10 sec and then stained cytoplasm with eosin working solution for 10 min. The sections were dehydrated with serial diluted alcohol at concentration 95% (3 times), 100% (2 times) for 5 min each followed by xylene for 5 min each (2 times). Slides were finally coverslipped using DPX mountant (BDH).

3.3.9 Statistics

Results were expressed as mean \pm standard error of mean (S.E.M.). Data were analyzed using program SigmaStat Version 3.5 (Systat Software, Inc., USA.). Weight gain and food consumption were analyzed using two way repeated measures ANOVA while relative organ weight (ROW), serum and plasma biochemical parameters, and hematological parameters were analyzed using one-way ANOVA. Post hoc testing was performed for inter-group comparisons. *P*-values less than 0.05 ($P < 0.05$) were considered statistically significant.

3.4 Results

3.4.1 Acute toxicity study

The effects of single oral administration of the rambutan rind extract in male Wistar rats were shown in Table 3.1. In this study, an oral administration of the rambutan rind extract at 1000, 2000, 3000, and 4,000 mg/kg did not cause mortality in the rats. Mortality occurred at 5000 mg/kg of the rambutan rind extract with the mortality rate of 12.5% (one death out of eight rats). The rambutan rind extract did not result in signs of toxic effect at the dose up to 3000 mg/kg. Some toxic effects were found when rats received more than 4000 mg/kg of the rambutan rind extract. The

main behavioral signs of toxicity observed were hypo-activity and convulsion. For this route the no-observed-adverse-effect level (NOAEL) for the oral dose of the rambutan rind extract was 3000 mg/kg and the lowest-observed-adverse-effect level (LOAEL) was 4000 mg/kg. Therefore, the acute toxicity data indicated that the evaluated oral LD₅₀ of the rambutan rind extract was then greater than 5000 mg/kg in male Wistar rats.

The body weight gain of male Wistar rats on day 7th and day 14th after single oral administration of the rambutan rind extract was significantly decreased in all groups treated with the rambutan rind extract when compared with the control group (Table 3.2). Average food consumption on week 1 was significantly decreased in the group treated with 1000, 3000, and 4000 mg/kg of the rambutan rind extract compared to the control while average food consumption on week 2 was significantly decreased in the group treated with 1000 and 4000 mg/kg of the rambutan rind extract compared to the control (Table 3.3).

Relative organ weights of the spleen, kidneys, and testes in male Wistar rats treated with single doses of the rambutan rind extract 5000 mg/kg up to were not different from the control group. Relative organ weight of the heart was significantly decreased in the groups treated with 1000, 2000, and 5000 mg/kg of the rambutan rind extract. Significant decreases in mean relative organ weight of liver occurred in all groups treated with the rambutan rind extract compared to the control (Table 3.4)

Serum biochemical parameters of male Wistar rats on day 14 after single oral administration of the rambutan rind extract were shown in Table 3.5. There were no significant differences in serum TG, TC, AST, and ALT levels while serum

glucose was significantly decreased in the groups treated with 3000 and 4000 mg/kg of the rambutan rind extract compared to the control.

For histopathological findings, no morphological changes in the kidneys were found in all rats treated with the rambutan rind extract. Fatty changes in liver specimens were shown in the control group (2 out of 8 rats), the rambutan rind extract at the doses of 1000 mg/kg (2 out of 8 rats), 2000 mg/kg (2 out of 8 rats), 3000 mg/kg (3 out of 8 rats), 4000 mg/kg (4 out of 8 rats), and 5000 mg/kg (4 out of 7 rats), respectively (Figure 3.1). However, the incidences and severities of these changes did not differ between the control group and the rats treated groups with the rambutan rind extract.

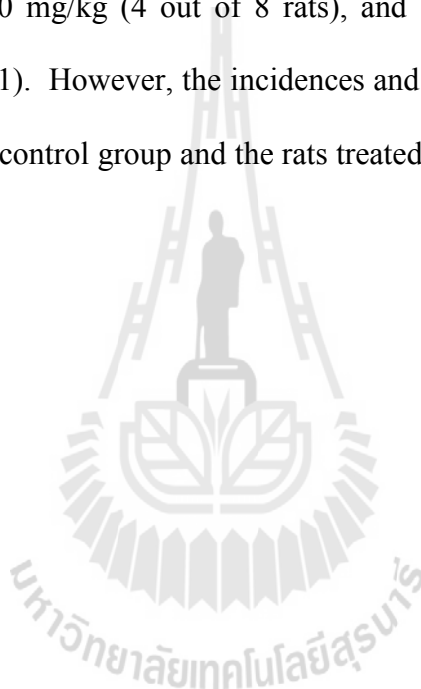


Table 3.1 Acute toxicity effects of single oral administration of the rambutan rind extract in male Wistar rats.

Group	D/T	Effects	
		Mortality latency (h)	Signs of toxicity
Control	0/8	-	None
1000 mg/kg rambutan rind extract	0/8	-	None
2000 mg/kg rambutan rind extract	0/8	-	None
3000 mg/kg rambutan rind extract	0/8	-	None
4000 mg/kg rambutan rind extract	0/8	-	hypo-activity (3/8)
5000 mg/kg rambutan rind extract	1/8	< 1	hypo-activity (4/8), convulsion (1/8)

D/T: number of deaths/total number treated; mortality latency: time to death (in hours) following the orally administration;

none: no toxic signs during the observation period.

* Convulsion was found in the rat that died later.

Table 3.2 Weight gain on day 7 and day 14 after single oral administration of the rambutan rind extract.

Group	Weight gain (g) from day 0	
	On day 7	On day 14
Control	25.00 ± 2.86	38.75 ± 3.75
1000 mg/kg rambutan rind extract	13.13 ± 3.02*	24.38 ± 2.76*
2000 mg/kg rambutan rind extract	11.88 ± 2.46*	24.38 ± 3.70*
3000 mg/kg rambutan rind extract	11.43 ± 2.82*	20.00 ± 2.86*
4000 mg/kg rambutan rind extract	11.67 ± 4.40*	17.50 ± 5.62*
5000 mg/kg rambutan rind extract	11.43 ± 2.82*	28.57 ± 4.96*

Values are expressed as mean ± S.E.M. (n=8 per group, except for rambutan rind extract 5000 mg/kg group; n=7)

*Significantly different from control, $P < 0.05$ (Repeated two way ANOVA; Duncan's Method).

Table 3.3 Average food consumption on week 1 and week 2 after single oral administration of the rambutan rind extract.

Group	Average food consumption (g/rat/day)	
	Week 1	Week 2
Control	17.15 ± 0.32	16.21 ± 0.37
1000 mg/kg rambutan rind extract	13.67 ± 0.81*	13.62 ± 0.42*
2000 mg/kg rambutan rind extract	15.96 ± 0.44	15.15 ± 0.22
3000 mg/kg rambutan rind extract	15.04 ± 0.51*	14.76 ± 0.25
4000 mg/kg rambutan rind extract	13.19 ± 0.88*	13.62 ± 0.41*
5000 mg/kg rambutan rind extract	15.39 ± 1.29	15.17 ± 1.32

Values are expressed as mean ± S.E.M. (n=8 per group, except for rambutan rind extract 5000 mg/kg group; n=7)

*Significantly different from control, $P < 0.05$ (Repeated two way ANOVA; Duncan's Method).

Table 3.4 Effects of single oral administration of the rambutan rind extract on the relative organ weight (ROW) of male Wistar rats.

Group	ROW (g per 100 g body weight)					
	Heart	Liver	Kidneys	Spleen	Testes	
Control	0.35 ± 0.01	3.44 ± 0.07	0.64 ± 0.01	0.22 ± 0.01	1.18 ± 0.03	
1000 mg/kg rambutan rind extract	0.32 ± 0.01*	3.07 ± 0.09*	0.64 ± 0.01	0.23 ± 0.02	1.15 ± 0.09	
2000 mg/kg rambutan rind extract	0.31 ± 0.01*	3.13 ± 0.12*	0.60 ± 0.01	0.23 ± 0.01	1.18 ± 0.04	
3000 mg/kg rambutan rind extract	0.33 ± 0.01	3.02 ± 0.07*	0.61 ± 0.01	0.23 ± 0.01	1.23 ± 0.05	
4000 mg/kg rambutan rind extract	0.33 ± 0.01	2.96 ± 0.10*	0.62 ± 0.02	0.24 ± 0.02	1.24 ± 0.04	
5000 mg/kg rambutan rind extract	0.32 ± 0.01*	3.06 ± 0.07*	0.61 ± 0.01	0.22 ± 0.01	1.07 ± 0.03	

Values are expressed as mean ± S.E.M.

*Significantly different from control, $P < 0.05$ (One way ANOVA; Duncan's Method).

Table 3.5 Serum biochemical parameters on day 14 after single oral administration of the rambutan rind extract in male

Wistar rats.

Parameters	Rambutan rind extract (mg/kg)					
	control (n = 8)	1000 (n = 8)	2000 (n = 8)	3000 (n = 8)	4000 (n = 8)	5000 (n = 7)
Glucose (mg/dl)	194.10 ± 8.84	189.64 ± 7.18	177.86 ± 11.73	147.500 ± 11.73*	137.86 ± 7.25*	182.14 ± 6.11
TG (mg/dl)	68.86 ± 9.87	67.81 ± 8.04	82.86 ± 8.89	52.86 ± 5.08	50.313 ± 6.34	63.93 ± 5.30
TC (mg/dl)	76.00 ± 3.87	75.63 ± 2.56	74.69 ± 3.96	70.42 ± 2.49	65.94 ± 2.42	78.21 ± 5.52
AST (U/l)	321.50 ± 21.72	275.00 ± 29.18	211.00 ± 17.54	255.50 ± 32.92	320.00 ± 37.69	246.00 ± 46.83
ALT (U/l)	51.67 ± 15.85	42.14 ± 4.35	28.57 ± 6.77	47.08 ± 12.99	47.08 ± 8.59	33.21 ± 5.67

Values are expressed as mean ± S.E.M.

*Significantly different from control, $P < 0.05$ (One way ANOVA; Duncan's Method).

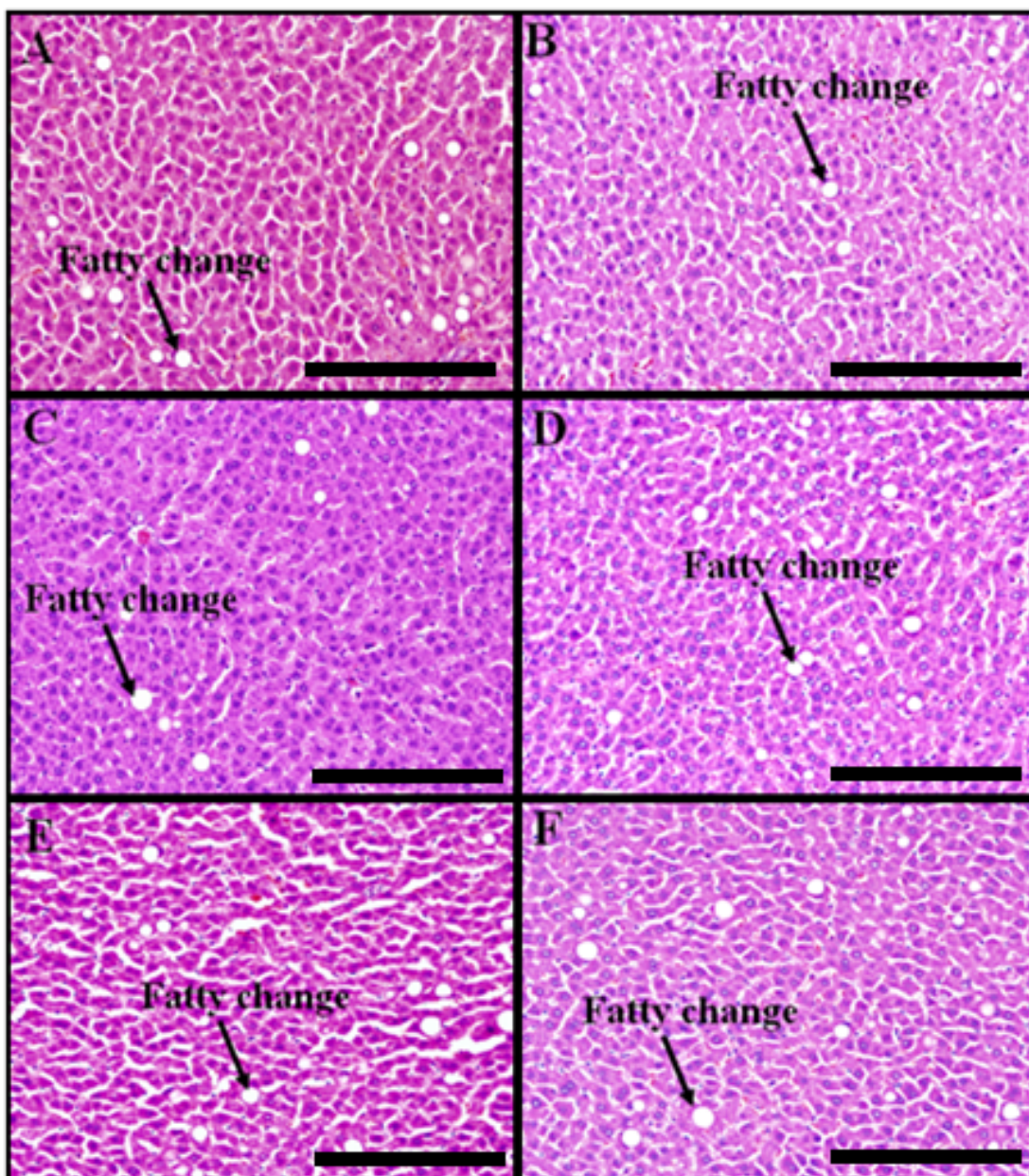


Figure 3.1 Liver sections were stained with haematoxylin and eosin showing the effect of the rambutan rind extract on acute toxicity study of male Wistar rat. (A) control, (B) 1000 mg/kg, (C) 2000 mg/kg, (D) 3000 mg/kg, (E) 4000 mg/kg, and (F) 5000 mg/kg of the rambutan rind extract. The arrow showed fatty change. Scale bars = 100 μm.

3.4.2 Sub-chronic toxicity study

Toxicity effects of sub-chronic oral administration of the rambutan rind extract in male Wistar rats were shown in Table 3.6. No mortality and toxic signs were shown in the rats daily orally administered with 500 and 1000 mg/kg of the rambutan rind extract for 30 days. Mortality (12.5% or one death out of eight rats) together with signs of toxicity (hypo-activity) was found at 2000 mg/kg of the rambutan rind extract. Therefore, the NOAEL for sub-chronic oral administration of the rambutan rind extract was 1000 mg/kg and the LOAEL was 2000 mg/kg.

The body weight gain and average food consumption were significantly decreased in male Wistar rats in all studied doses of the rambutan rind extract when compared to the control group throughout 30 days of the study (Tables 3.7 and 3.8). Relative organ weights of the liver, kidneys, and spleen of the rambutan rind extract treated groups were not different from the control group. Relative organ weights of testes in 1000 mg/kg of the rambutan rind extract group and of the heart in 2000 mg/kg of the rambutan rind extract group were significantly higher than the control (Table 3.9).

The plasma biochemical parameter levels of glucose, TC, AST, ALT, and creatinine in rats administered with the rambutan rind extract were not different from the control. Plasma TG and BUN were significantly decreased in rats treated with 500, 1000, and 2000 mg/kg of the rambutan rind extract compared to the control (Table 3.10). The hematological parameter levels of RBC, Hb, Hct, MCV, MCH, RDW, WBC, monocyte, and basophil in rats administered with the rambutan rind extract were not different from the control. Significant decrease in the MCHC level was found at dose of 500 mg/kg of the rambutan rind extract compared to the control.

Significant decrease in the lymphocyte level was found at dose of 2000 mg/kg of the rambutan rind extract compared to the control. The rambutan rind extract caused significant increases in the platelet count at doses of 1000 and 2000 mg/kg, neutrophil at a dose of 2000 mg/kg and eosinophil at doses of 500 and 1000 mg/kg when compared to the control (Table 3.11).

Histopathological finding, no morphological changes in the kidneys and the liver (Figure 3.2) was found in all rats treated with the rambutan rind extract.



Table 3.6 Toxicity effects of sub-chronic oral administration of the rambutan rind extract in male Wistar rats.

Group	Effects		
	D/T	Mortality latency (day)	Signs of toxicity
Control	0/8	-	None
500 mg/kg rambutan rind extract	0/8	-	None
1000 mg/kg rambutan rind extract	0/8	-	None
2000 mg/kg rambutan rind extract	1/8	6	hypo-activity (4/8)

D/T: number of deaths/total number treated; mortality latency: time to death (in day) following the orally administration; none: no toxic signs during the observation period.

Table 3.7 Effects of sub-chronic oral administration of the rambutan rind extract on weight gain in male Wistar rats.

Group	Weight gain (g) from day 0			
	On day 7	On day 14	On day 21	On day 28
Control	26.25 ± 6.04	46.25 ± 7.81	62.50 ± 12.08	83.75 ± 11.42
500 mg/kg rambutan rind extract	15.00 ± 5.71	18.75 ± 6.83*	27.50 ± 9.20*	37.50 ± 8.02*
1000 mg/kg rambutan rind extract	-1.25 ± 7.68*	12.50 ± 10.25*	25.00 ± 9.48*	37.50 ± 9.42*
2000 mg/kg rambutan rind extract	-2.86 ± 6.55*	11.43 ± 7.24*	21.43 ± 7.97*	27.14 ± 11.72*

Values are expressed as mean ± S.E.M. (n=8 per group, except for the 2000 mg/kg rambutan rind extract group; n=7)

*Significantly different from control, $P < 0.05$ (Repeated two way ANOVA; Duncan's Method).

Table 3.8 Effects of sub-chronic oral administration of the rambutan rind extract on average food consumption in male Wistar rats.

Group	Average food consumption (g/rat/day)			
	Week 1	Week 2	Week 3	Week 4
Control	18.27 ± 0.83	19.72 ± 1.46	17.98 ± 1.03	19.12 ± 0.56
500 mg/kg rambutan rind extract	16.49 ± 1.27	15.61 ± 0.73*	14.98 ± 0.43*	12.67 ± 0.71*
1000 mg/kg rambutan rind extract	13.18 ± 1.09*	15.58 ± 1.03*	15.48 ± 0.84*	14.75 ± 0.61*
2000 mg/kg rambutan rind extract	12.88 ± 0.44*	13.88 ± 2.22*	12.79 ± 1.99*	11.24 ± 1.75*

Values are expressed as mean ± S.E.M. (n=8 per group, except for the 2000 mg/kg rambutan rind extract group; n=7)

*Significantly different from control, $P < 0.05$. (Repeated two way ANOVA; Duncan's Method).

Table 3.9 Effects of sub-chronic oral administration of the rambutan rind extract on the relative organ weight (ROW) of male

Wistar rats.

Group	ROW (g per 100 g body weight)					
	Heart	Liver	Kidneys	Spleen	Testes	
Control	0.45 ± 0.03	4.13 ± 0.39	0.80 ± 0.05	0.25 ± 0.02	1.05 ± 0.03	
500 mg/kg rambutan rind extract	0.45 ± 0.01	3.24 ± 0.09	0.75 ± 0.02	0.23 ± 0.01	1.19 ± 0.06	
1000 mg/kg rambutan rind extract	0.49 ± 0.03	4.11 ± 0.40	0.83 ± 0.06	0.27 ± 0.03	1.24 ± 0.05*	
2000 mg/kg rambutan rind extract	0.55 ± 0.03*	3.70 ± 0.36	0.85 ± 0.06	0.26 ± 0.02	1.12 ± 0.13	

Values are expressed as mean ± S.E.M.

*Significantly different from control, $P < 0.05$ (One way ANOVA; Duncan's Method).

Table 3.10 Effects of sub-chronic oral administration of the rambutan rind extract on plasma biochemical parameters of male

Wistar rats.

Parameters	Rambutan rind extract (mg/kg)			
	control (n = 8)	500 (n = 8)	1000 (n = 8)	2000 (n = 8)
Glucose (mg%)	170.75 ± 8.45	157.25 ± 8.79	168.50 ± 5.69	153.00 ± 14.70
TG (mg/dl)	146.88 ± 10.00	70.00 ± 4.74*	78.75 ± 4.60*	52.86 ± 6.86*
TC (mg/dl)	59.38 ± 3.42	67.50 ± 2.86	63.13 ± 3.02	67.86 ± 2.31
AST (U/l)	222.67 ± 38.90	272.40 ± 6.00	227.86 ± 12.08	191.40 ± 27.11
ALT (U/l)	44.00 ± 7.33	45.43 ± 6.67	39.88 ± 3.81	40.86 ± 9.60
Creatinin (mg%)	0.35 ± 0.02	0.41 ± 0.02	0.40 ± 0.02	0.39 ± 0.02
BUN (mg/dl)	20.00 ± 0.79	16.98 ± 0.92*	16.70 ± 0.94*	14.70 ± 0.89*

Values are expressed as mean ± S.E.M.

*Significantly different from control, $P < 0.05$ (One way ANOVA; Duncan's Method).

Table 3.11 Effects of sub-chronic oral administration of the rambutan rind extract on hematological parameters of male

Wistar rats.

Parameters	Rambutan rind extract (mg/kg)			
	control (n = 8)	500 (n = 8)	1000 (n = 8)	2000 (n = 8)
RBC ($10^6/\mu\text{L}$)	8.04 \pm 0.30	8.23 \pm 0.11	8.50 \pm 0.09	8.08 \pm 0.11
Hb (g/dL)	15.63 \pm 0.60	16.13 \pm 0.32	16.63 \pm 0.20	15.94 \pm 0.04
Hct (%)	44.75 \pm 1.59	46.63 \pm 0.73	47.13 \pm 0.62	45.14 \pm 0.55
MCV (fL)	55.40 \pm 0.28	56.58 \pm 0.61	55.34 \pm 0.47	55.60 \pm 0.44
MCH (pg/cell)	19.58 \pm 0.15	19.63 \pm 0.25	19.56 \pm 0.16	19.79 \pm 0.23
MCHC (g/dL)	35.39 \pm 0.21	34.73 \pm 0.15*	35.26 \pm 0.09	35.59 \pm 0.26
RDW (%)	11.98 \pm 0.23	12.11 \pm 0.35	12.08 \pm 0.20	12.50 \pm 0.38
Platelet ($10^3/\mu\text{L}$)	613.00 \pm 43.83	688.13 \pm 30.82	762.29 \pm 27.17*	765.50 \pm 43.53*

Values are expressed as mean \pm S.E.M. *Significantly different from control, $P < 0.05$ (One way ANOVA; Duncan's Method).

Table 3.11 Effects of sub-chronic oral administration of the rambutan rind extract on hematological parameters of male

Wistar rats (Cont.).

Parameters	Rambutan rind extract (mg/kg)		
	control (n = 8)	500 (n = 8)	1000 (n = 8)
WBC ($10^3/\mu\text{L}$)	2.05 ± 0.17	2.00 ± 0.37	1.61 ± 0.16
Neutrophils (%)	8.29 ± 1.65	6.57 ± 1.02	13.71 ± 1.39*
Lymphocyte (%)	86.25 ± 1.52	85.63 ± 2.36	80.88 ± 1.68*
Monocyte (%)	3.25 ± 1.01	4.63 ± 1.28	2.25 ± 1.03
Eosinophill (%)	0.38 ± 0.74	1.50 ± 0.29*	1.38 ± 0.53*
Basophill (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
			2.14 ± 0.52
			24.67 ± 3.78*
			69.71 ± 5.91*
			1.67 ± 0.41
			0.25 ± 0.17
			0.00 ± 0.00

Values are expressed as mean ± S.E.M. *Significantly different from control, $P < 0.05$ (One way ANOVA; Duncan's Method).

RBC = red blood cells; Hb = hemoglobin; Hct = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular

Hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red blood cell distribution width;

WBC = white blood cells

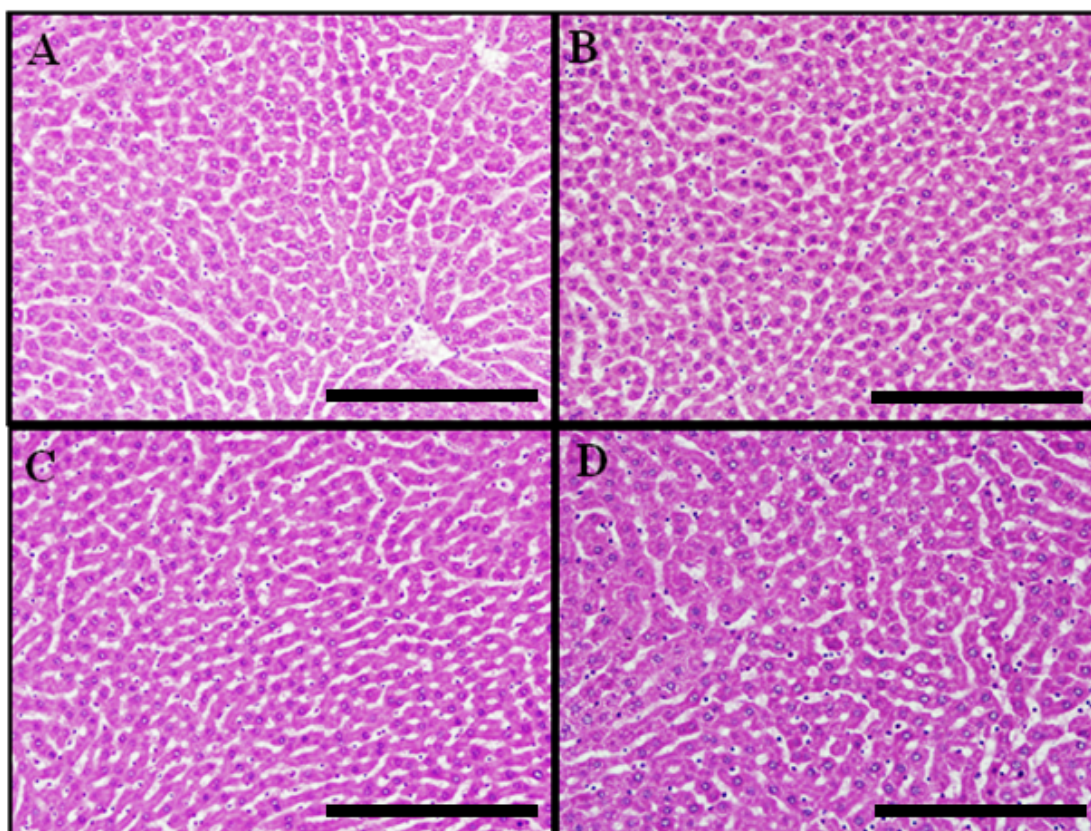


Figure 3.2 Liver sections were stained with haematoxylin and eosin showing the effect of the rambutan rind extract on sub-chronic toxicity study of male Wistar rat. (A) control, (B) 500 mg/kg, (C) 1000 mg/kg, and (D) 2000 mg/kg of the rambutan rind extract. Scale bars = 100 μ m.

3.5 Discussion and conclusion

The use of medicine plants as alternative treatments have been increasing worldwide. Although, medicine plants have biological activities that are beneficial to human but the potential toxicity of these bioactive substances have not been established (Yam et al., 2009). Thus, the safety and efficacy of medicine plants must be studied thoroughly to maximize their benefits for human. The rambutan rind extract

has long been used in traditional medicine (Palanisamy et al., 2008). Previous studies of the rambutan rind extract have exhibited their biological activities *in vitro* (Palanisamy et al., 2008, 2011; Thitilertdecha et al., 2008; Nawawi et al., 1999), but there was no study on the toxicity of the rambutan rind extract. In the present acute toxicity study, rats orally administered up to the dose of 3000 mg/kg of the rambutan rind extract did not exhibit any signs of adverse effects and the LOAEL was 4000 mg/kg. Rambutan rind extract at the dose of 5000 mg/kg (p.o.) exhibited adverse effects and mortality (12.5%). This study suggested that the evaluated oral LD₅₀ of the rambutan rind extract was then greater than 5000 mg/kg. In the sub-chronic toxicity study, rat orally administered 1000 mg/kg of the rambutan rind extract did not exhibit any signs of adverse effects and the LOAEL was 2000 mg/kg.

The decreases in body weight gain in both acute and sub-chronic toxicity studies of the rambutan rind extract were in line with the decrease in average food consumed by the rats. A decrease in body weight gain and food consumptions may have resulted from behavioral signs of toxicity, stress, or physiological variation in rats such as metabolism (Jaijoy et al., 2011; Rahman and Siddiqui, 2006; Raza et al., 2002; Teo et al., 2002). The rambutan rind extract was significantly decreased the relative organ weight of heart (1000, 2000, and 5000 mg/kg) and liver (1000, 2000, 3000, 4000, and 5000 mg/kg) in the acute toxicity study. In the sub-chronic toxicity study, the relative organ weight of heart and testes were significantly increased in rats treated with 2000 and 1000 mg/kg of the rambutan rind extract. There was no significant difference in the relative organ weight of kidneys and spleen. However, all of the increases or decreases were slight changes and the differences could have been due to the variation in size of internal organs of the animals (Chunlaratthanaphorn

et al., 2007) and the decrease of internal organ weight was simple and sensitive indices of toxicity after exposure to toxic substances (Raza et al., 2002; Tan et al., 2008; Teo et al., 2002). The rambutan rind extract did not induce any toxic effect on the kidneys and the spleen going by this indicator, since relative weights of the organs were not significantly different from control values.

The rambutan rind extract (3000 and 4000 mg/kg, p.o.) significantly decreased serum glucose in the acute toxicity study. A decrease in serum glucose levels in treated rats demonstrated that the rambutan rind extract decrease food consumptions may have resulted from behavioral signs of toxicity, stress, or physiological variation in rats such as metabolism (Jaijoy et al., 2011; Rahman and Siddiqui, 2006; Raza et al., 2002; Teo et al., 2002). Further, the serum glucose levels serves as an index of carbohydrate metabolism in the system (Rahman and Siddiqui, 2006). Sub-chronic administration of the rambutan rind extract to rats had no effect on plasma glucose, TC, AST, ALT, and creatinine levels. Plasma TG and BUN levels were significantly decreased in rats treated with 500, 1000 and 2000 mg/kg of the rambutan rind extract for 30 days, respectively. A reduction in plasma triglyceride levels in the rambutan rind extract treated rats revealed that the rambutan rind extract had the greatest potential for protection against atherosclerosis (its accompanying risk of cardiovascular diseases) and may be correlated with a potentially decrease in plasma glucose levels (without a significant difference), which in turn may depress the mobilization of fat (Mansi, Amneh, and Nasr, 2007). The levels of BUN were minor changes and still within the normal range (5.00-25.00 mg/dl) (Angkhasirisap et al., 2002). Since the levels of creatinine and BUN were the index of kidney function, it suggests that the rambutan rind extract does not induce toxicity to the kidneys. These

observations were further confirmed by the histopathological assessment of the kidneys.

Apart from that, histological analysis was done to further confirm the alteration in cell structure of the liver. In the present study, acute and sub-chronic oral administration of the rambutan rind extract did not adversely affect the morphology of livers which agrees with the present results of AST and ALT levels and relative organ weight of the liver. Thus, the rambutan rind extract has no potential toxicity to liver.

Hematological parameter levels, such as RBC, Hb, Hct, MCV, MCH, RDW, WBC, monocyte, and basophil in rats orally administered with the rambutan rind extract for 30 days were not different from the control group. When compared to control, significant increases in platelet count was found at doses of 1000 and 2000 mg/kg, neutrophil was found at dose of 2000 mg/kg and eosinophil was found at the doses of 500 and 1000 mg/kg of the rambutan rind extract. Significant decreases in MCHC and lymphocyte were found in rats administered with the rambutan rind extract (500 and 1000 mg/kg, respectively) compared to the control. The rambutan rind extract caused decreases in lymphocyte and MCHC and increases in neutrophil, eosinophil, and platelet count, which might be due to the effect of stress condition in treated rats. Stress is defined as a state of threatened homeostasis, following exposure to extrinsic or intrinsic adverse forces (Chrousos, 1992). Stress has affected various aspects of the immune function. It has been indicated that the innate non-specific immune response as well as the specific immune response may be altered by stress (Rabinowitz et al., 2002). Thus, stress in the rambutan rind extract treated rats may activate of animal defense mechanisms and immune system.

In conclusion, this is the first report of acute and sub-chronic toxicity studies of the rambutan rind extract. The present studies provide useful information on the acute and sub-chronic toxicity profiles of oral use of the hydroethanolic extract of the fruit rind of rambutan. The present findings of acute and sub-chronic toxicity in rats will help for future clinical studies of the medicinal safety and *in vivo* experimental studies of the pharmacological potential of medicine plant. Further study may focus on chronic toxicity study of the rambutan rind extract in order to evaluate its long term effects.

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CHAPTER IV

EFFECTS OF THE RAMBUTAN RIND EXTRACT ON LIPASE, ALPHA-AMYLASE AND ALPHA- GLUCOSIDASE ACTIVITIES *IN VITRO* AND PLASMA BIOCHEMICAL PARAMETERS IN LIPID EMULSION- INDUCED HYPERLIPIDEMIA RATS

4.1 Abstract

Obesity has become a major health problem worldwide. One anti-obesity therapeutic approach is to reduce gastrointestinal fatty acids production and absorption through the inhibition of fat-digesting enzyme such as pancreatic lipase enzyme. This study investigated the effects of the rambutan rind extract on lipase, alpha-amylase, and alpha-glucosidase activities *in vitro*, and biochemical parameters after the oral administration of lipid emulsion in rats. For *in vitro* experiments, the percentage of yield, total phenolic content, antioxidant activity determined using a DPPH scavenging assay (50% inhibition concentration or IC₅₀), and total anthocyanin of the rambutan rind extract were determined. Assay for the inhibitory effects of the rambutan rind extract, tannin, and orlistat on pancreatic lipase, alpha-amylase, and alpha-glucosidase activities were determined. For *in vivo* experiments, rats (n=40) were orally administered of the rambutan rind extract (1000 and 2000 mg/ 4 ml/kg), tannin (1000 mg/ 4 ml/kg), and orlistat (45 mg/ 4 ml/kg) dissolved in lipid emulsion

while the control group received lipid emulsion alone (4 ml/kg). Blood samples were collected from the tail vein at 0, 1, 2, 3, and 4 h after the oral administration of each treatment. The plasma triglyceride (TG), total cholesterol (TC), glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were measured. The results showed that the rind of rambutan extracted by 85% ethanol had a yield of 18.05%. The rambutan rind extract contained total phenolic content (416.18 ± 0.01 mg gallic acid/g dry extract), total anthocyanin content (14.90 ± 0.8 μ g/g dry extract), with IC_{50} value of 0.288 ± 0.04 mg/ml extract. *In vitro* study, the rambutan rind extract, tannin, and orlistat showed potent inhibitory effects on pancreatic lipase, alpha-amylase, and alpha-glucosidase activities. Maximum percent inhibition of pancreatic lipase (72.68%), alpha-amylase (97.30%), and alpha-glucosidase (95.79%) activities were demonstrated at 80, 2.5, and 2.5 mg/ml of the rambutan rind extract, respectively. *In vivo* study, the rambutan rind extract (1000 and 2000 mg/kg, p.o.), tannin (1000 mg/kg, p.o.), and orlistat (45 mg/kg, p.o.) significantly suppressed the increased plasma level of TG caused by lipid emulsion administration. The present findings suggested that the rambutan rind extract and tannin displayed beneficial effects in the treatment of obesity, possibly by lipase, alpha-amylase, and alpha-glucosidase activities inhibition. In addition, the rambutan rind extract and tannin could suppress the plasma levels of TG in lipid emulsion-induced hyperlipidemia rats. Thus, the phenolic compounds including tannin found in the rambutan rind extract may play a key role in lipase, alpha-amylase, and alpha-glucosidase inhibitory activities, and hypotriglyceridemic effect.

4.2 Introduction

Obesity is characterized by the accumulation of excess fat in adipose tissues and results of changes in lifestyles, especially in eating habits (Lois and Kumar, 2009). Approximately 2.3 billion adults were expected to be overweight and approximately 700 million people were expected to be obese in 2015 (WHO, 2011). Obesity is one of the independent risk factors for serious health problems associated with chronic diseases such as hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, congestive heart failure, stroke, osteoarthritis, pulmonary dysfunction (obstructive sleep apnea and hypoventilation syndrome), certain types of cancer (breast, endometrium, colon, prostate), gastrointestinal diseases (fatty liver, cirrhosis, gastroesophageal reflux, gallstones), menstrual abnormalities, increase in surgical risk, impaired fertility, increased pregnancy risks, brain disease (Alzheimer's disease), anxiety, depression, and suicide (Devlin et al., 2000; Kopelman, 2000; Roth et al., 2004; Wickelgren, 1998). The anti-obesity drugs (orlistat and sibutramine) are currently available on the market (Chaput et al., 2007). Orlistat is an intestinal lipase inhibitor that decreases the digestibility of dietary fat and increase fat excretion, while sibutramine is a serotonin and noradrenaline uptake inhibitor that reduces appetite and increases energy expenditure (Bray, 2000; Chaput et al., 2007). Both drugs have side effects such as hypertension, dry mouth, constipation, headache, and insomnia (Bray, 2001; Davidson et al., 1999). The development of inhibitors of nutrient digestion and absorption, which reduce energy intake through gastrointestinal mechanisms without altering any central mechanisms, is one of the most important strategies in the treatment of obesity (Birari and Bhutani, 2007). Dietary fat is not directly absorbed by the intestine unless the fat has been subjected to the action of pancreatic lipase (Yun,

2010). Pancreatic lipase is a key enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerols to monoacylglycerols and fatty acids (Mukherjee, 2003; Shi and Burn, 2004; Thomson et al., 1997). Therefore, pancreatic lipase is one of the most widely studied mechanisms for determining natural products potential efficacy as anti-obesity agents (Birari and Bhutani, 2007). A wide variety of plant extracts possess pancreatic lipase inhibitory effects, including *Glycyrrhiza glabra* roots (Birari et al., 2011), *Theobroma cacao* (Gu et al., 2011), *Panax japonicus* rhizomes (Han et al., 2005), *Cassia nomame* (Hatano et al., 1997), *Actinidia arguta* roots (Jang et al., 2008), green tea leaves (Juhel et al., 2000), *Dioscorea nipponica* Makino (Kwon et al., 2003), *Panax quinquefolium* leaves (Liu et al., 2008), *Salacia reticulata* (Yoshikawa et al., 2002), berries (McDougall, Kulkarni, and Stewart, 2009), *Cuminum cyminum* (Milan et al., 2008), grape seed (Moreno et al., 2003), peanut shell (Moreno et al., 2006), *Mangifera indica* (Moreno et al., 2006), oolong tea (Nakai et al., 2005), *Nelumbo nucifera* (Ono et al., 2006), apple (Sugiyama et al., 2007), *Glycyrrhiza uralensis* (Won et al., 2007), *Cassia minosoides* (Yamamoto et al., 2000), and Chinese herbal medicines (Zheng et al., 2010).

Obese individuals develop resistance to the cellular action of insulin, characterized by an impaired ability of insulin to inhibit glucose output from the liver and to promote glucose uptake in fat and muscle (Saltiel and Kahn, 2001; Hribal et al., 2002). Insulin resistance is a key etiological factor for type 2 diabetes mellitus (Qatanani and Lazar, 2007). Diabetes mellitus is a metabolic disorder of multiple etiologies that is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolisms resulting from defects in insulin secretion, insulin action, or both. The diabetes mellitus is primarily classified into insulin-

dependent diabetes mellitus (type 1 diabetes) and non- insulin-dependent diabetes mellitus (type 2 diabetes) (Andrade-Cetto, Becerra-Jiménez, and Cárdenas-Vázquez, 2008). The prevalence of type 2 diabetes is increasing globally (Zimmet, Alberti, and Shaw, 2001). Post-prandial hyperglycemia plays an important role in the development of type 2 diabetes (Baron, 1998). Alpha-amylase and alpha-glucosidase are the key enzymes involved in the metabolism of carbohydrates. Alpha-amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, which are ultimately converted into monosaccharides by alpha-glucosidase before being absorbed into the intestinal epithelium and entering blood circulation (Shinde et al., 2008). Therefore, one of the therapeutic approaches for decreasing post-prandial hyperglycemia is to prevent absorption of glucose by the inhibition of carbohydrate-hydrolyzing enzymes, such as alpha-amylase and alpha-glucosidase (Kim, Wang, and Rhee, 2004; Shobana, Sreerama, and Malleshi, 2009). Many available synthetic drugs such as acarbose, voglibose, and miglitol are widely used as inhibitors of these enzymes in patients with type 2 diabetes (Luo et al., 2001; Wehmeier and Piepersberg, 2004). However, these inhibitors are reported to cause several side effects, such as liver disorders, flatulence, abdominal pain, renal tumours, and diarrhea (Fujisawa et al., 2005; Hiroyuki, Tomohide, and Kazunori, 2001; Shobana et al., 2009). Therefore, it becomes necessary to identify the alpha-amylase and alpha-glucosidase inhibitors from natural sources that have lesser side-effects. Effects of plant extracts on alpha-amylase and alpha-glucosidase inhibitory activities have been demonstrated by many studies, such as soybeans (Ademiluyi and Oboh, 2011), *Bergenia ciliata* rhizomes (Bhandari et al., 2008), *Terminalia chenula* Retz. Fruits (Gao et al., 2007), *Theobroma cacao* (Gu et al., 2011), *Lagerstroemia speciosa* leaves (Hou et al., 2009), sorghum,

foxtail millet, and proso millet (Kim, Hyun, and Kim, 2011), pine (Kim et al., 2005), *Grateloupia elliptica* (Kim et al., 2008), *Ecklonia cava* (Lee et al., 2010), *Carpesium abrotanoides* (Mayur et al., 2010), *Tournefortia hartwegiana* (Ortiz-Andrade et al., 2007), *Mormodica balsamina* L., *Senna italica* Mill., *Cassia abbreviata* Oliv., *Tinospora fragosa*, *Waltheria indica* L., and *Gymnosporia buxifolia* (Shai et al., 2010), *Syzygium cumini* seed kernel (Shinde et al., 2008), *Andrographis paniculata* (Subramanian, Asmawi, and Sadikun, 2008), chestnut astringent skin (Tsujita and Takaku, 2008), tea fruits peel (Wang et al., 2011), and soybean leaves (Yuk et al., 2011).

The fruit rind of rambutan was reported to contain a large variety of substances possessing antioxidant activity such as ascorbic acid, anthocyanins, flavonoids, tannins, ellagic acid, corilagin, and geraniin (Khonkarn et al., 2010; Palanisamy et al., 2008; Palanisamy et al., 2011a; Palanisamy et al., 2011b; Thitilertdecha et al., 2008; Thitilertdecha et al., 2010; Wall, 2006; Worngsiri et al., 1993). Apart from its high antioxidant activity, antibacterial (Palanisamy et al., 2008; Thitilertdecha et al., 2008), anti-herpes simplex virus type 1 (Nawawi et al., 1999), and antiproliferative activities (Khonkarn et al., 2010) of the rambutan rind extract have been reported. It has been recently reported an anti-hyperglycemic activity of the total phenolic content and geraniin enriched rambutan rind extract (Palanisamy et al., 2011a; Palanisamy et al., 2011b). Flavonoids and olean-type triterpene oligoglycosides from the rambutan rind extract showed their ability to inhibit fatty acid synthase activity, *in vitro* (Zhao et al., 2011). Therefore, this study investigated the effects of the crude extract from the fruit rind of rambutan on lipase, alpha-amylase, and alpha-glucosidase activities *in vitro*

and plasma biochemical parameters (TG, TC, glucose, AST, and ALT) in lipid emulsion-induced hyperlipidemia rats.

4.3 Materials and methods

4.3.1 Plant material

The rambutan rind extract obtained from stock extract in chapter 3 was used in the experiments conducted in this chapter.

4.3.2 Determination of total phenolic contents

The total phenolic compounds of the rambutan rind extract were measured according to the Folin-Ciocalteu reagent method that was adapted from the method of Minussi et al. (2003). Briefly, the rambutan rind extract was dissolved in 10% ethanol. The reaction mixtures consisted of 200 μ l the rambutan rind extract solution and 4 ml of 2% sodium carbonate (Na_2CO_3 , BDH Ltd., UK) and were mixed. Two minutes later, 200 μ l of Folin-Ciocalteu reagent was added and incubated at room temperature for 30 min. The mixtures were measured absorbance by using a spectrophotometer (CECIL 1011, England) at 750 nm. The total phenolic compounds were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry extract. All determinations were performed in triplicate.

4.3.3 Determination of antioxidant activity

Antioxidants are considered important nutraceuticals on account of many health benefits (Droge, 2002; Lee, Koo, and Min, 2004; Valko et al., 2007). There is increasing interest in natural antioxidant products for use as medicines and food

additives (Willcox et al., 2004). Antioxidants played an important role in lowering oxidative stresses caused by reactive oxygen species (ROS) (Nordberg and Arner, 2001; Sánchez-Moreno, Larrauri, and Saura-Calixto, 1999). ROS including superoxide anion radical, hydroxyl radical and hydrogen peroxide are generated under physiological and pathological stresses in human body (Nordberg and Arner, 2001). Several assays have been frequently used to estimate antioxidant activity in fruits, vegetables and their products and foods for clinical studies including reducing power, chelating iron, and scavenging reactive oxygen species of superoxide anion, hydroxyl radicals, and hydrogen peroxide (H₂O₂) (Zhao et al., 2008). Thus, some methods determine the ability of antioxidants to scavenge free radicals generated in the reaction, such as the TEAC (trolox equivalent antioxidant capacity; Arts et al., 2004), ORAC (oxygen radical absorbance capacity; Ou et al., 2001; Prior et al., 2003), ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1999; Guo et al., 2003), DPPH (1,1-diphenyl-2-picrylhydrazyl; Brand-Williams et al., 1995; Gil et al., 2002), and ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)) (Leong and Shui, 2002; Miller and Rice-Evans, 1997) assays. DPPH is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge (Brand-Williams et al., 1995; Eklund et al., 2005; Gil et al., 2002). DPPH radical has an absorption band at 515 nm which disappears upon reduction by an antiradical compound (Brand-Williams et al., 1995; Gil et al., 2002). Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay (Alma et al., 2003; Karioti et al., 2004; Kordali et al., 2005).

The free radical scavenging activity of the rambutan rind extract was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method that was adapted

from the method of Blois (1958). Briefly, 4 ml of 0.5 mM DPPH in methanol, 800 μ l of the rambutan rind extract at different concentrations and 4 ml of 0.1 M tris HCl buffer (pH 7.0) were added in the test tubes. After that, the mixtures were then mixed and incubated at room temperature for 30 min. The absorbance of the mixtures was measured at 517 nm by a spectrophotometer. All determinations were performed in triplicate. The ability to scavenge the DPPH radical was calculated as percent DPPH scavenging using the following equation:

$$\% \text{ DPPH scavenging} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

where: A_0 is the absorbance of the control and A_1 is the absorbance of the mixture containing extracts. The DPPH radical scavenging activity (%) was plotted against the plant extract concentration (mg/mL) to determine the concentration of extract necessary to decrease DPPH radical scavenging by 50% (IC_{50}). This part of work was conducted by Science and Technology Service Center, Faculty of Science, Chiang Mai University, Thailand.

4.3.4 Determination of total anthocyanin contents

The total anthocyanin contents of the rambutan rind extract were adapted from the method of Lohachoompol, Srzednicki, and Craske (2004). Briefly, the rambutan rind extract was dissolved in 25 ml of methanol:HCl (0.1 M) at a ratio of 85:15. The mixtures were mixed and diluted to the appropriate concentration for measurement of absorbance in the spectrophotometer using 1 cm path length quartz cells at 538 nm. Total anthocyanin contents were expressed as anthocyanidin equivalents in micrograms per gram of dry extract. All determinations were performed

in triplicate. This part of work was conducted by Science and Technology Service Center, Faculty of Science, Chiang Mai University, Thailand.

4.3.5 Materials for enzymes activities

4.3.5.1 Materials for porcine pancreatic lipase activity

Porcine pancreatic lipase enzyme solution

Porcine pancreatic lipase enzyme solution (3.33% w/v) was prepared by adding 0.5 g of porcine pancreatin (Sigma Chemical Co., St. Louis, MO, USA) to 15 ml of ice cold phosphate buffer (50 mM, pH 8.0).

1% (v/v) Triolein in Tween 40 suspension

1% (v/v) Triolein in Tween 40 suspension was prepared by adding 1 ml of glyceryl trioleate (Sigma) to 99 ml Tween 40 (Sigma) and then sonicated by using ultrasonic processor (Sonics Vibra Cell™, model VCX 750, Sonics & Materials Inc., New Town, USA) at 40 W for 3 min.

1 M Phosphate buffer solution (pH 7.4)

1 M Phosphate buffer solution (pH 7.4) was prepared by adding 106.5 g of di-sodium hydrogen phosphate anhydrous (Na_2HPO_4 ; BDH) and 39.7 g of sodium dihydrogen orthophosphate 1-hydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; BDH) to 800 ml of double deionized distilled (DDD) water. This solution was stirred on a hot plate magnetic stirrer (VELP Scientifica, Europe) at 50-60 °C for 1 h, left to be cool down to room temperature and then adjusted to pH 7.4 with 10 M HCl or 10 M NaOH. This solution was adjusted volume to 1000 ml with DDD water in volumetric flask. To make 100 ml of 50 mM phosphate buffer (pH 8.0), 5 ml of 1 M phosphate buffer

solution was added to 95 ml of DDD water. This solution was then adjusted to pH 8.0 with 1 M HCl or 2 M NaOH.

0.05 N Sodium hydroxide solution

Sodium hydroxide solution (0.05 N) was prepared by adding 2 g of sodium hydroxide anhydrous pellets (NaOH, Carlo Erba Reagents, Italy) to 1000 ml of DDD water.

4.3.5.2 Materials for alpha-amylase enzyme activity

Alpha-amylase enzyme solution

Alpha-amylase enzyme solution (0.01% w/v) was prepared by adding 0.01 g of porcine pancreatic alpha-amylase (Type VI-B, 500 KU; Sigma) to 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. This enzyme was prepared in cooled sodium phosphate buffer.

20 mM Sodium phosphate buffer solution

20 mM Sodium phosphate buffer solution (pH 6.9) containing 6.7 mM sodium chloride was prepared by adding 2 ml of 1 M phosphate buffer (pH 7.4) to 98 ml of DDD water and then added 0.0392 g of sodium chloride (Sigma). This solution was then adjusted to pH 6.9 with 1 M HCl or 2 M NaOH.

1% w/v Starch solution

Starch solution (1% w/v) was prepared by boiling and stirring 1 g of potato starch (Carlo Erba) in 60 ml of DDD water for 15 min. This solution was then adjusted volume to 100 ml with DDD water in volumetric flask.

Color reagent solution

Color reagent solution was prepared in DDD water and contained:

5.31 M Potassium Sodium Tartrate Tetrahydrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$; Sigma)

2 M Sodium hydroxide anhydrous pellets (NaOH; Carlo Erba)

96 mM 3,5-Dinitrosalicylic acid (DNS) ($\text{C}_7\text{H}_4\text{N}_2\text{O}_7$; Fluka Analytical, Switzerland)

This solution was prepared by adding 30 g of potassium sodium tartrate tetrahydrate, 1.10 g of DNS, and 1.6 g of NaOH to 70 ml of DDD water. This solution was stirred on a hot plate magnetic stirrer (VELP Scientifica, Europe) at 45-50 °C for 4-5 h, left to be cool down to room temperature and then adjusted volume to 100 ml with DDD water in volumetric flask and kept in amber bottle.

4.3.5.3 Materials for alpha-glucosidase enzyme

Alpha-glucosidase enzyme solution

Alpha-glucosidase enzyme solution (3 U/ml) was prepared by adding 4.89 mg solid of alpha-glucosidase from Baker's yeast (Type I; Sigma) to 34.23 ml of ice-cool DDD water. This enzyme was aliquoted in separate microtubes and stored at -80 °C. To make 5 ml of 0.3 U/ml alpha-glucosidase enzyme solution, 0.5 ml of 3 U/ml alpha-glucosidase enzyme solution was added to 4.5 ml of ice-cold DDD water.

Potassium phosphate buffer (PPB) solution

Potassium phosphate buffer solution (1 M, pH 6.8) was prepared by adding 1.361 g of potassium phosphate monobasic (KH_2PO_4 ; Sigma) in 100 ml of

DDD water. This solution was then adjusted to pH 6.8 with 10 M HCl or 10 M NaOH. To make 100 ml of 0.067 M potassium phosphate buffer (pH 6.8), 6.7 ml of 1 M potassium phosphate buffer (pH 6.8) was added to 93.3 ml of DDD water. This solution was then adjusted to pH 6.8 with 1 M HCl or 2 M NaOH.

10 mM 4-Nitrophenyl-alpha-D-glucopyranoside (PNPG) solution

4-Nitrophenyl-alpha-D-glucopyranoside solution (10 mM) was prepared by adding 0.0046 g of 4-Nitrophenyl-alpha-D-glucopyranoside (Sigma) to 5 ml of DDD water.

3 mM L-glutathione (GSH) solution

L-glutathione solution (3 mM) was prepared by adding 0.151 g of L-glutathione (Sigma) to 5 ml of DDD water.

0.1 M Sodium carbonate solution

Sodium carbonate solution (0.1 M) was prepared by adding 1.06 g of sodium carbonate (Na_2CO_3 ; BDH) to 100 ml of DDD water.

4.3.6 Animals

Male Wistar rats (241.38 ± 10.56 g) were obtained from Institutional Animal Care, SUT. They were maintained under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 20 ± 1 °C) with free access to food and water. This study was conducted under permit of the SUT Animal Care and Use Committee.

4.3.7 Methods for studying enzyme inhibition *in vitro*

4.3.7.1 *In vitro* assay for measuring the inhibition of porcine pancreatic lipase enzyme

Lipase activity was determined by measuring the release rate of oleic acid from triolein using titrimetric method that was adapted from the methods of Huerta et al. (2007), Sharma et al. (2005), and Wrolstad et al. (2005). The amount of oleic acid released during the reaction was determined by direct titration with NaOH to a phenolphthalein end point. Briefly, the rambutan rind, tannin (Fluka), and orlistat (Xenical, Roche Diagnostics GmbH., Germany) were dissolved in DDD water to give concentrations ranging from 5 to 80 mg/ml, 1.25 to 20 mg/ml, and 40 mg/ml, respectively. The reaction mixtures consisted of 2.5 ml of the rambutan rind extract, tannin, or orlistat, 3 ml of 1% Triolein in Tween 40, and 1 ml of 50 mM phosphate buffer (pH 8.0). The mixtures were swirled and incubated in water bath (model WB-22, WiseBath, Korea) at 37 °C for 30 min. After that, 1 ml of 3.33% (w/v) porcine pancreatic lipase enzyme was added to the mixtures. The mixtures were then incubated in water bath at 37 °C for 1 h. The reaction was stopped by adding 3 ml of 95% ethanol solution and 2 to 3 drops of phenolphthalein indicator. Titration was performed with 0.05 N sodium hydroxide solution using burette and pH meter (model C830, Consort, Belgium) until a light pink color appeared. Individual control (A) and blank (a) were conducted in a similar way by replacing sample with 2.5 ml DDD water, with or without porcine pancreatic lipase enzyme, respectively. Individual test 1 (B) and test 2 (b) were prepared as mentioned above with or without porcine pancreatic lipase enzyme, respectively. Blank (a) and test 2 (b) without porcine pancreatic lipase enzyme solution were replaced by 1 ml phosphate buffer (50 mM,

pH 8). Orlistat (Roche) was used as positive control at concentration of 40 mg/ml. All determinations were performed in triplicate. The inhibition percentage of porcine pancreatic lipase enzyme activity was assessed by following formula:

$$\text{Inhibition (\%)} = \left[\frac{(A-a) - (B-b)}{(A-a)} \right] \times 100$$

where: A and B were the volume of NaOH used to reach the titration end point of control and sample with porcine pancreatic lipase enzyme, respectively. a and b were the volume of NaOH used to reach the titration end point of blank and sample without porcine pancreatic lipase enzyme, respectively.

4.3.7.2 *In vitro* assay for measuring the inhibition of alpha-amylase enzyme

Alpha-amylase activity was determined by colorimetric method using starch as the substrate. This method was adapted from method of Nickavar, Abolhasani, and Izadpanah (2008). Briefly, the rambutan rind extract, tannin (Fluka), and a positive control acarbose (Glucobay[®] 50, PT Bayer Indonesia, Jakarta, Indonesia) were dissolved in DDD water to give concentrations ranging from 0.15626 to 80 mg/ml, 1.25 to 40 mg/ml, and 10 mg/ml, respectively. The reaction mixtures consisted of 50 µl the rambutan rind extract, tannin, or acarbose (Bayer) and 50 µl of 0.01% (w/v) alpha-amylase enzyme solution were mixed in microtube and incubated in water bath at 25 °C for 30 min. After that, 100 µl of 1% (w/v) starch solution was added to these mixtures, mixed and incubated in water bath at 25 °C for 3 min. The color reagent solution (100 µl) was added to these mixtures. The mixtures were then mixed and incubated in water bath at 85 °C for 15 min. After that, the mixture was

removed from the water bath and allowed to ice-cooled. Thereafter, 900 μl of DDD water was added to the mixtures. The absorbance of the assay mixture was measured by Benchmark Plus Microplate Spectrophotometer (BIO-RAD Laboratories Ltd., Japan) at 540 nm. Individual test 1 and blank 1 were conducted in a similar way by replacing sample with 50 μl DDD water, with or without alpha-amylase enzyme, respectively. Individual test 2 and blank 2 were prepared as mentioned above with or without alpha-amylase enzyme, respectively. Blank 1 and blank 2 without alpha-amylase enzyme solution were replaced by 50 μl sodium phosphate buffers (20 mM, pH 6.9). Acarbose (Bayer) was used as positive control at concentration of 10 mg/ml (Subramanian et al., 2008). All determinations were performed in triplicate. The inhibition percentage of alpha-amylase enzyme was assessed by following formula:

$$\text{Inhibition (\%)} = \left[\frac{(\Delta A_{\text{Control}} - \Delta A_{\text{Sample}})}{\Delta A_{\text{Control}}} \right] \times 100$$

$$\Delta A_{\text{Control}} = A_{\text{Test1}} - A_{\text{Blank1}}$$

$$\Delta A_{\text{Sample}} = A_{\text{Test2}} - A_{\text{Blank2}}$$

where: A_{Test1} and A_{Test2} were defined as the absorbance of DDD water and sample with alpha-amylase enzyme. A_{Blank1} , and A_{Blank2} were defined as the absorbance of DDD water and sample without alpha-amylase enzyme.

4.3.7.3 *In vitro* assay for measuring the inhibition of alpha-glucosidase enzyme

Alpha-glucosidase activity was determined by measuring the rate of the release of *p*-nitrophenol from PNPG using colorimetric method that was adapted from Si et al. (2010). Briefly, the mixture consisted of 25 μl of 3 mM GSH,

250 μl of 0.067 M PPB (pH 6.8), and 25 μl of 0.3 U/ml of alpha-glucosidase enzyme. Then, the mixture was added by 100 μl of the rambutan rind extract, tannin (Fluka), or acarbose (Bayer) dissolved in DDD water to give concentrations ranging from 0.15625 to 20 mg/ml and 1.25 to 40 mg/ml, 10 mg/ml, respectively. The mixture was incubated in water bath at 37 °C for 10 min. After that, 25 μl of 10 mM PNPG was added and incubated at 37 °C for 10 min. The reaction was stopped by adding 400 μl of 0.1 M sodium carbonate solution. The absorbance of the assay mixture was measured by Benchmark Plus Microplate Spectrophotometer (BIO-RAD) at 400 nm. Individual test 1 and blank 1 were conducted in a similar way by replacing sample with 100 μl DDD water, with or without alpha-glucosidase enzyme, respectively. Individual test 2 and blank 2 were prepared as mentioned above with or without alpha-glucosidase enzyme, respectively. Blank 1 and blank 2 without alpha-glucosidase enzyme solution were replaced by 25 μl ice-cold DDD water. Acarbose (Bayer) was used as positive control at concentration of 10 mg/ml (Subramanian et al., 2008). All determinations were performed in triplicate. The inhibition percentage of alpha-glucosidase enzyme was assessed by following formula:

$$\text{Inhibition (\%)} = \left[\frac{(\Delta A_{\text{Control}} - \Delta A_{\text{Sample}})}{\Delta A_{\text{Control}}} \right] \times 100$$

$$\Delta A_{\text{Control}} = A_{\text{Test1}} - A_{\text{Blank1}}$$

$$\Delta A_{\text{Sample}} = A_{\text{Test2}} - A_{\text{Blank2}}$$

where: A_{Test1} and A_{Test2} were defined as the absorbance of DDD water and sample with alpha-glucosidase enzyme. A_{Blank1} , and A_{Blank2} were defined as the absorbance of DDD water and sample without alpha-glucosidase enzyme.

4.3.8 Method for determination of plasma biochemical parameters after the oral administration of lipid emulsion in rats

This study was adapted from the method of Han et al. (2005) and Lo et al. (2007). Forty rats were selected by stratified randomization and then divided into five groups of eight each. Rats were fasted overnight and then given a single oral dose of the rambutan rind extract (1000 and 2000 mg/kg), tannin (1000 mg/kg) (Fluka), and orlistat (45 mg/kg) (Roche) dissolved in lipid emulsion (20% Intralipid, Sino-Swed Pharmaceutical Corp., China) at a dosing volume of 4 ml/kg while the control group received lipid emulsion alone (4 ml/kg). One hundred milliliters of the lipid emulsion (20% Intralipid, Sino-Swed, China) is composed of purified soybean oil (200 g), purified egg phospholipids (12 g), and glycerol anhydrous (22 g). Blood samples were collected from the tail vein at 0, 1, 2, 3, and 4 h after the oral administration of each treatment using heparinized microtubes and centrifuged (model Z 233M-2, Hermle Labortechnik GmbH., Germany) at 2000 rcf for 5 minutes. The plasma was separated and frozen at -20 °C until further assay for the plasma biochemistry parameters. The plasma triglyceride (TG), total cholesterol (TC), glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were measured by automated analyzer (Hitachi 911, Japan).

4.3.9 Statistics

Results were expressed as mean \pm S.E.M. Plasma biochemical parameters were analyzed using two way repeated measures ANOVA for differences between groups and times followed by Duncan's post hoc test (SigmaStat version 3.5, Systat Software, Inc., USA.). Post hoc testing was performed for inter-group

comparisons. *P*-values less than 0.05 ($P < 0.05$) were considered statistically significant.

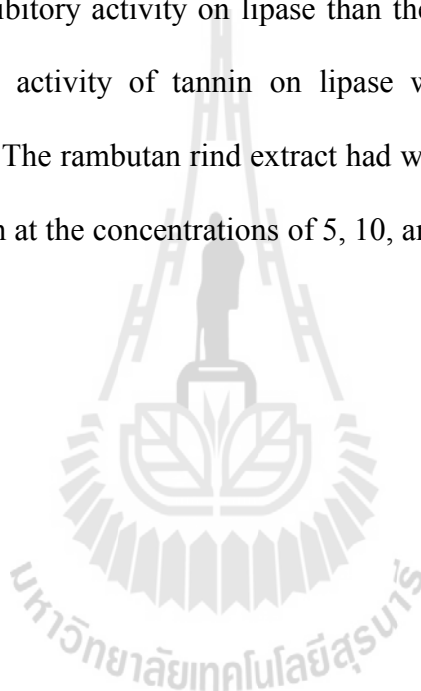
4.4 Results

In this experiment, the percentage yield, total phenolic content, antioxidant activity determined using a DPPH scavenging assay (50% inhibition concentration or IC_{50}), total anthocyanin content, lipase, alpha-amylase, and alpha-glucosidase inhibitory activities *in vitro* of the rambutan rind extract were determined. Our findings indicated that the rind of the rambutan extracted by 85% ethanol had a yield of 18.05%. Total phenolic content of the rambutan rind extract was 416.18 ± 0.01 mg gallic acid/g dry extract, while total anthocyanin content was 14.90 ± 0.8 μ g/g dry extract. The rambutan rind extract exhibited a dose dependent inhibition of DPPH activity, with an IC_{50} value of 0.288 ± 0.04 mg/ml extract.

4.4.1 Pancreatic lipase enzyme inhibitory activity of the rambutan rind extract and tannin

The present study tested the rambutan rind extract and tannin for inhibitory action against porcine pancreatic lipase enzyme *in vitro* in the assays system using triolein as a substrate. The results confirmed that the rambutan rind extract contains lipase inhibitors. Dose-dependent inhibition of the rambutan rind extract and tannin on porcine pancreatic lipase enzyme activity over the observed concentration range (Figures 4.1A and 4.1B) was demonstrated. From the 2 sets of pancreatic lipase enzyme assay, orlistat (40 mg/ml, positive control) exhibited strong inhibition on porcine pancreatic lipase enzyme activity (63.76% and 61.21%) as

shown in Figures 4.1A and 4.1B. The rambutan rind extract showed 6.26%, 7.88%, 9.65%, 33.46%, and 72.68% inhibition of porcine pancreatic lipase enzyme activity at the concentrations of 5, 10, 20, 40, and 80 mg/ml, respectively (Figure 4.1A). Tannin showed 22.42%, 27.27%, 41.82%, 42.42%, and 52.27% inhibition of porcine pancreatic lipase enzyme activity at the concentrations of 1.25, 2.50, 5, 10, and 20 mg/ml, respectively (Figure 4.1B). At the same concentration (40 mg/ml), orlistat exhibited stronger inhibitory activity on lipase than the rambutan rind extract. At 20 mg/ml, the inhibitory activity of tannin on lipase was stronger than that of the rambutan rind extract. The rambutan rind extract had weaker inhibitory effects against the enzyme than tannin at the concentrations of 5, 10, and 20 mg/ml.



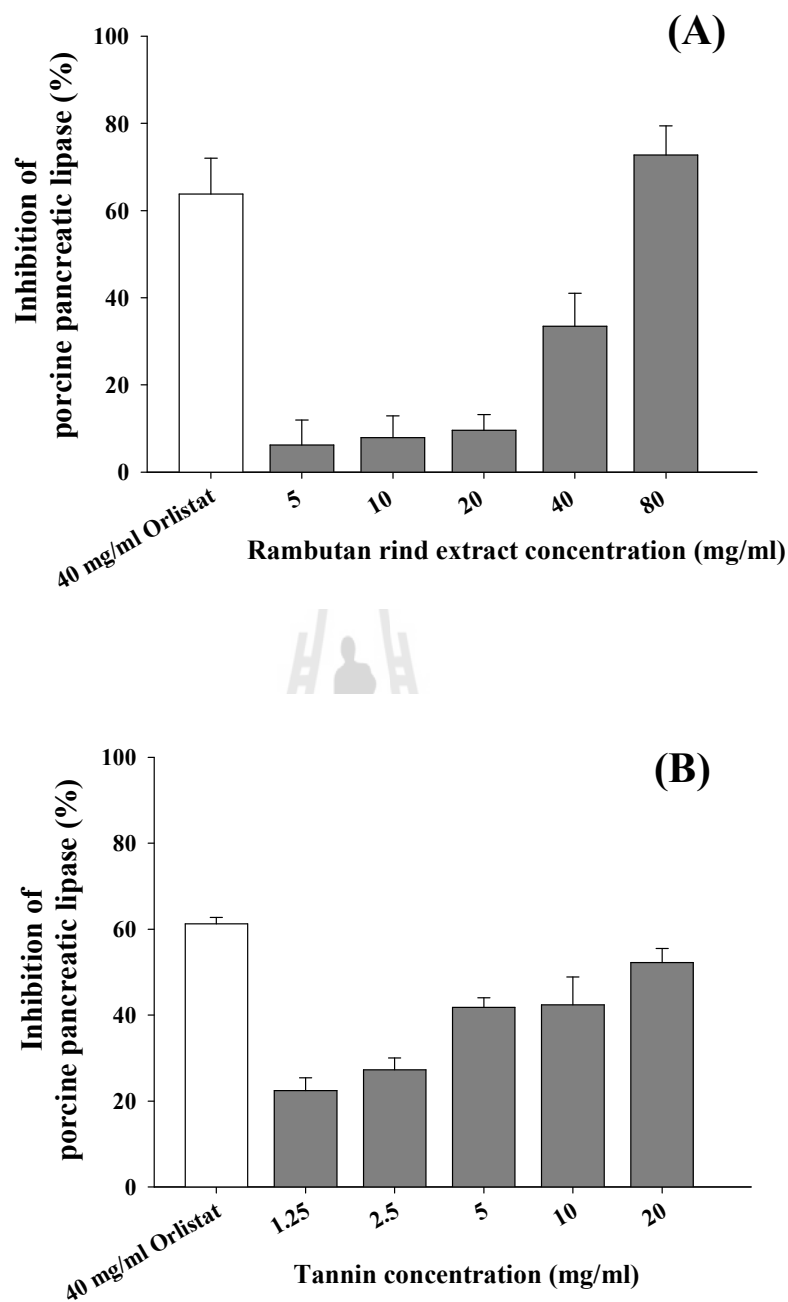


Figure 4.1 Inhibitory effects of various concentrations of the rambutan rind extract (A), tannin (B), and orlistat on porcine pancreatic lipase enzyme activity *in vitro*. Values are expressed as mean \pm S.E.M. of triplicate experiments.

4.4.2 Alpha-amylase enzyme inhibitory activity of the rambutan rind extract and tannin

The present study tested the rambutan rind extract and tannin for inhibitory action against alpha-amylase enzyme *in vitro* in the assays system using starch as a substrate. The results confirmed that the rambutan rind extract contains alpha-amylase inhibitors. Over the observed concentration range, dose dependent inhibition of the rambutan rind extract, but not tannin, on alpha-amylase enzyme activity (Figures 4.2A and 4.2B) was demonstrated. From the 2 sets of alpha-amylase enzyme assay, acarbose (10 mg/ml, positive control) exhibited strong inhibition on alpha-amylase enzyme activity (95.95% and 97.87%) as shown in Figures 4.2A and 4.2B. The rambutan rind extract showed 15.10%, 50.65%, 86.02%, 93.28%, 97.30%, 92.09%, 89.95%, 81.40%, 92.35%, and 72.44% inhibition of the alpha-amylase enzyme activity at the concentrations of 0.15625, 0.3125, 0.625, 1.25, 2.50, 5, 10, 20, 40 and 80 mg/ml, respectively (Figure 4.2A). Tannin showed 85.66%, 95.65%, 92.63%, 86.58%, 84.63%, and 69.61% inhibition of the alpha-amylase enzyme activity at the concentrations of 1.25, 2.50, 5, 10, 20, and 40 mg/ml, respectively (Figure 4.2B). The maximum percentage inhibition of alpha-amylase enzyme activity of the rambutan rind extract and tannin were obtained at the concentration of 2.50 mg/ml (Figures 4.2A and 4.2B). The same concentration of 10 mg/ml acarbose exhibited the strongest inhibitory activity on alpha-amylase among the rambutan rind extract and tannin (Figures 4.2A and 4.2B). The rambutan rind extract had stronger inhibitory effects against the enzyme than tannin at the same concentrations (1.25, 2.5, 10, and 40 mg/ml). At 5 and 20 mg/ml, the rambutan rind extract had weaker inhibitory effects against the enzyme than tannin.

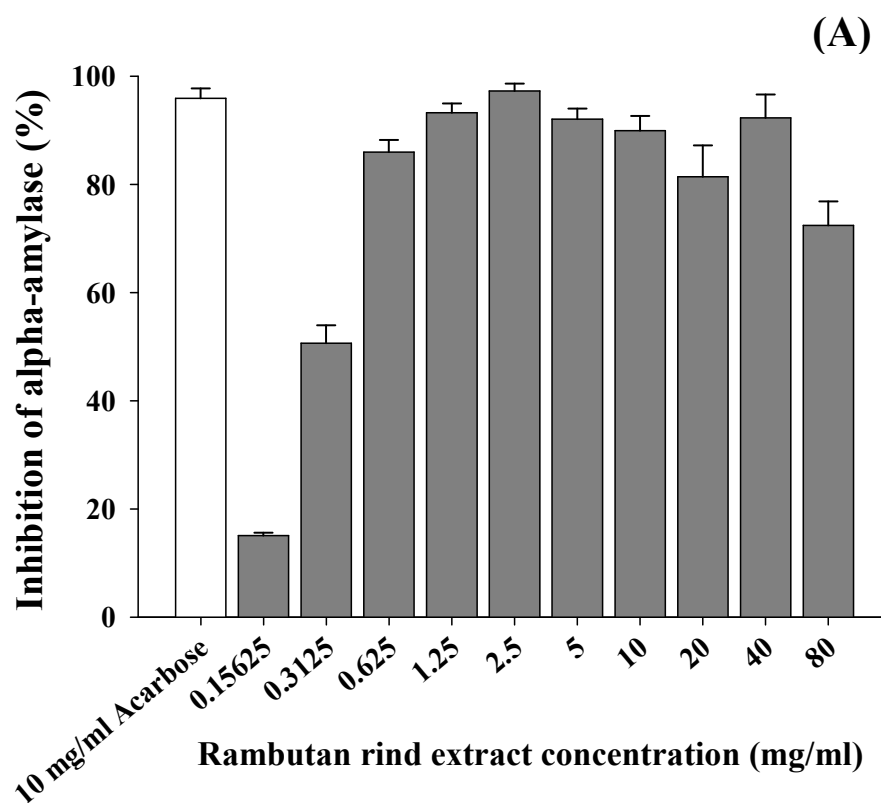


Figure 4.2 Inhibitory effects of various concentrations of the rambutan rind extract (A), tannin (B) and acarbose on alpha-amylase enzyme activity *in vitro*. Values are expressed as mean \pm S.E.M. of triplicate experiments.

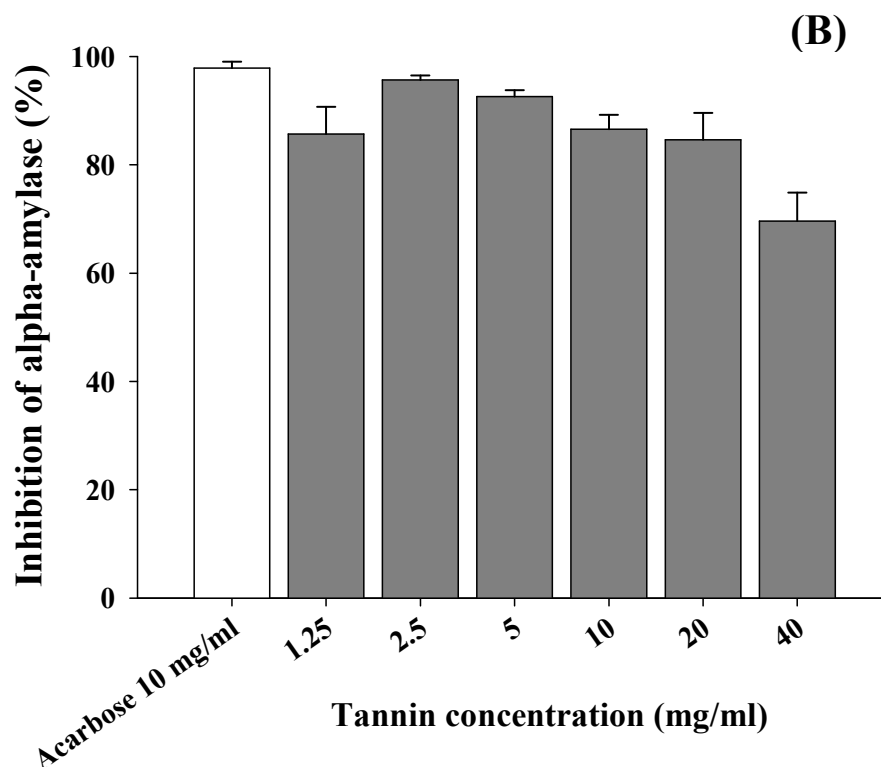


Figure 4.2 Inhibitory effects of various concentrations of the rambutan rind extract (A), tannin (B) and acarbose on alpha-amylase enzyme activity *in vitro* (Cont.). Values are expressed as mean \pm S.E.M. of triplicate experiments.

4.4.3 Alpha-glucosidase enzyme inhibitory activity of the rambutan rind extract and tannin

The present study tested the rambutan rind extract and tannin for inhibitory action against alpha-glucosidase enzyme *in vitro* in the assays system using PNPG as a substrate. The results confirmed that the rambutan rind extract contains alpha- glucosidase inhibitors. Dose dependent inhibition on alpha-glucosidase enzyme activity was observed at 0.15625, 0.3125, 0.625, 1.25, and 2.5 mg/ml of the rambutan rind extract and 1.25, 2.5, and 5 mg/ml of tannin. At the doses of 5, 10, and 20 mg/ml

of the rambutan rind extract and 10, 20, and 40 mg/ml of tannin, alpha-glucosidase enzyme inhibitory activity was slightly decreased (Figures 4.3A and 4.3B). From the 2 sets of alpha-glucosidase enzyme assay, acarbose (10 mg/ml, positive control) exhibited strong inhibition on alpha-amylase enzyme activity (79.47% and 65.93%) as shown in Figures 4.3A and 4.3B. The rambutan rind extract showed 67.18%, 70.98%, 85.10%, 91.65%, 95.79%, 89.52%, 80.22%, and 64.68% inhibition of alpha-glucosidase enzyme activity at the concentrations of 0.15625, 0.3125, 0.625, 1.25, 2.50, 5, 10, and 20 mg/ml, respectively (Figure 4.3A). Tannin showed 73.73%, 84.22%, 96.66%, 89.53%, 76.53%, and 37.79% inhibition of alpha-glucosidase enzyme activity at the concentrations of 1.25, 2.50, 5, 10, 20, and 40 mg/ml, respectively (Figure 4.3B). The maximum percentage inhibition of alpha-glucosidase enzyme activity of the rambutan rind extract and tannin at different concentrations were obtained at the concentrations of 2.50 and 5 mg/ml, respectively. At the same concentrations (10 mg/ml), acarbose exhibited weaker inhibitory activity on alpha-glucosidase than the rambutan rind extract and tannin. The rambutan rind extract had stronger inhibitory effects against the enzyme than tannin at the same concentrations (1.25 and 2.5 mg/ml). At 5, 10, and 20 mg/ml, the rambutan rind extract had weaker inhibitory effects against the enzyme than tannin.

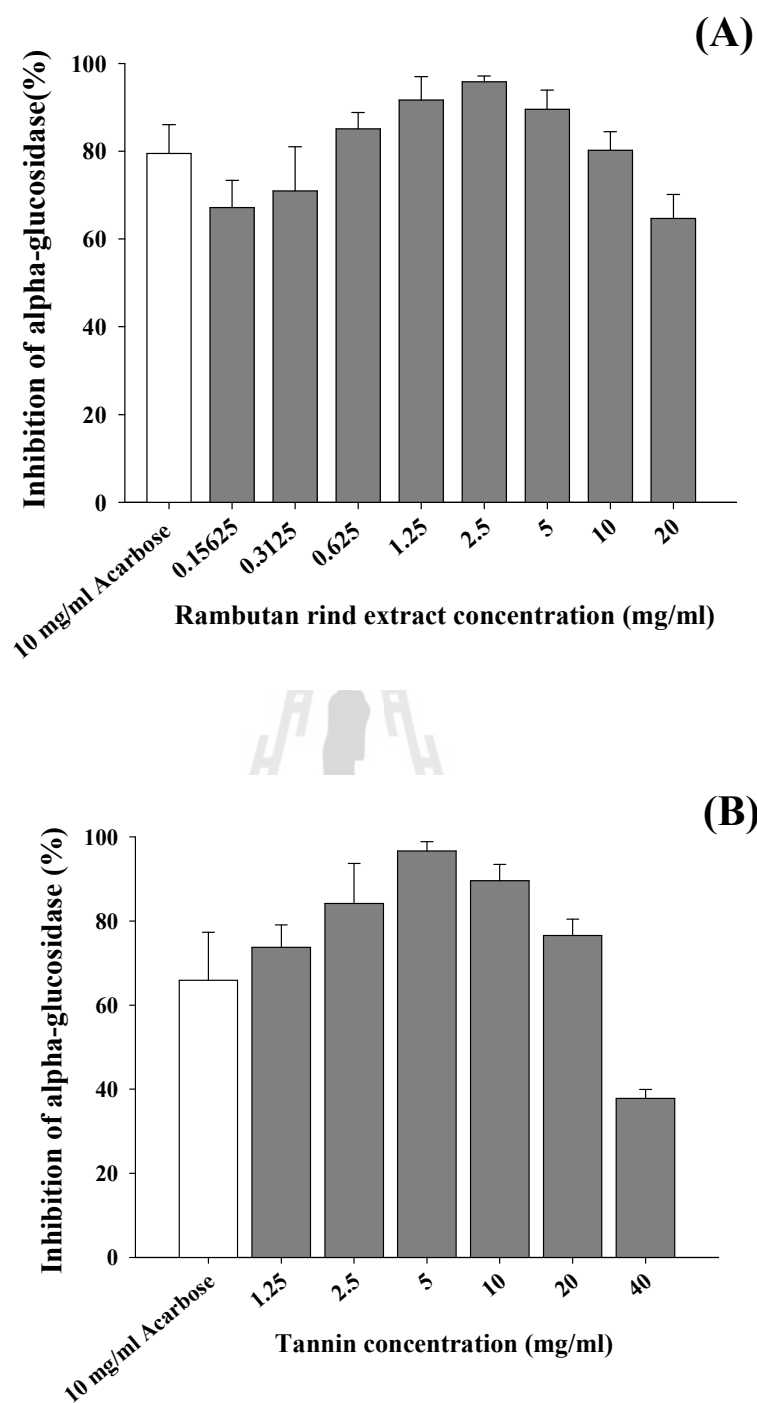


Figure 4.3 Inhibitory effects of various concentrations of the rambutan rind extract (A), tannin (B) and acarbose on alpha-glucosidase enzyme activity *in vitro*. Values are expressed as mean \pm S.E.M. of triplicate experiments.

4.4.4 Effects of the rambutan rind extract and tannin on plasma biochemical parameters after the oral administration of lipid emulsion in rats

This *in vivo* experiment showed the time course characteristics of the plasma TG, TC, glucose, AST, and ALT levels when lipid emulsion was orally administered to rats alone or with rambutan rind extract, orlistat, and tannin. The plasma levels of TG after orally administration of lipid emulsion alone (4 ml/kg, control group) or lipid emulsion with either orlistat (45 mg/ 4 ml/kg, positive control), tannin (1000 mg/ 4 ml/kg), the rambutan rind extract (1000 mg/ 4 ml/kg) or the rambutan rind extract (2000 mg/ 4 ml/kg) were shown in Figure 4.4. The plasma TG levels of the control group increased after the oral administration of the lipid emulsion alone, reaching a maximum level at 2 h (141.19 ± 10.79 mg/dl) later, which significantly differ from 0 h (105.88 ± 6.01 mg/dl) within group. Significant increases in the plasma TG levels were found at 2 h (112.22 ± 8.70 mg/dl) and 4 h (104.50 ± 4.35 mg/dl) after orally administered of the lipid emulsion with orlistat (45 mg/ 4 ml/kg) compared to 0 h (89.83 ± 9.05 mg/dl) within the group. The lipid emulsion with tannin (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma TG levels at 4 h (102.30 ± 3.69 mg/dl) when compared to 0 h (69.44 ± 4.84 mg/dl) within the group. The lipid emulsion with rambutan rind extract (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma TG levels at 3 h (103.40 ± 10.18 mg/dl) and 4 h (106.70 ± 4.17 mg/dl) when compared to 0 h (70.40 ± 8.34 mg/dl) within the group while 2000 mg/ 4 ml/kg of the lipid emulsion with rambutan rind extract did not cause any difference. Orlistat (45 mg/ 4 ml/kg, p.o.), tannin (1000 mg/ 4 ml/kg, p.o.), and the rambutan rind extract (1000 and 2000 mg/ 4 ml/kg, p.o.) significantly suppressed

the increase in plasma TG levels 2 h after administration when compared to the lipid emulsion alone at the same time point. The administration of orlistat (45 mg/ 4 ml/kg) significantly reduced the plasma TG levels compared with that of the lipid emulsion alone group at 1 and 2 h after administration. Tannin administered to the rats at a dose 1000 mg/ 4 ml/kg significantly reduced the plasma TG levels compared with that of the lipid emulsion alone group at 1, 2, and 3 h after administration.



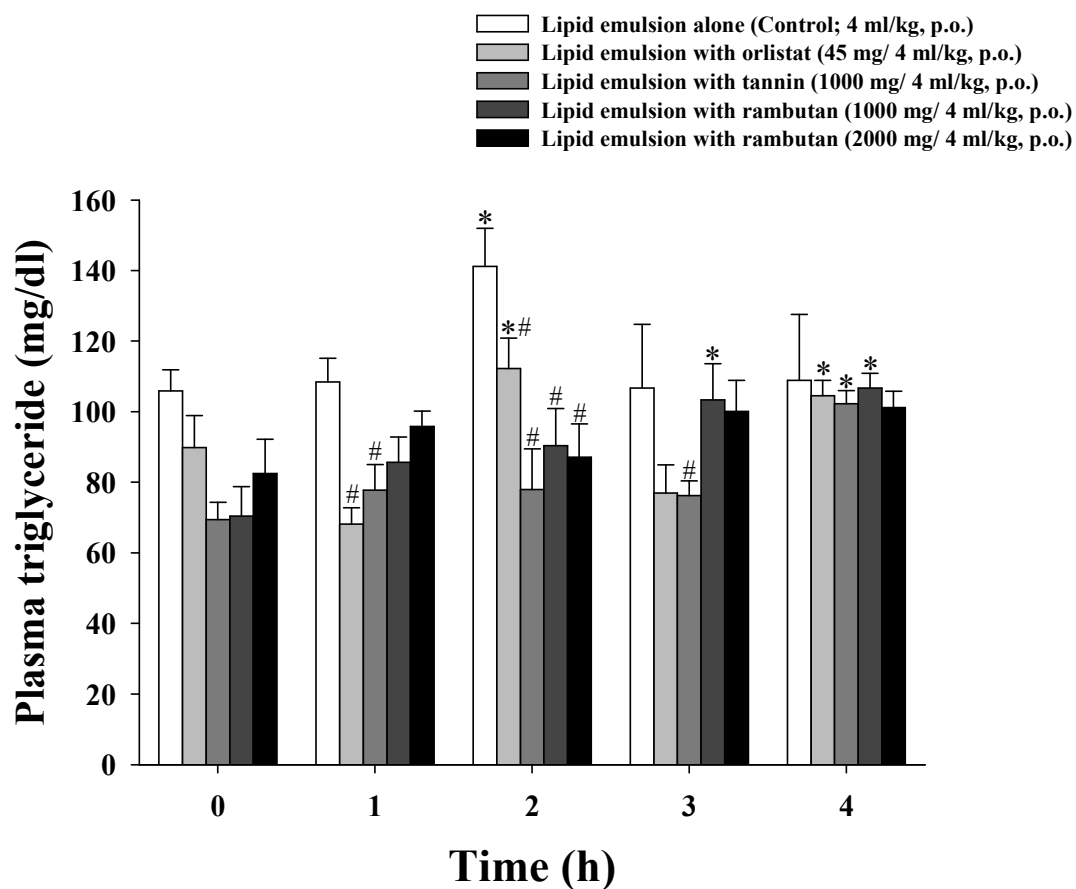


Figure 4.4 Effects of the rambutan rind extract, orlistat, and tannin on rat plasma TG levels after oral administration of lipid emulsion. Values are expressed as mean \pm S.E.M. of 8 rats. * Significantly difference ($P<0.05$) when compared to 0 h within the group, # Significantly difference ($P<0.05$) when compared to lipid emulsion alone treated group at the same time point.

The plasma levels of TC after orally administration of lipid emulsion alone (4 ml/kg, control group) or lipid emulsion with either orlistat (45 mg/ 4 ml/kg, positive control), tannin (1000 mg/ 4 ml/kg), the rambutan rind extract (1000 mg/ 4 ml/kg) or the rambutan rind extract (2000 mg/ 4 ml/kg) were shown in Figure 4.5. The plasma TC levels of control group increased after the oral administration of the lipid emulsion alone, reaching a maximum level at 4 h (85.29 ± 5.36 mg/dl) later, which significantly differ from 0 h (57.20 ± 5.70 mg/dl) within group. Significant increases in the plasma TC levels were found at 3 h (76.21 ± 8.54 mg/dl) and 4 h (78.57 ± 8.57 mg/dl) after orally administered of the lipid emulsion with orlistat compared to 0 h (61.88 ± 6.74 mg/dl) within the group. The lipid emulsion with tannin (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma TC levels at 2 h (78.88 ± 8.61 mg/dl) when compared to 0 h (61.88 ± 7.43 mg/dl) within the group. The lipid emulsion with rambutan rind extract (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma TC levels at 2 h (78.57 ± 9.93 mg/dl), 3 h (87.08 ± 9.93 mg/dl), and 4 h (71.50 ± 4.30 mg/dl) when compared to 0 h (58.44 ± 8.60 mg/dl) within the group while 2000 mg/ 4 ml/kg of the lipid emulsion with rambutan rind extract significantly increased the plasma TC levels at 2 h (77.00 ± 5.02 mg/dl) when compared to 0 h (60.50 ± 6.09 mg/dl) within the group. Orlistat, tannin, and the rambutan rind extract did not suppress the increase in plasma TC levels caused by lipid emulsion.

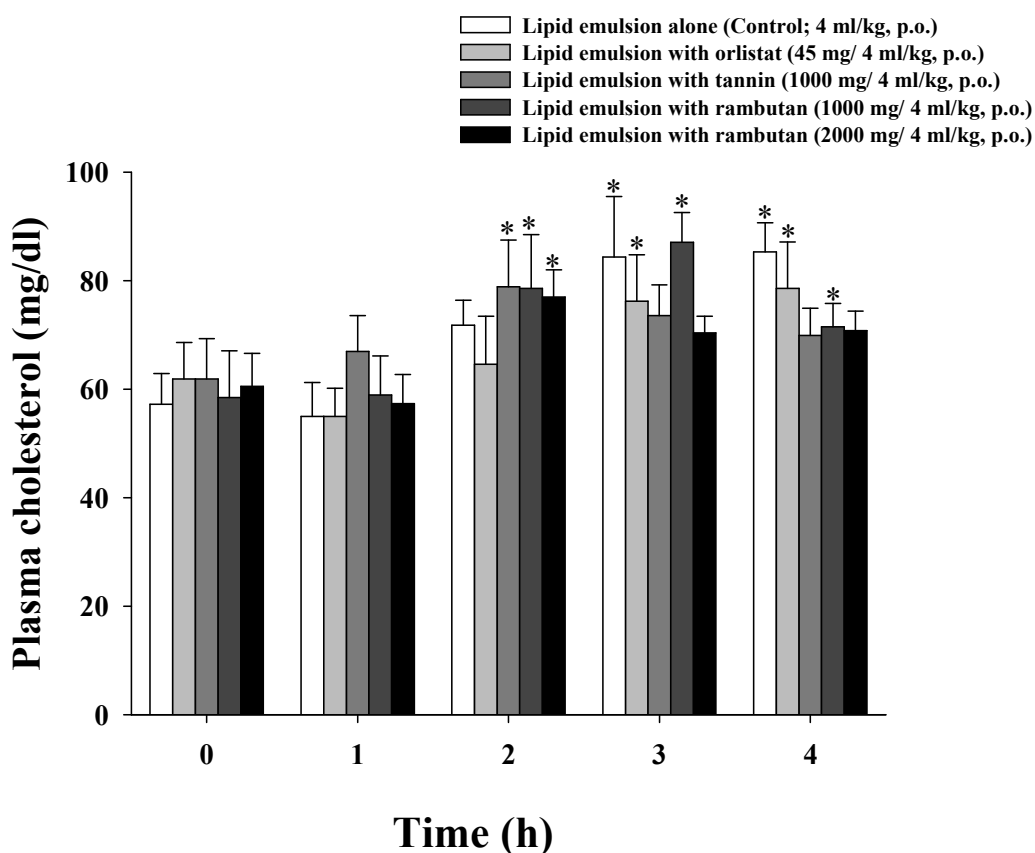


Figure 4.5 Effects of the rambutan rind extract, orlistat, and tannin on rat plasma TC levels after oral administration of lipid emulsion. Values are expressed as mean \pm S.E.M. of 8 rats. * Significantly difference ($P<0.05$) when compared to 0 h within the group, # Significantly difference ($P<0.05$) when compared to lipid emulsion alone treated group at the same time point.

The plasma levels of glucose after orally administration of lipid emulsion alone (4 ml/kg, control group) or lipid emulsion with either orlistat (45 mg/ 4 ml/kg, positive control), tannin (1000 mg/ 4 ml/kg), the rambutan rind extract (1000 mg/ 4 ml/kg) or the rambutan rind extract (2000 mg/ 4 ml/kg) were shown in Figure 4.6. Lipid emulsion alone did not cause any change in the plasma levels of glucose.

Significantly decreased the plasma glucose levels were found at 1 h (74.25 ± 5.33 mg/dl), 2 h (74.67 ± 4.85 mg/dl), and 4 h (71.50 ± 3.18 mg/dl) after orally administered of the lipid emulsion with orlistat (45 mg/ 4 ml/kg) compared to 0 h (95.33 ± 6.54 mg/dl) within the group. The lipid emulsion with tannin (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma glucose levels at 2 h (98.00 ± 21.69 mg/dl) when compared to 0 h (94.29 ± 4.19 mg/dl) within the group. The lipid emulsion with rambutan rind extract (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma glucose levels at 2 h (112.20 ± 11.44 mg/dl) and 3 h (96.25 ± 6.91 mg/dl) when compared to 0 h (90.75 ± 10.52 mg/dl) within the group while 2000 mg/ 4 ml/kg of the lipid emulsion with rambutan rind extract significantly increased the plasma glucose levels at 2 h (89.83 ± 11.71 mg/dl) when compared to 0 h (82.50 ± 5.50 mg/dl) within the group. The administration of the lipid emulsion with tannin (1000 mg/ 4 ml/kg, p.o.) and the lipid emulsion with the rambutan rind extract (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma glucose levels compared with that of the lipid emulsion alone group at 2 h after administration, while 2000 mg/ 4 ml/kg of the rambutan rind extract significantly decreased the plasma glucose levels compared with that of the lipid emulsion alone group at 3 h after administration.

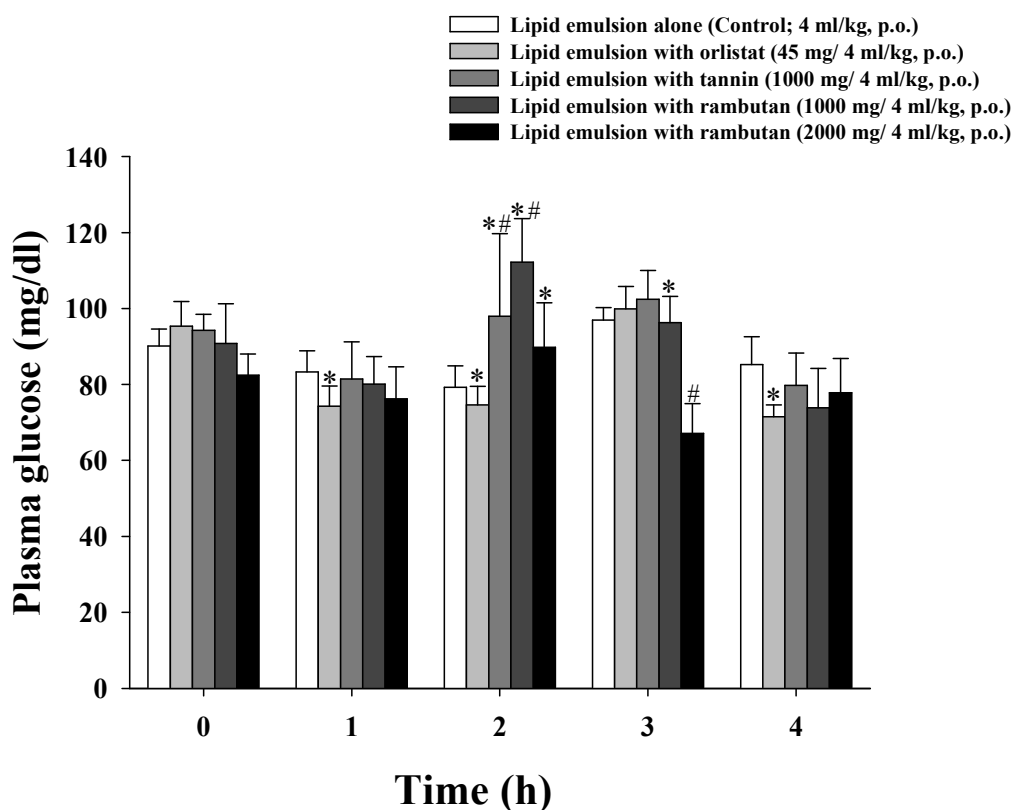


Figure 4.6 Effects of the rambutan rind extract, orlistat, and tannin on rat plasma glucose levels after oral administration of lipid emulsion. Values are expressed as mean \pm S.E.M. of 8 rats. * Significantly difference ($P<0.05$) when compared to 0 h within the group, # Significantly difference ($P<0.05$) when compared to lipid emulsion alone treated group at the same time point.

The plasma levels of AST after orally administration of lipid emulsion alone (4 ml/kg, control group) or lipid emulsion with either orlistat (45 mg/ 4 ml/kg, positive control), tannin (1000 mg/ 4 ml/kg), the rambutan rind extract (1000 mg/ 4 ml/kg) or the rambutan rind extract (2000 mg/ 4 ml/kg) were shown in Figure 4.7. Administration of lipid emulsion alone and lipid emulsion with tannin did not cause

any change in the plasma levels of AST. The lipid emulsion with orlistat (45 mg/ 4 ml/kg, p.o.) significantly decreased the plasma AST levels at 4 h (106.86 ± 8.29 U/L) when compared to 0 h (185.17 ± 13.80 U/L) within the group, while 1000 mg/ 4 ml/kg of the lipid emulsion with rambutan rind extract significantly decreased the plasma AST levels at 4 h (135.93 ± 8.67 U/L) when compared to 0 h (185.63 ± 16.88 U/L) within the group. Significant decreases in the plasma AST levels were found at 1 h (110.00 ± 15.78 U/L), 2 h (122.83 ± 9.55 U/L), 3 h (115.50 ± 22.68 U/L), and 4 h (120.31 ± 6.89 U/L) after orally administered of the lipid emulsion with rambutan rind extract (2000 mg/ 4 ml/kg) compared to 0 h (180.71 ± 16.37 U/L) within the group. The administration of the rambutan rind extract (2000 mg/ 4 ml/kg, p.o.) significantly decreased the plasma AST levels compared with that of the lipid emulsion alone group at 1 and 2 h after administration.



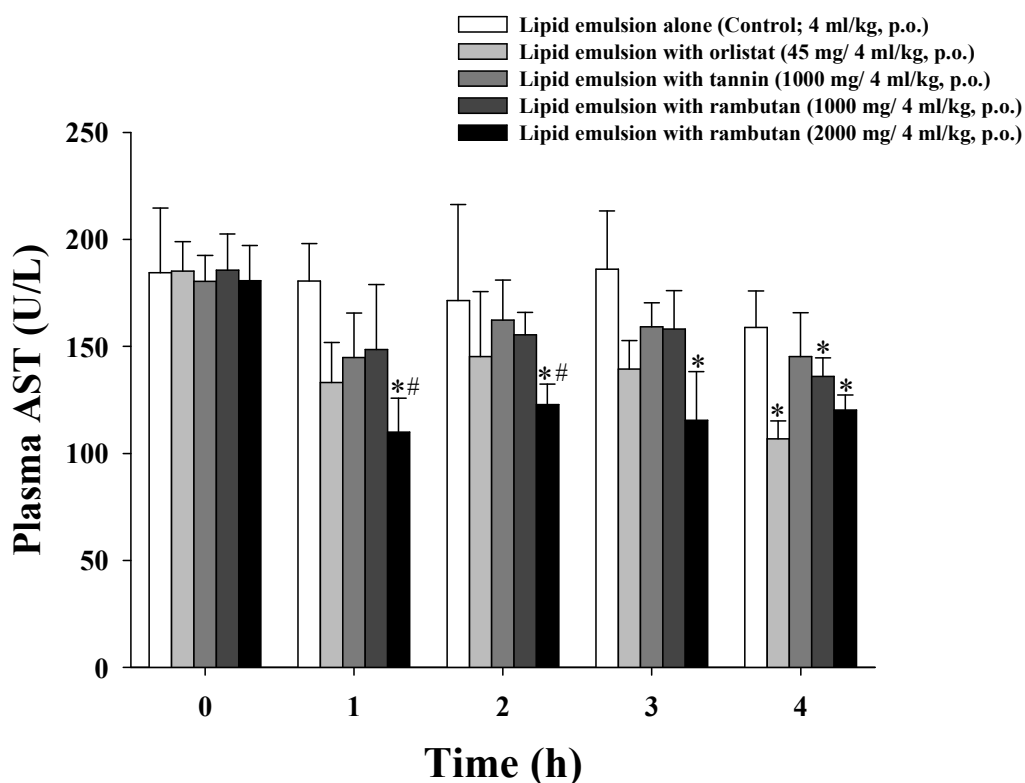


Figure 4.7 Effects of the rambutan rind extract, orlistat, and tannin on rat plasma AST levels after oral administration of lipid emulsion. Values are expressed as mean \pm S.E.M. of 8 rats. * Significantly difference ($P < 0.05$) when compared to 0 h within the group, # Significantly difference ($P < 0.05$) when compared to lipid emulsion alone treated group at the same time point.

The plasma levels of ALT after orally administration of lipid emulsion alone (4 ml/kg, control group) or lipid emulsion with either orlistat (45 mg/ 4 ml/kg, positive control), tannin (1000 mg/ 4 ml/kg), the rambutan rind extract (1000 mg/ 4 ml/kg) or the rambutan rind extract (2000 mg/ 4 ml/kg) were shown in Figure 4.8. The plasma ALT levels of control group were increased at 2 h (60.94 ± 7.02 U/L) and

3 h (66.00 ± 8.82 U/L) after the oral administered of the lipid emulsion alone, reaching a maximum levels at 3 h (66.00 ± 8.82 U/L), which significantly differ from 0 h (42.50 ± 2.28 U/L) within group. Administration of lipid emulsion with orlistat did not cause any change in the plasma levels of ALT. Significant increases in the plasma ALT levels were found at 2 h (56.29 ± 6.69 U/L) and 4 h (57.36 ± 2.86 U/L) after orally administered of the lipid emulsion with tannin (1000 mg/ 4 ml/kg) when compared to 0 h (39.29 ± 2.73 U/L) within the group. The lipid emulsion with rambutan rind extract (1000 mg/ 4 ml/kg, p.o.) significantly increased the plasma ALT levels at 3 h (70.83 ± 9.72 U/L) when compared to 0 h (38.50 ± 5.98 U/L) within the group, while 2000 mg/ 4 ml/kg of the lipid emulsion with rambutan rind extract significantly increased the plasma ALT levels at 4 h (57.36 ± 4.65 U/L) when compared to 0 h (37.71 ± 2.02 U/L) within the group. Orlistat (45 mg/ 4 ml/kg, p.o.) and the rambutan rind extract (1000 and 2000 mg/ 4 ml/kg, p.o.) significantly suppressed the increase in plasma ALT levels at 2 h after administration when compared to the lipid emulsion alone at the same time point.

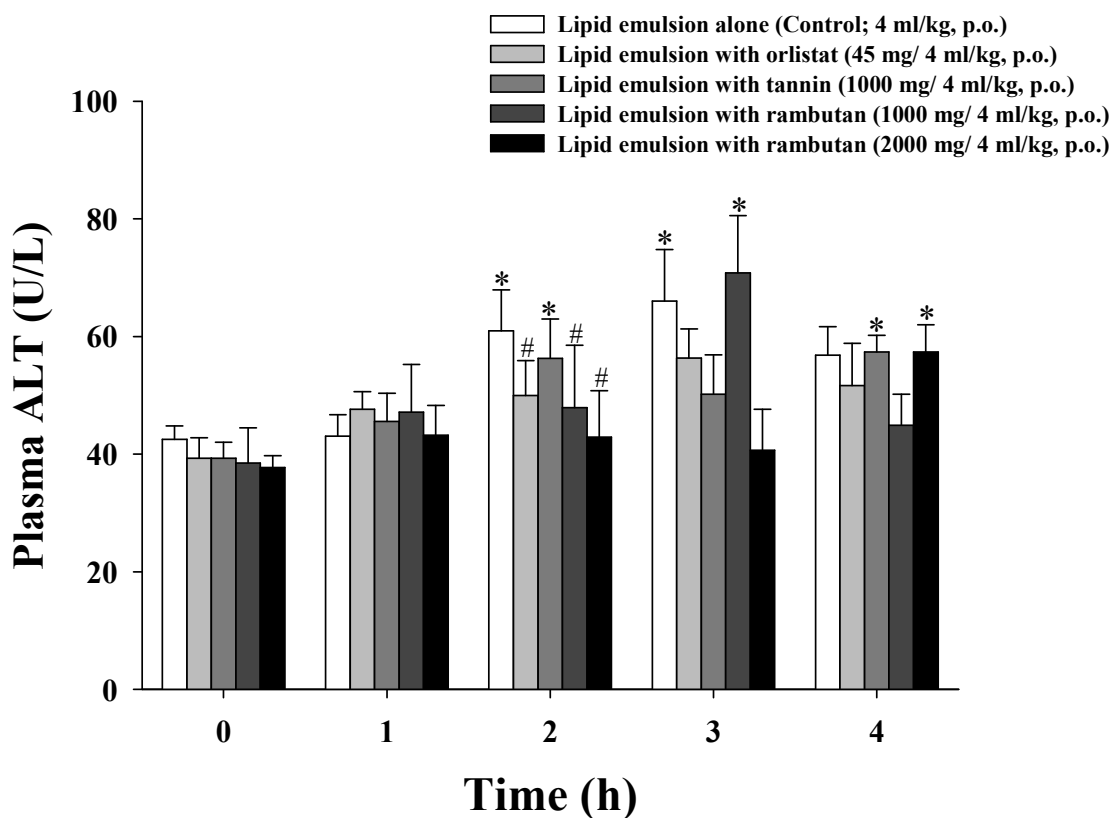


Figure 4.8 Effects of the rambutan rind extract, orlistat, and tannin on rat plasma ALT levels after oral administration of lipid emulsion. Values are expressed as mean \pm S.E.M. of 8 rats. * Significantly difference ($P<0.05$) when compared to 0 h within the group, # Significantly difference ($P<0.05$) when compared to lipid emulsion alone treated group at the same time point.

4.5 Discussion and conclusion

The percentage yield of the rambutan rind extract obtained from this study was 18.05% which is similar to that found in previous study by Khonkarn et al. (2010), Palanisamy et al. (2011a; 2011b). Ethanolic extract of the rambutan rind produced significant antioxidant activity ($IC_{50} = 0.288 \pm 0.04$ mg/ml extract), indicating that the rambutan rind extract exhibited a potential free radical scavenging activity (Palanisamy et al., 2008; Thitilerdecha et al., 2008). This can be attributed to the total phenolic content (416.18 ± 0.01 mg gallic acid/g dry extract) present in the rambutan rind extract. Total phenolic content is very important plant constituents as their hydroxyl groups confer scavenging ability (Mayur et al., 2010). They are known as powerful chain breaking antioxidants. Thus, total phenolic content found in the rambutan rind extract tends to correspond with free radical scavenging activity (Auger et al., 2004; Bagchi et al., 2006). Anthocyanin and tannins are potent polyphenolic antioxidants found in the rambutan rind extract. Total anthocyanin content of the rambutan rind extract obtained from this study was 14.90 ± 0.8 μ g/g dry extract. Sun et al. (2011) reported that the rambutan rind extracted by using ethanol:water:acetic acid (60:39:1) extraction had total anthocyanin content of 181.3 mg/100 g of fresh extract. Wongsiri et al. (1993) revealed that the rambutan rind extracted by using water extraction had high tannin content (21.18%). In the study of rats fed with high cholesterol diets supplemented with 1000 mg/kg tannic acid (tannin), tannin significantly increased in the excretion of neutral, acidic, and total fecal sterols, and lowered plasma levels of triglyceride and cholesterol, and also reduced cholesterol in liver (Park et al., 2002). Tannin might be one of bioactive compounds found in the rambutan rind extract that responsible for anti-obesity effect of the rambutan rind

extract. Therefore, tannin was selected to be tested in both *in vitro* and *in vivo* experiments in this chapter.

Obesity is characterized by the accumulation of excess fat in adipose tissues and has become a major health problem worldwide (Lois and Kumar, 2009). One anti-obesity therapeutic approach is to reduce gastrointestinal fatty acids production and absorption through the inhibition of fat-digesting enzyme such as pancreatic lipase enzyme. Pancreatic lipase is the most important enzyme in the digestion of dietary fat (Mukherjee, 2003; Shi and Burn, 2004; Thomson et al., 1997). Orlistat, a potent inhibitor of gastric, pancreatic, and carboxylester lipase (Sharma et al., 2005), was used as positive control for the study of inhibitory effects against pancreatic lipase enzyme. Orlistat is the only drug authorized for the treatment of obesity within an adequate energy intake, which acts by inhibiting the lipolytic activity of pancreatic lipase enzyme (Bray, 2000; Chaput et al., 2007). Orlistat inhibit the absorption of fat and promote excretion of ingested fat leading to weight loss (Shi et al., 2005). Orlistat (250 µg/ml) was reported to inhibit porcine pancreatic lipase by 95.7% using the colorimetric method (Zhang et al., 2008). Orlistat (45 mg/kg) was reported to reduce the levels of plasma triacylglycerols after oral administration of lipid emulsion to rats (Han et al., 2005). With the aim of finding new compounds more potent or with less secondary effects than orlistat, new natural products are being identified and screened for their pancreatic lipase inhibitory potential. Some plants extract containing total phenolic content possessed pancreatic lipase inhibitory effects such as *Glycyrrhiza glabra* roots (Birari et al., 2011), green tea leaves (Juhel et al., 2000), *Dioscorea nipponica* Makino (Kwon et al., 2003), *Panax quinquefolium* leaves (Liu et al., 2008), *Salacia reticulata* (Yamamoto et al., 2002), berries (McDougall, Kulkarni, and

Stewart, 2009), *Theobroma cacao* (Gu et al., 2011), grape seed (Moreno, et al., 2003), and apple (Sugiyama et al., 2007). Our findings indicated that the rambutan rind extract and tannin inhibited lipase enzyme activity in a dose-dependent manner. At 40 mg/ml, orlistat showed stronger inhibition of pancreatic lipase enzyme activity than the rambutan rind extract (63.76% and 33.46%). The rambutan rind extract at 80 mg/ml showed stronger inhibition of pancreatic lipase enzyme activity than 40 mg/ml orlistat (72.68% and 63.76%). At the same concentrations (5, 10, and 20 mg/ml), tannin showed stronger inhibition of this enzyme than the rambutan rind extract. Thus, the rambutan rind extract showed potentially promising effects for weight control by blocking the digestion and absorption of dietary lipids. The rambutan rind extract could be candidate for anti-obesity treatment. However, more data are needed to define effects, optimal dose required, and mechanism of action, as well as their possible side of toxic effects.

Obese individuals develop resistance to the cellular action of insulin, characterized by an impaired ability of insulin to inhibit glucose output from the liver and to promote glucose uptake in fat and muscle (Saltiel and Kahn, 2001; Hribal et al., 2002). Insulin resistance is a key etiological factor for type 2 diabetes mellitus (Qatanani and Lazar, 2007). Diabetes mellitus is a metabolic disorder of multiple etiologies that is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolisms resulting from defects in insulin secretion, insulin action, or both. Therefore, alpha-amylase and alpha-glucosidase are the key enzymes involved in the metabolism of carbohydrates, which is a common management in postprandial hyperglycemia. Acarbose is an alpha-glucosidase and an alpha-amylase inhibitor that can prevent carbohydrates absorption (Fujisawa et al.,

2005; Hiroyuki, Tomohide, and Kazunori, 2001; Saito et al., 1998; Shobana et al., 2009). Acarbose reportedly can cause a number of adverse effects. Hence, there is an urgent need to identify indigenous natural resources with the ability delay to prevent glucose absorption that have no or less side effects. Carbohydrate digestion inhibitors are under development to improve glycemic control and these may also induce some weight loss (Tucci, Boyland, and Halford, 2010). Many studies have been demonstrated that total phenolic content enriched extracts of plants have free radical scavenging-linked antioxidant activity and high alpha-amylase and alpha-glucosidase inhibitory activities (Ademiluyi and Oboh, 2011; Bhandari et al., 2008; Kim, Hyun, and Kim, 2011; Lee et al., 2010; Tsujita, Takaku, and Suzuki, 2008; Yuk et al., 2011). Acarbose was used as positive control for the study of inhibitory effects of alpha-amylase and alpha-glucosidase on the absorption of carbohydrates. Acarbose (4 μ M) strongly inhibited the activities of alpha-amylase and sucrase (98% and 63%), *in vitro* (Tucci, Boyland, and Halford, 2010 quoted in Samulitis et al., 1987). Acarbose (10 mg/ml) strongly inhibited the activities of alpha-amylase and alpha-glucosidase (50.10% and 70%), *in vitro* (Subramanian et al., 2008). Studies in rats, acarbose was reported to cause a dose-dependent reduction of weight gain (Tucci, Boyland, and Halford, 2010 quoted in Puls et al., 1981). Administration of acarbose (150 mg/day) to women with polycystic ovary syndrome significantly reduced body weight and body mass index (Penna et al., 2005; Sönmez et al., 2005; Tuğrul et al., 2008). Our studies demonstrated that the rambutan rind extract and tannin inhibited alpha-amylase and alpha-glucosidase enzymes activities. At the same concentration (10 mg/ml), acarbose showed stronger inhibition of alpha-amylase activity than the rambutan rind extract and tannin (95.95%, 89.95%, and 86.58%), respectively. The rambutan rind extract

had stronger inhibitory effects against the enzyme than tannin at the same concentrations (1.25, 2.5, 10, and 40 mg/ml). At 5 and 20 mg/ml, the rambutan rind extract had weaker inhibitory effects against the enzyme than tannin. The maximum percentage inhibition of alpha-amylase enzyme activity by the rambutan rind extract and tannin were obtained at concentrations of 2.50 mg/ml (97.30% and 95.65%). At the same concentration (10 mg/ml), acarbose exhibited weaker inhibitory activity on alpha-glucosidase than the rambutan rind extract and tannin (79.47%, 80.22%, and 89.53%), respectively. The rambutan rind extract had stronger inhibitory effects against the enzyme than tannin at the same concentrations (1.25 and 2.5 mg/ml). At 5, 10, and 20 mg/ml, the rambutan rind extract had weaker inhibitory effects against the enzyme than tannin. The maximum percentage inhibition of alpha-glucosidase enzyme activity of the rambutan rind extract and tannin were obtained at 2.50 and 5 mg/ml (96.66% and 95.79%), respectively. Similar findings were observed by Palanisamy et al. (2011a; 2011b), total phenolic content and geraniin from the rambutan rind extract were reported to have alpha-amylase and alpha-glucosidase enzymes inhibitory activities. Thus, the rambutan rind extract showed potentially promising effects for weight control by blocking the digestion and absorption of dietary carbohydrates.

In vivo study, lipid emulsion alone significantly increased the plasma TG levels and reached a peak at 2 h. The rambutan rind extract (1000 and 2000 mg/ 4 ml/kg), orlistat (45 mg/ 4 ml/kg), and tannin (1000 mg/ 4 ml/kg) significantly suppressed the increase in plasma TG levels caused by lipid emulsion alone. Lipid emulsion alone significantly increased the plasma TC levels and reached a peak at 4 h. No significant difference in plasma TC levels was found in all treatments compared to

lipid emulsion alone at each time point. The results of both *in vivo* and *in vitro* studies suggested that the rambutan rind extract and tannin may prevent lipid emulsion and lipase interactions. Inhibition of pancreatic lipase by the rambutan rind extract and tannin could restrict fat digestion by suppression of the hydrolysis of the triglycerides contained in lipid emulsion and could suppress absorption of these triglycerides. As a result, reduction of the plasma TG levels in the rats was occurred. Lipid emulsion alone did not cause any change in plasma glucose levels. Tannin (1000 mg/ 4 ml/kg) and the rambutan rind extract (1000 mg/ 4 ml/kg) significantly increased plasma glucose levels at 2 h while the rambutan rind extract (2000 mg/ 4 ml/kg) significantly decreased plasma glucose levels at 3 h compared to lipid emulsion alone. Hypoglycemic effects of the rambutan rind extract (2000 mg/ 4 ml/kg) may be due to their inhibition effects on alpha-amylase and alpha-glucosidase enzymes. The results from this study suggested that the rambutan rind extract (2000 mg/ 4 ml/kg) was a potent inhibitor of alpha-amylase and alpha-glucosidase *in vitro* which lead to a reduction in the intestinal absorption of carbohydrates in hypoglycemia *in vivo*. The possible mechanism is the inhibition of alpha-amylase and alpha-glucosidase enzymes can reduce the postprandial increase of blood glucose after a mixed carbohydrate diet (Tundis, Loizzo, and Menichini, 2010). Another possible mechanisms underlying the effect of the rambutan rind extract (2000 mg/ 4 ml/kg) on hypoglycemic may involve a reduction of plasma non-esterified fatty acid concentration, improved insulin sensitivity, and stimulation of glucagon-like peptide-1 (GLP-1) secretion in the lower small intestine (Jacob et al., 2009). Reducing postprandial hyperglycemia prevent glucose uptake into adipose tissue to inhibit synthesis and accumulation of TG (Maury et al., 1993). The inhibition of digestion

and absorption of dietary fat and carbohydrate can prevent the obesity (Tucci et al., 2010). The rambutan rind extract (2000 mg/ 4 ml/kg) significantly decreased the plasma AST levels at 1 and 2 h after administration when compared to the lipid emulsion alone. Lipid emulsion alone significantly increased the plasma ALT levels and reached a peak at 3 h. Orlistat and the rambutan rind extract (1000 and 2000 mg/ 4 ml/kg) significantly suppressed the increment of the plasma ALT at 2 h by lipid emulsion alone. The possible mechanism of the effect of orlistat and the rambutan rind extract on the reduction of AST and ALT levels may relate to a reduction of plasma free fatty acid (FFA) levels, a decrease in FFA flux into the liver, and an increase in hepatic insulin sensitivity (Zelber-Sagi et al., 2006). Furthermore, orlistat and the rambutan rind extract could suppress the increased of FFA levels which reduced a characteristic response of liver to the proinflammatory cytokine tumor necrosis factor- α (TNF- α) which protected hepatocyte injury and affecting the integrity of liver cells. (Amin and Nagy, 2009; Rodriguez-Hernandez et al., 2011; Vozarova et al., 2002).

In conclusion, the present findings suggested that the the rambutan rind extract and tannin displayed beneficial effects in the prevention and treatment of obesity, possibly by inhibiting pancreatic lipase, alpha-amylase, and alpha-glucosidase activities. In addition, the rambutan rind extract and tannin could suppress the plasma levels of TG in lipid emulsion-induced hyperlipidemia rats. Thus, the phenolic compounds including tannin found in the rambutan rind extract may play key roles in lipase, alpha-amylase, and alpha-glucosidase inhibitory activities, and hypotriglyceridemic effect.

4.6 References

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CHAPTER V

**EFFECTS OF THE RAMBUTAN RIND EXTRACT ON
FOS EXPRESSION IN THE HYPOTHALAMUS AND THE
NUCLEUS OF THE SOLITARY TRACT IN MALE
WISTAR RATS**

5.1 Abstract

Obesity is the results from positive energy homeostasis in which energy intake exceeds energy expenditure. The hypothalamus has been focused on as the center of the brain that controls appetite, energy expenditure, and body weight. Rambutan (*Nephelium lappaceum* L.) is a medicinal plant containing phenolic compounds (tannins, flavonoids and anthocyanins) that has potential to prevent obesity. However, there is little known about central effects of the rambutan rind extract. Therefore, the present study was designed to investigate the effect of the rambutan rind extract on a neuronal activation marker Fos expression in the rat hypothalamus and the nucleus of the solitary tract (NTS). Adult male rats (n = 16) received either double deionized distilled (DDD) water (1 ml/kg) or 2000 mg/ml/kg of the rambutan rind extract by oral gavage. Ninety minutes later, all rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). Blood samples were collected and plasma levels of triglyceride (TG), total cholesterol (TC), glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using automated analyzer. After blood

collection, the rat brains were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and coronal sectioned through the level of the arcuate (ARC), paraventricular (PVN), lateral hypothalamic (LHA), and perifornical (PFA) nuclei of the hypothalamus and the NTS using a cryostat. Free floating sections of these areas were processed for Fos immunohistochemistry. The results revealed that the plasma levels of AST and ALT in rat administered with the rambutan rind extract were not different from the control group. In comparison to the control, the rambutan rind extract significantly reduced plasma glucose, TG, and TC. The rambutan rind extract induced Fos expression only in the ARC. A significant increase in the number of Fos-positive neurons was shown in the area of the ARC ranging from -3.60 to -3.96 mm from bregma following the rambutan rind extract compared to the control. The numbers of Fos-positive neurons following the rambutan rind extract administration in the medialposterior region of this area of the ARC were higher than in the lateralposterior region, but no significant difference was found. In both regions, the rambutan rind extract significantly increased Fos positive neurons when compared to the control. The present findings suggested that rambutan rind extract had a central effect on the regulation of body weight and food intake by activation of the medialposterior and the lateroposterior arcuate neurons. However, further investigations are needed to identify which neuronal types in the ARC are activated by the rambutan rind extract and to investigate physiological effects of the rambutan rind extract on body weight control.

5.2 Introduction

Obesity is defined as a conditions characterized by excess body fat that is quantified by the elevation in body weight of patients (Seidell and Flegal, 1997). In general, it is accepted that obesity results from positive energy homeostasis in which energy intake exceeds energy expenditure (Wood et al., 1998). Energy homeostasis is regulated by neural and hormonal (adiposity and satiety signals) signals that are integrated in the brain (Seeley and Woods, 2003). The hypothalamus has been focused on as the center of the brain that controls appetite, energy expenditure, and body weight (Lawrence, Turnbull, and Rothwell, 1999; Schwartz, 2000). Many hypothalamic nuclei are important in the regulation of food intake and energy homeostasis, including the arcuate (ARC), paraventricular (PVN), ventromedial (VMH), lateral hypothalamic (LHA) and perifornical (PFA) nuclei (Lawrence, Turnbull, and Rothwell, 1999; Schwartz, 2000; Valassi et al., 2008; Williams et al., 2001). Cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) have been reported to act as satiety signals, reducing food intake (Lee et al., 1997; Valassi et al., 2008). These satiety signals are sent to the nucleus of the solitary tract (NTS) in the caudal medulla of brainstem (Woods and Seeley, 2002; Woods, 2004). Leptin and insulin have been reported to act as adiposity signals, reduces feeding and body weight (Elias et al., 1998; Schwartz, 2000; Vettor, 2002). Adiposity signals enter the brain at the level of the hypothalamus (Schwartz, 2000; Valassi et al., 2008). Both the NTS and the hypothalamus are involved in the trafficking and integration of the satiety signals and the regulation of food intake (Schwartz, 2000; Williams et al., 2001). The ARC is essential in the regulation of body weight and food intake. Two types of neurons in the arcuate: the orexigenic

neuropeptide Y and agouti-related peptide (NPY/AGRP) neurons and the anorexigenic pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (POMC/CART) neurons, which are predominate in the medial and the lateral regions of the ARC (Valassi et al., 2008; Williams et al., 2001). When adiposity signals reach the ARC, anorexigenic peptides are released which activate a catabolic circuit. In contrast, the activation of anabolic pathway leads to the release of orexigenic peptides and occurs when adiposity signal concentrations in the brain are low (Valassi et al., 2008).

The study of Kim et al. (2005) reported that administrated crude saponin of Korean red ginseng for 3 weeks significantly reduced number of the NPY-immunoreactive neurons of the PVN, LHA and VMN of the hypothalamus, body weight, food intake, parametrical adipose tissues and serum leptin level in high fat diet-induced obesity rats compared to the control. The effect of adlay (*Coxi lachrym-jobi*) seed extract for 4 weeks have been shown in high fat diet-induced obese rats. Adlay seed extract containing proteins, lipids, carbohydrates and vitamin B₁ significantly decreased NPY and leptin receptor immunoreactivities neurons of the PVN and ARC of the hypothalamus, food intake, body weight, weights of epididymal and peritoneal fat, and serum levels of triglyceride and cholesterol in high fat diet-induced obese rats compared to the control (Kim, Yun, and Lee, 2007). Fos is a protein product of the *c-fos* immediate-early gene has been used as a marker for the activation of neurons in the brain since electrical or chemical stimulation results in markedly enhanced expression of *c-fos* mRNA as well as protein expression (Bullitt, 2004; Nikolaev et al., 2002). Xiong et al. (2010) reported that administrated of ginsenoside Rb1 for 4 weeks significantly reduced number of the Fos-positive neurons

in the hindbrain NTS and the ARC and VMH of the hypothalamus, food intake, body weight gain, body fat content, and significant increase energy expenditure in high fat diet-induced obese rats compared to the control. Rambutan (*Nephelium lappaceum* L.) is a medicinal plant containing phenolic compounds (tannins, flavonoids, and anthocyanins) (Palanisamy et al., 2008; Thitilertdecha et al., 2008, 2010; Wall, 2006), which has the pharmacological effects such as antioxidant and antibacterial activities (Palanisamy et al., 2008; Thitilertdecha et al., 2008), anti-herpes simplex virus type 1 (Nawawi et al., 1999), antiproliferative (Khonkarn et al., 2010), and anti-hyperglycemic activities (Palanisamy et al., 2011a, 2011b). However, there is little known about central effects of the rambutan rind extract. Therefore, the present study was designed to investigate the effect of the rambutan rind extract on a neuronal activation marker Fos expression in the rat hypothalamus and NTS.

5.3 Materials and methods

5.3.1 Plant material

The rambutan rind extract obtained from stock extract in chapter 3 was used in the experiments conducted in this chapter.

5.3.2 Animals

Male Wistar rats (243.75 ± 13.46 g) were obtained from Institutional Animal Care, SUT. They were maintained under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 20 ± 1 °C) with free access to food and water. This study was conducted under permit of the SUT Animal Care and Use Committee.

5.3.3 Materials

0.9% Normal saline solution

0.9% Normal saline solution was prepared by adding 9 g of sodium chloride (NaCl; Sigma Chemical Co., St. Louis, MO, USA) to 900 ml of deionized distilled water (DI). This solution was adjusted volume to 1000 ml with DI water in a volumetric flask.

Heparinsed saline solution

Heparinsed saline (5 IU/ml) solution was prepared by adding 1 ml of a 5000 units/ml heparin solution (LEO Phamaceutical product, Ballerup, Denmark) to 999 ml of 0.9% normal saline solution.

1 M Phosphate buffer solution (pH 7.4)

1 M Phosphate buffer solution (pH 7.4) was prepared by adding 106.5 g of di-sodium hydrogen phosphate anhydrous (Na_2HPO_4 ; BDH Ltd., UK) and 39.7 g of sodium dihydrogen orthophosphate 1-hydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; BDH) to 800 ml of double deionized distilled (DDD) water. This solution was stirred on a hot plate magnetic stirrer (VELP Scientifica, Europe) at 50-60 °C for 1 h, left to be cool down to room temperature and then adjusted to pH 7.4 with 10 M HCl or 10 M NaOH. This solution was adjusted volume to 1000 ml with DDD water in a volumetric flask.

0.1 M Phosphate buffer solution (pH 7.4)

0.1 M Phosphate buffer solution (pH 7.4) was prepared by adding 100 ml of 1 M phosphate buffer solution to 900 ml of DI water. This solution was adjusted to pH 7.4 with 10 M HCl or 10 M NaOH.

4% Paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4)

4% Paraformaldehyde solution was prepared by adding 40 g of paraformaldehyde (Acros Organics, New Jersey, USA) to 900 ml of 0.1 M phosphate buffer solution. This solution was stirred on a hot plate magnetic stirrer (VELP Scientifica, Europe) at 150-200 °C for 1-2 h, left to be cool down to room temperature and then adjusted to pH 7.4 with 10 M HCl or 10 M NaOH. This solution was adjusted volume to 1000 ml with DI water in a volumetric flask.

Anti-freeze cryoprotectant solution

Anti-freeze cryoprotectant solution was 0.1 M phosphate buffer (pH 7.4) containing 30% w/v sucrose ($C_{12}H_{22}O_{11}$; Ajax Finechem Pty Ltd., Australia), 30% v/v ethanediol ($C_2H_6O_2$; Ajax Finechem) and 1% w/v polyvinylpyrrolidone (C_6H_9NO , PVP-40; Sigma). This solution was adjusted volume to 100 ml with DI water in volumetric flask.

5 M Sodium chloride solution

5 M Sodium chloride solution was prepared by adding 292.2 g of sodium chloride (NaCl; Sigma) to 600 ml of DDD water. This solution was then adjusted volume to 1000 ml with DDD water in volumetric flask.

0.1 M Sodium phosphate buffer (PBS) solution

0.1 M Sodium phosphate buffer solution was prepared by adding 30 ml of 5 M sodium chloride to 970 ml of 0.1 M phosphate buffer (pH 7.4). This solution was then adjusted to pH 7.4 with 10 M HCl or 10 M NaOH.

Washing solution (PBS-T) solution

Washing solution was prepared by adding 3 ml of 0.3% v/v Triton X-100 (Panreac Analytical Reagent & Fine Chemical, Spain) to 997 ml of 0.1 M sodium phosphate buffer (pH 7.4). This solution was then adjusted to pH 7.4 with 10 M HCl or 10 M NaOH.

Hydrogen peroxide solution

Hydrogen peroxide solution was 0.1 M sodium phosphate buffer (pH 7.4) containing 1% v/v hydrogen peroxide (H₂O₂; Merck Schuchardt OHG., Hohenbrunn, Germany) and 20% v/v methanol (CH₄O; BDH). This solution was prepared immediately prior to use.

Preincubation buffer

Preincubation buffer was a washing solution containing 5% v/v Normal Goat Serum.

Primary antibody

Rabbit polyclonal anti-Fos (*c-fos* Ab-5, Calbiochem; USA) was diluted 1:5,000 in preincubation buffer.

Secondary antibody

Secondary antibody was a washing solution containing 1% v/v biotinylated anti-rabbit immunoglobulin and 3% v/v normal goat serum (Vectastain Elite ABC Kit rabbit IgG's, Vector Laboratories, Burlingame, USA).

Avidin-biotinylated horseradish peroxidase complex (ABC)

ABC solution was a washing solution containing 2% v/v Avidin and 2% v/v biotinylated horseradish peroxidase (Vectastain Elite ABC Kit rabbit IgG's). This solution was left to incubate for at least 30 minutes prior to use.

0.1 M Acetate buffer (stop solution)

0.1 M Acetate buffer was prepared by adding 0.82 g of sodium acetate (CH_3COONa ; BDH) to 100 ml of DDD water. This solution was then adjusted to pH 6.0 with acetic acid.

Ni-DAB- H_2O_2 Solution

Ni-DAB- H_2O_2 solution was 0.1 M sodium phosphate buffer solution (pH 7.4) containing 0.05% w/v ammonium nickel (II) sulfate hexahydrate ($\text{NiN}_2\text{H}_8\text{S}_2\text{O}_8 \cdot 6\text{H}_2\text{O}$; Sigma), 2% v/v diaminobenzidine (DAB) (3,3'-Diaminobenzidine tetrahydrochloride hydrate, $\text{C}_{12}\text{H}_{14}\text{N}_4 \cdot 4\text{HCl} \cdot x\text{H}_2\text{O}$; Sigma), and 0.05% v/v hydrogen peroxide (H_2O_2 ; Merck). This solution was then filtered through No.1 Whatman filter paper (Whatman) prior to use.

Chrome alum gelatin

Chrome alum gelatin solution was prepared in DI water containing 1% w/v gelatine pellets (Ajax) and 0.1% w/v chromium (II) potassium sulfate dodecahydrate ($\text{CrK}_2\text{O}_8\text{S}_2 \cdot 12\text{H}_2\text{O}$; Fluka).

5.3.4 Methods

Sixteen rats were selected by stratified randomization and then divided into two groups of eight rats. Rats were fasted overnight and then given a single oral dose of 2000 mg/kg of the rambutan rind extract dissolved in DDD water at a dosing volume of 1 ml/kg while the control group received DDD water (1 ml/kg). Ninety minutes after each treatment, all rats were anesthetized with pentobarbital sodium (Nembutal, Ceva Sante Animale, Libourne, France) at a dose of 60 mg/kg (i.p.). Blood samples (3 ml from each rat) were collected *via* cardiac puncture into heparinized tubes and centrifuged at 2000 rcf for 5 min, obtained plasma was stored -20 °C until further analysis. Plasma biochemical parameters [triglyceride (TG), total cholesterol (TC), glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] were determined using automated analyzer (Hitachi 911, Japan).

After blood collection, the rats were perfused through the left ventricle of the heart with 200-250 ml of ice-cold heparinized saline at a flow rate of 40 ml/min using peristaltic pump (model SP 311, VELP Scientifica, Europe). Immediately after starting the pump, the right atrium was cut to allow an escape route for the blood and perfusion fluid. After the atrium effluent was clear, the rats were perfused with 300-350 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at a flow rate of 40 ml/min to fix the brain. After that, the brain were removed and soaked in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C. Brains were then transferred into 30% sucrose in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight or until the brain sank at 4 °C and 30% sucrose in 0.1 M

phosphate buffer (pH 7.4) at 4 °C for 24 h. After that, the brains were covered with powdered dry ice unit frozen and then stored at -20 °C until sectioned.

The brains regions of the hypothalamus [PVN, LHA, PFA, and ARC] and the NTS in the caudal medulla of brainstem were identified according to the rat brain stereotaxic atlas of Paxinos and Watson (2009). Frozen brains were sectioned coronally (30 µm thickness) through the level of the PVN (bregma -1.08 to -1.92 mm; Figure 5.1), LHA (bregma -2.16 to -3.84 mm; Figure 5.1), PFA (bregma -2.64 to -3.60 mm; Figure 5.1), and ARC (bregma -1.80 to -4.36 mm; Figure 5.1) and 40 µm thickness for coronal sections of the NTS (bregma -11.16 to -14.76 mm) using a cryostat (Microm HM 525, Microm International GmbH., Germany). Free floating sections were then stored in an anti-freeze cryoprotectant at -20 °C.



Table 5.1 Abbreviations of the hypothalamic brain regions. Nomenclature and abbreviations are from stereotaxic atlas of the rat brain (Paxinos and Watson, 2009).

Abbreviation	Full name
ArcD	Arcuate hypothalamic nucleus, dorsal part
ArcL	Arcuate hypothalamic nucleus, lateral part
ArcLP	Arcuate hypothalamic nucleus, lateroposterior part
ArcM	Arcuate hypothalamic nucleus, medial part
ArcMP	Arcuate hypothalamic nucleus, medialposterior part
10Cb	10 th cerebellar lobule (nodule)
chp	Choroid plexus
f	Fornix
MCLH	Magnocellular nucleus of the lateral hypothalamus
MRe 3V	Mammillary recess of the 3 rd ventricle
PaAP	Paraventricular hypothalamic nucleus, anterior parvicellular part
PaDC	Paraventricular hypothalamic nucleus, dorsal cap
PaLM	Paraventricular hypothalamic nucleus, lateral magnocellular part
PaMM	Paraventricular hypothalamic nucleus, medial magnocellular part
PaMP	Paraventricular hypothalamic nucleus, medial parvicellular part
PaV	Paraventricular hypothalamic nucleus, ventral part
PeFLH	Perifornical part of the lateral hypothalamus
PeF	Perifornical nucleus
PLH	Peduncular part of the lateral hypothalamus
TuLH	Tuberal part of the lateral hypothalamus
3V	3 rd ventricle
4V	4 th ventricle

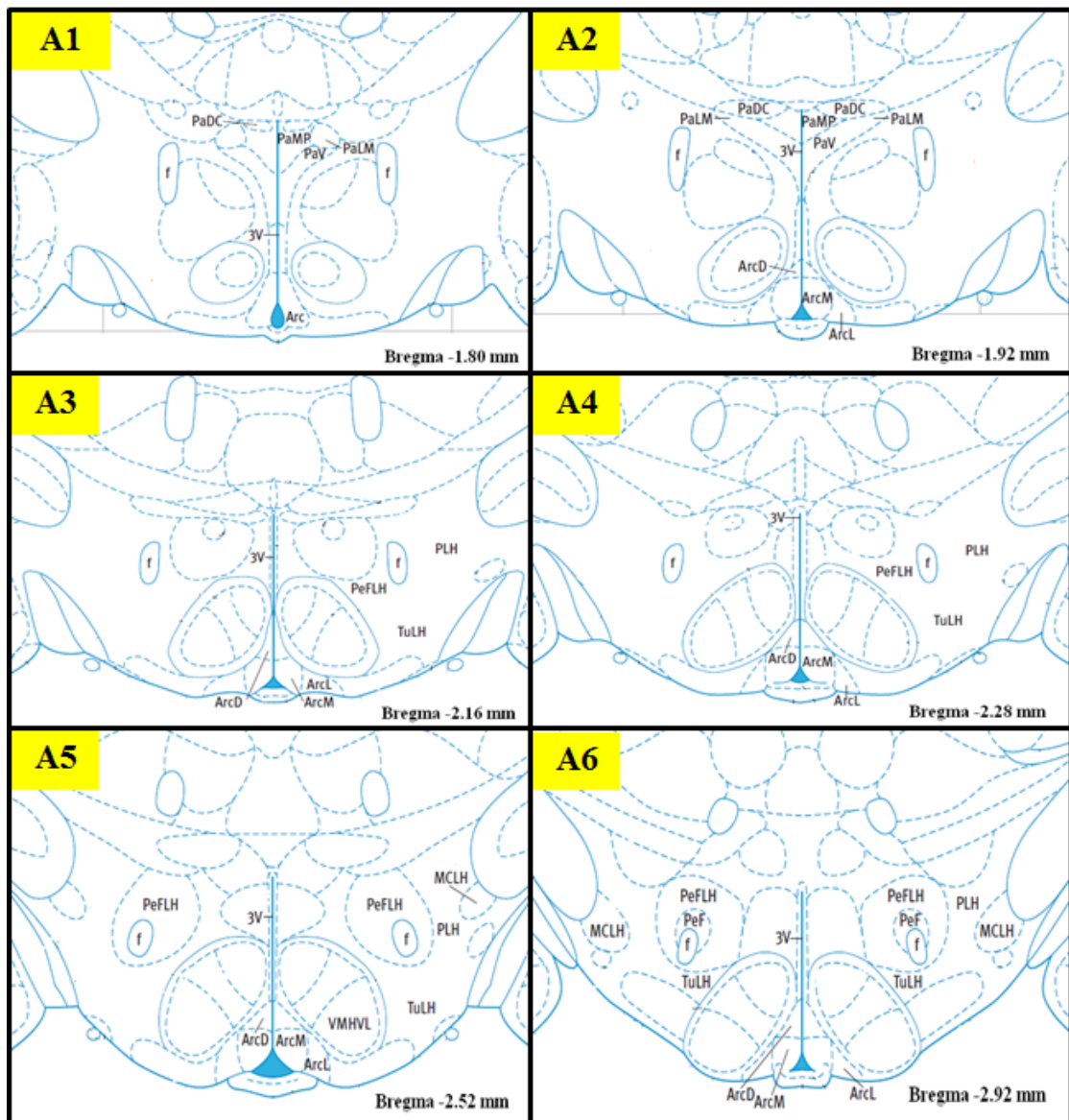


Figure 5.1 Schematic diagrams of coronal sections illustrating the brains regions of the ARC (-1.80 to -4.36 mm from bregma) from A1-A18. Coronal illustrations are redrawn, with the given coordinates, from the stereotaxic atlas of the rat brain (Paxinos and Watson, 2009). For abbreviations, see Table 5.1.

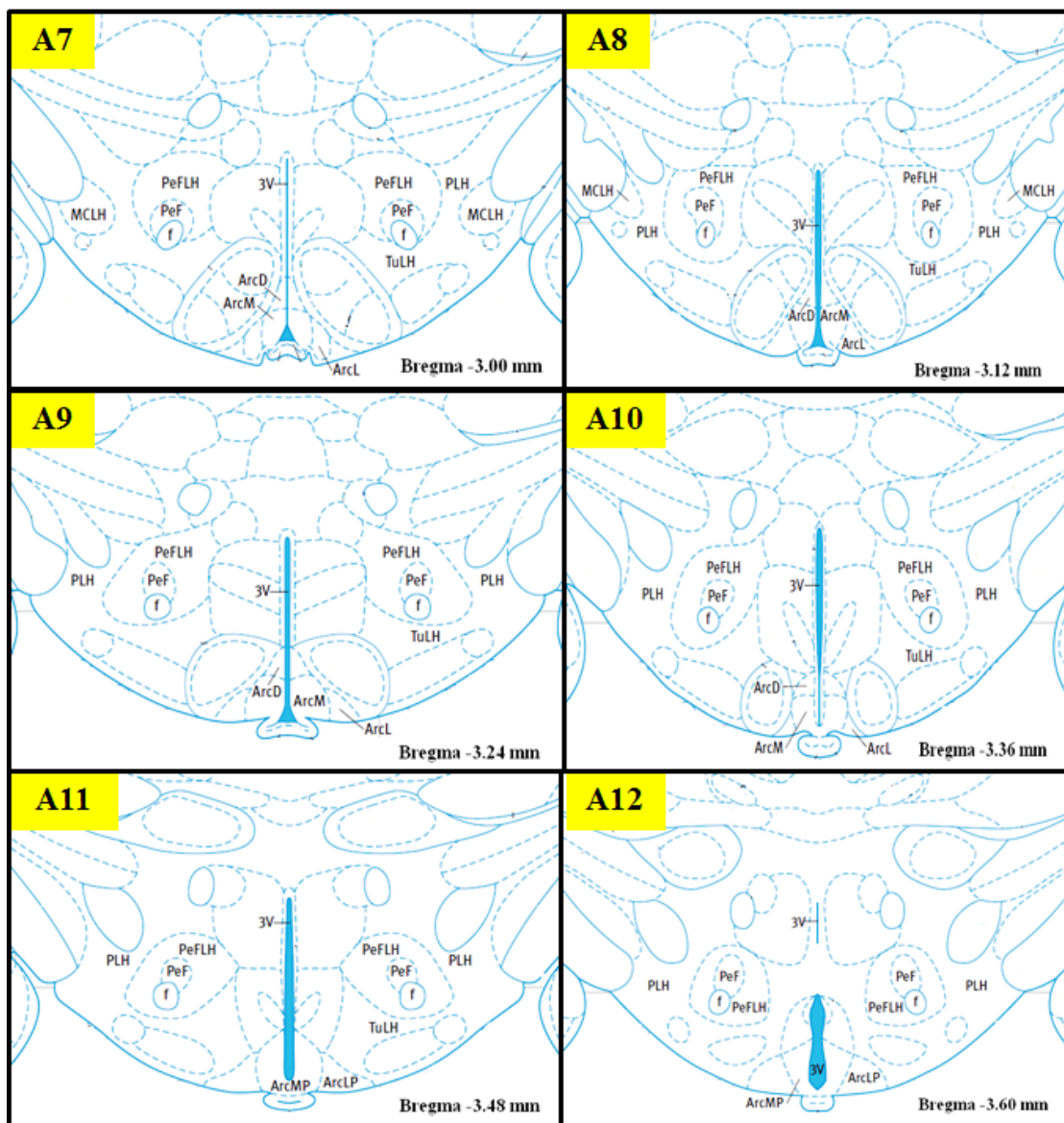


Figure 5.1 Schematic diagrams of coronal sections illustrating the brains regions of the ARC (-1.80 to -4.36 mm from bregma) from A1-A18 (Cont.). Coronal illustrations are redrawn, with the given coordinates, from the stereotaxic atlas of the rat brain (Paxinos and Watson, 2009). For abbreviations, see Table 5.1.

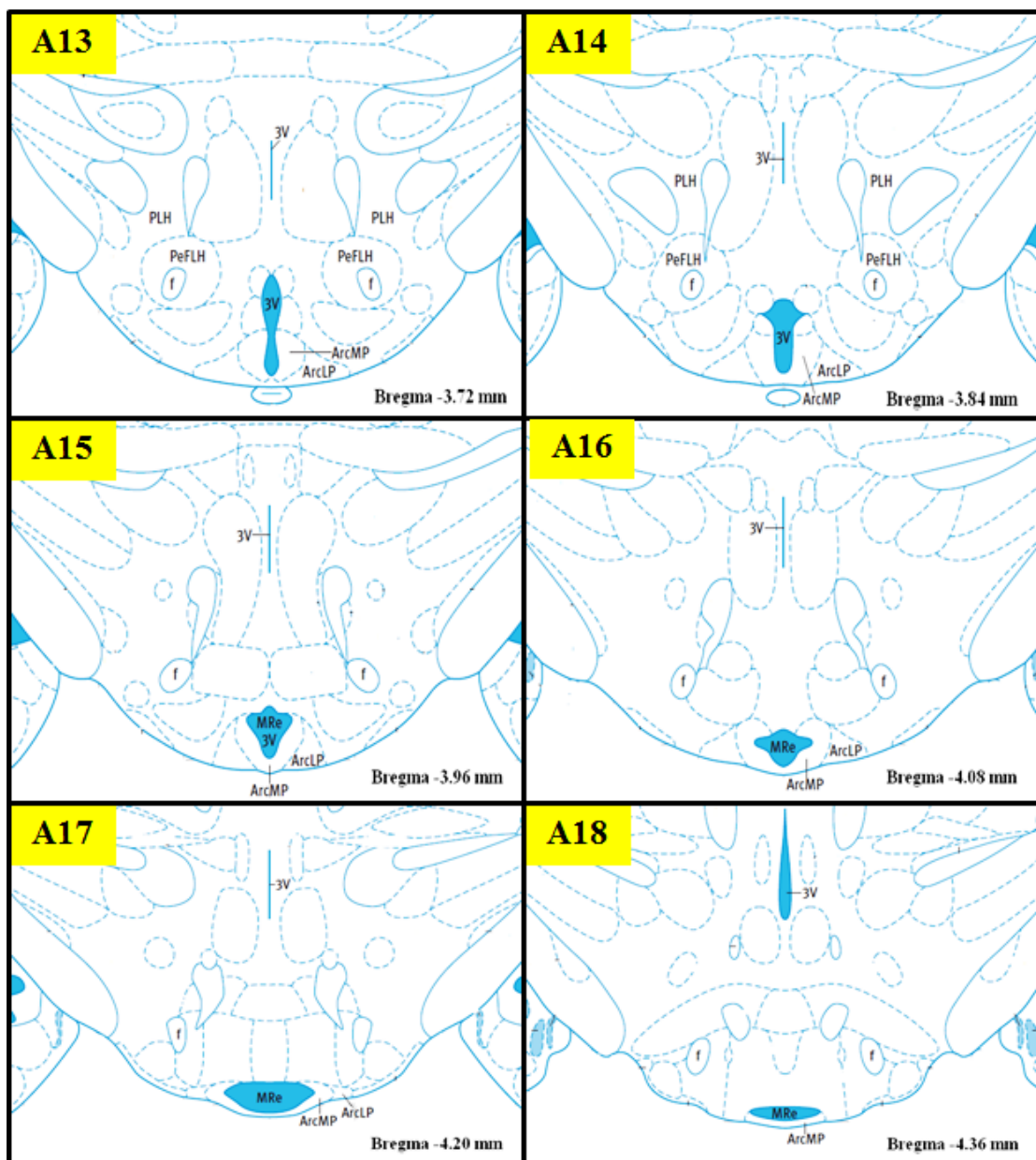


Figure 5.1 Schematic diagrams of coronal sections illustrating the brains regions of the ARC (bregma -1.80 to -4.36 mm) from A1-A18 (Cont.). Coronal illustrations are redrawn, with the given coordinates, from the stereotaxic atlas of the rat brain (Paxinos and Watson, 2009). For abbreviations, see Table 5.1.

5.3.4.1 Fos immunohistochemistry

Free-floating sections were immunostained for Fos according to the avidin-biotin complex (ABC) methods. Briefly, these sections were washed three times (x10 min) with 0.1 M phosphate buffer (pH 7.4), fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h, washed three times (x10 min) with 0.1 M phosphate buffer (pH 7.4) and then washed six times (x15 min) with washing solution. Endogenous peroxidase was then deactivated with hydrogen peroxide solution for 15 min, washed three times (x10 min) with washing solution and then blocked with preincubation buffer for 30 min. After that, the sections were incubated with c-Fos Ab-5 primary antibody (1:5,000 diluted in preincubation buffer) for 48 h in 500 µl labelled plastic bottles (Sterilin[®], Sterilin Ltd., Aberbargoed, UK) at 4 °C. Sections were then washed eight times (x5 min) with washing solution, incubated in secondary antibody solution for 1 h at room temperature and then washed three times (x10 min) with washing solution. After that, the sections were incubated in ABC complex solution for 1 h at room temperature and then washed two times (x10 min) with washing solution. The sections were rinsed with 0.1 M acetate buffer for 5 min and then incubated with Ni-DAB-H₂O₂ solution for approximately 10 min. The reaction was stopped by stop solution for 5 min. The sections were then washed in sodium phosphate buffer solution (pH 7.4) for 5 min, rinsed three times (x5 min) with 0.1 M phosphate buffer (pH 7.4) and mounted onto the chrome alum gelatin subbed slides. Slides were dried at 45 °C on the hotplate (Medex Nagel GmbH., Germany). After that, the sections were dehydrated with serial dilution alcohol (70%, 90%, 95%, 100%, and 100%, 5 min each) followed by xylene (2x5 min). Slides were finally

coverslipped using DPX mountant (BDH). This method was performed with gentle agitation on an orbital shaker VRN-360 (Gemmy Industrial Corp., Taiwan).

5.3.4.2 Quantitative analysis

Quantitative assessment of Fos-positive neuron was achieved by counting the number of Fos-positive cells in the PVN, LHA, PFA, and ARC and the NTS in the caudal medulla of brainstem. Cells with distinct blue-black nuclear Fos staining in PVN, LHA, PFA, and ARC and the NTS areas were manually counted under light microscopy (X10 objective, Nikon ECLIPSE 80i, Nikon Corporation Ltd., Japan) and images were captured and stored by DP72 software (Olympus, Tokyo, Japan).

5.3.5 Statistics

Results were expressed as mean \pm S.E.M. and analyzed by student's *t*-test (SigmaStat version 3.5). *P*-values less than 0.05 ($P < 0.05$) were considered statistically significant.

5.4 Results

In this experiment, the effects of the rambutan rind extract on the plasma biochemical parameters (TG, glucose, TC, AST, and ALT) and Fos expression in the hypothalamus (PVN, LHA, PFA, and ARC areas) and the NTS in the caudal medulla brainstem were determined. The effects of the rambutan rind extract on plasma biochemical parameters were shown in Figure 5.2. There were no significant difference in the plasma AST levels of the rambutan rind extract vs control ($91.39 \pm$

5.43 vs 86.33 ± 4.70 U/L) and ALT (36.86 ± 3.90 vs 29.52 ± 1.61 U/L). In comparison to the control, the rambutan rind extract significantly reduced plasma glucose (190.00 ± 4.89 vs 227.38 ± 11.89 mg/dl, rambutan rind extract vs control), TG (37.29 ± 5.28 vs 59.44 ± 5.71 mg/ml, rambutan rind extract vs control), and TC (66.04 ± 5.23 vs 76.67 ± 4.10 mg/dl, rambutan rind extract vs control) (Figure 5.2).

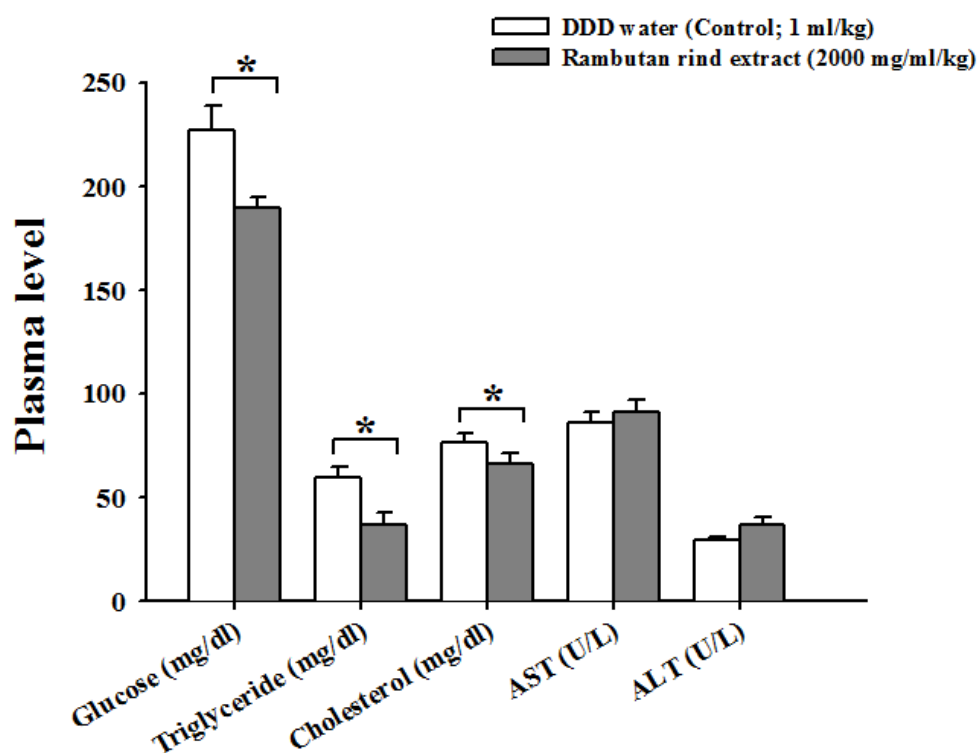


Figure 5.2 Plasma biochemical parameters of male Wistar rats orally administered with the rambutan rind extract. Values are expressed as mean \pm S.E.M.

* Significantly different from control group, $P < 0.05$ (Student's *t*-test).

The present results revealed that the rambutan rind extract induced Fos expression in the ARC in both control and the rambutan rind extract treated rats (Figure 5.3), but had no effect on Fos expression in the PVN, LHA, PFA, and NTS. The rambutan rind extract did not cause a significant difference in the number of Fos-

positive neurons in whole area of the ARC when compared with the control (Figure 5.4). A significant increase in the number of Fos-positive neurons was shown in the area of the ARC ranging from -3.60 to -3.96 mm from bregma following the rambutan rind extract compared to the control (38.17 ± 10.59 vs 8.75 ± 3.65 , rambutan rind extract vs control), as shown in Figure 5.5. There was no difference in other areas of the ARC (-1.80 to -3.48 mm and -4.08 to -4.36 mm from bregma). In comparison to control, the medialposterior and the lateralposterior regions of the area of the ARC ranging from -3.60 to -3.96 mm, the rambutan rind extract significantly increased in the numbers of Fos-positive neurons in both regions (Figure 5.6). Following the administration of the rambutan rind extract, the medialposterior region appeared to have higher number of Fos-positive neurons than the lateralposterior region, but no significant difference was found in comparison between these 2 regions (Figure 5.6).



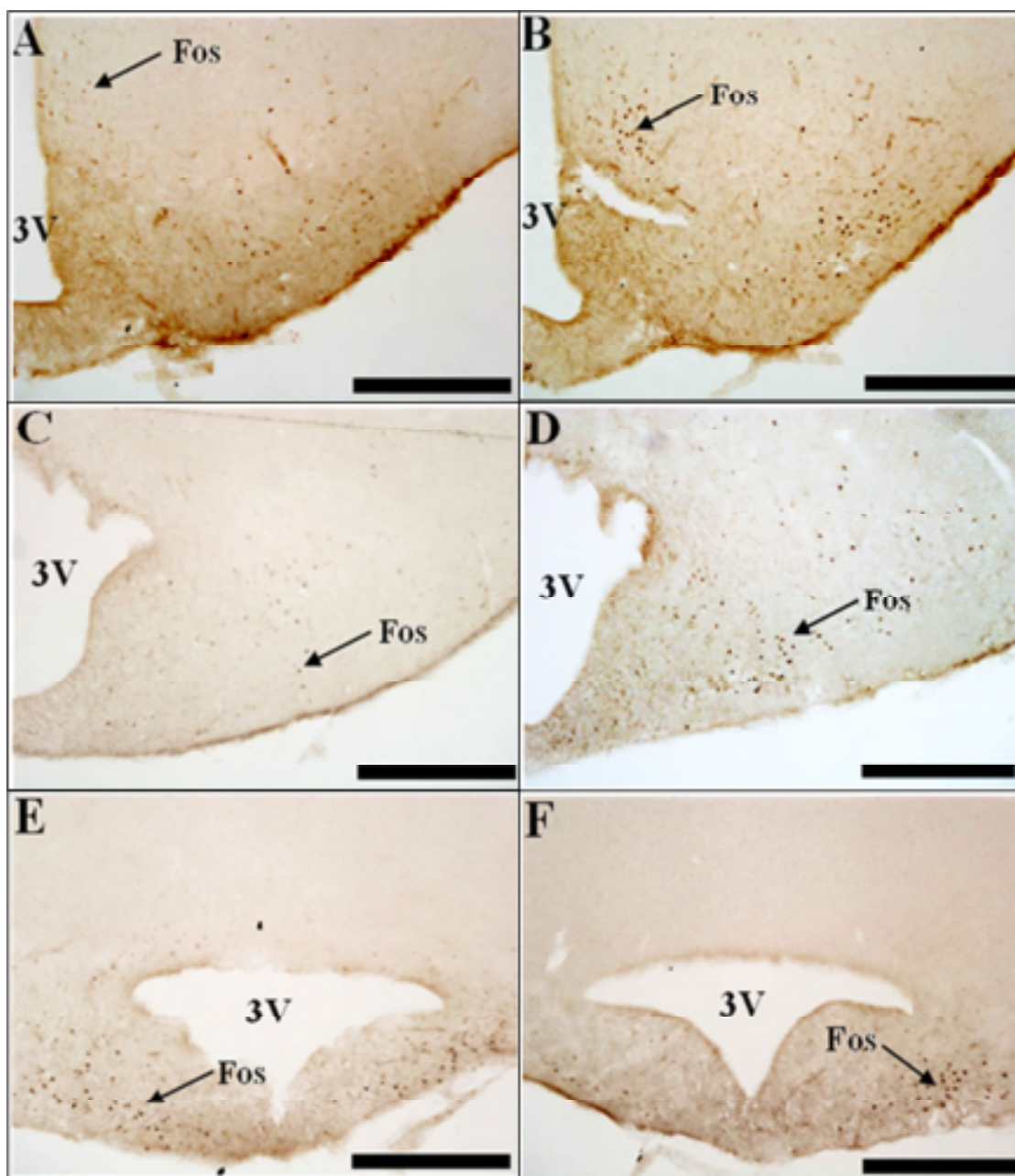


Figure 5.3 Effect of oral administration of the rambutan rind extract on Fos expression in the ARC. Photomicrographs illustrates Fos expression in the ARC after oral administration of either 1 ml/kg of DDD water (A, C, and E) or 2000 mg/ml/kg of the rambutan rind extract (B, D, and F) (A and B, -1.80 to -3.48 mm from bregma; C and D, -3.60 to -3.96 mm from bregma; E and F, -4.08 to -4.36 mm from bregma). Fos expression

in the area of the ARC ranging from -3.60 to -3.96 mm from bregma of the rambutan rind extract group is stronger than the control group. Abbreviations: 3V, third ventricle. Arrow indicates Fos-positive neurons. Scale bars = 100 μ m.

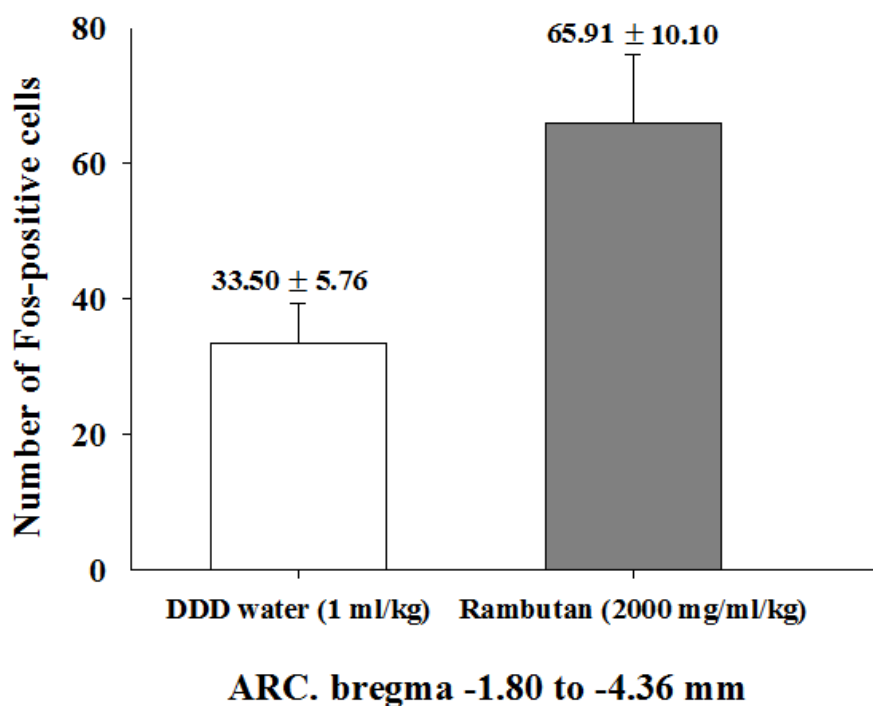


Figure 5.4 Effect of the rambutan rind extract on the number of Fos-positive neurons per section in the area of the ARC ranging from -1.80 to -4.36 mm from bregma. Values are expressed as mean \pm S.E.M. of average number of cells/section in the ARC.

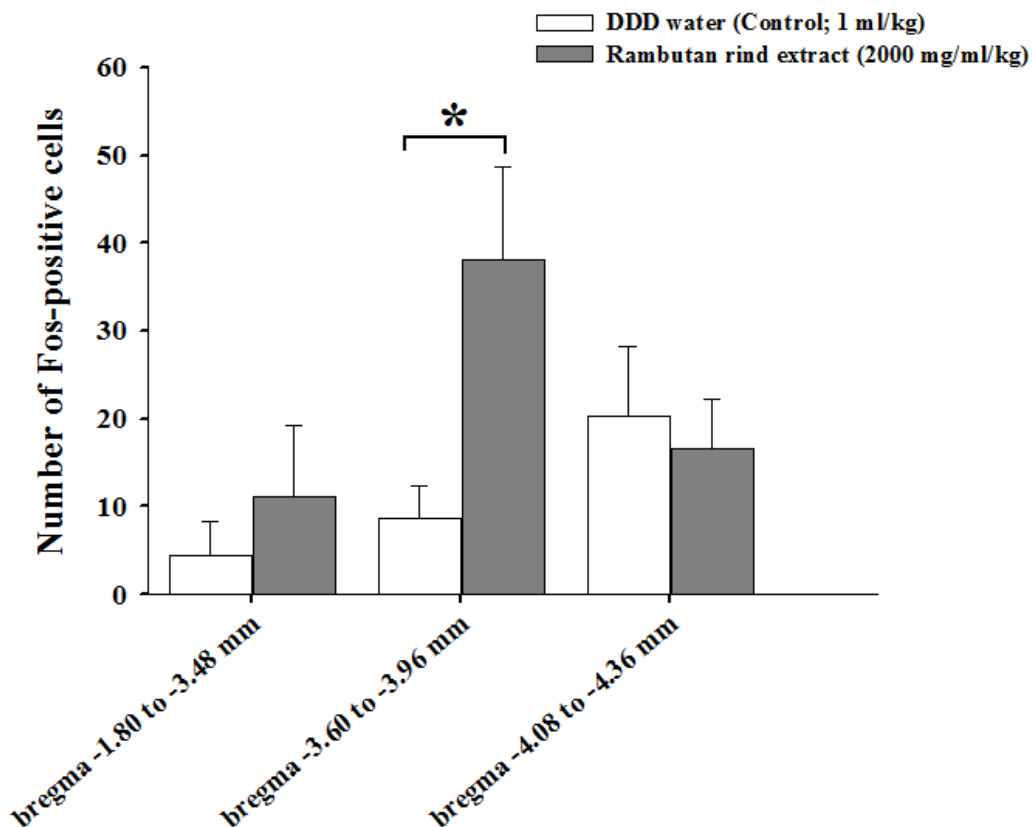


Figure 5.5 Effect of the rambutan rind extract on the number of Fos-positive neurons per section in 3 areas of the ARC (-1.80 to -3.48 mm, -3.60 to -3.96 mm, and -4.08 to -4.36 mm from bregma). Note that the number of Fos-positive neurons are increased significantly in the area of ARC ranging from -3.60 to -3.96 mm from bregma in the rambutan rind extract treated group compared to the control group ($P < 0.05$). Values are expressed as mean \pm S.E.M. of average number of cells/section in the ARC area. * Significantly different from the control group, $P < 0.05$ (Student's *t*-test).

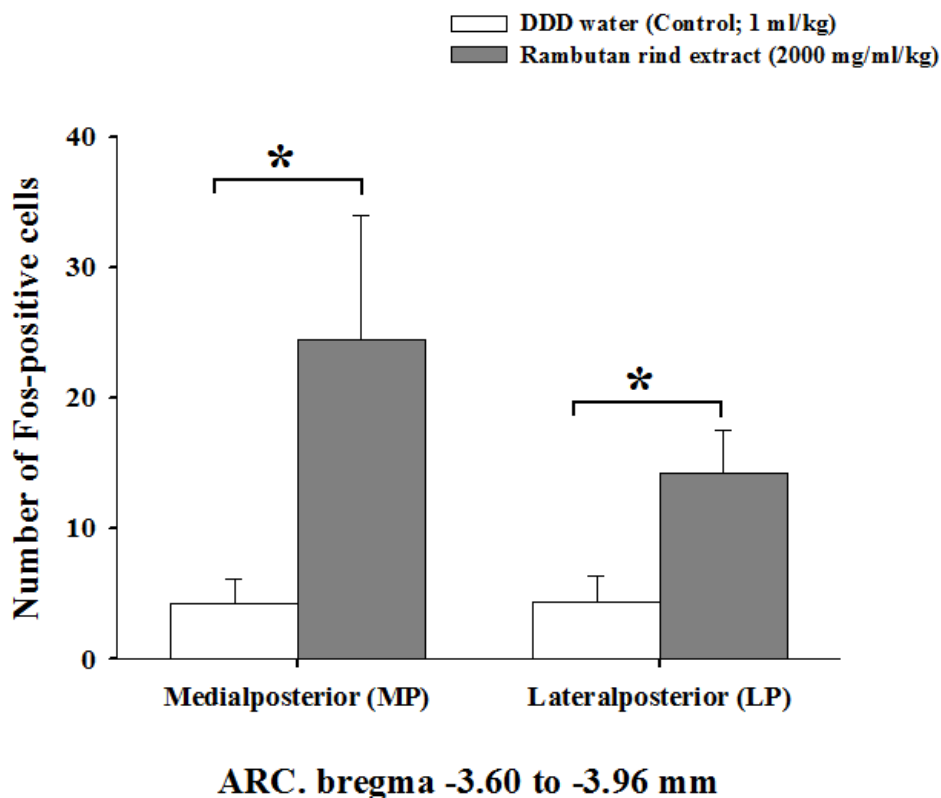


Figure 5.6 Effect of the rambutan rind extract on the number of Fos-positive neurons per section in the medialposterior and lateralposterior regions of the ARC area ranging from -3.60 to -3.96 mm from bregma. Values are expressed as mean \pm S.E.M. of average number of cells/section in each region of the ARC area. * Significantly different from control group, $P < 0.05$ (Student's t -test).

5.5 Discussion and conclusion

This study investigated the effects of the rambutan rind extract on the plasma biochemical parameters (TG, glucose, TC, AST, and ALT) and Fos expression in the hypothalamus (PVN, LHA, PFA, and ARC areas) and the NTS in the caudal medulla brainstem. Our findings indicated that the plasma levels of TG, TC, and glucose were significantly decreased while plasma levels of AST and ALT were not significant difference between rats administered with the rambutan rind extract (2000 mg/ml/kg) and the control group (1 ml/kg DDD water) for 90 minutes. The rambutan rind extract appeared nontoxic as evidenced by no difference in the plasma AST and ALT levels of the rambutan rind extract administration compared to the control. A reduction in plasma TG and TC levels in the rambutan rind extract treated rats revealed that the rambutan rind extract had the greatest potential for protection against obesity and atherosclerosis (its accompanying risk of cardiovascular diseases) and may be correlated with a potentially decrease in plasma glucose levels, which in turn may depress the mobilization of fat (Mansi et al., 2007). A decrease in plasma glucose levels in the rambutan rind extract treated rats demonstrated that the rambutan rind extract could produce some anti-hyperglycemic effect, similar results were observed by Palanisamy et al. (2011a; 2011b), that may be a result of inhibitory effects of the rambutan rind extract on alpha-amylase and alpha-glucosidase enzyme activities *in vitro* (results from chapter III).

The present results revealed that the rambutan rind extract induced Fos expression only in the ARC, but not in the PVN, LHA, PFA, and NTS. The rambutan rind extract did not cause a significant difference in the number of Fos-positive neurons in whole area of the ARC (-1.80 to -4.36 mm from bregma). The rambutan

rind extract significantly increased the number of Fos-positive neurons only in the area of the ARC ranging from -3.60 to -3.96 mm from bregma, compared to the control. When compared between medialposterior and lateralposterior regions of this area of the ARC, the rambutan rind extract significantly increased the number of Fos-positive neurons in both regions when compared to the control. The numbers of Fos-positive neurons induced by the rambutan rind extract in the medialposterior region of this area of the ARC were higher than the lateralposterior regions, but there was no significant difference between these two regions. These results revealed the central effect of the rambutan rind extract as the activation of the medialposterior and the lateroposterior arcuate neurons in the ARC area ranging from -3.60 to -3.96 mm from bregma was observed. From the present findings that the rambutan rind extract activated neuronal activation in both medialposterior and lateralposterior regions of the ARC, the rambutan rind extract may alter body weight and food intake by activating the ARC neurons since the ARC is essential in the regulation of body weight and food intake. However, it cannot be ruled out that orexigenic NPY/AGRP neurons or anorexigenic POMC/CART neurons were activated by the rambutan rind extract. In conclusion, the present findings suggested the hypoglycemic, hypocholesterolemic, and hypotriglycemic effects of the rambutan rind extract. The rambutan rind extract may have a central effect on changes of body weight and food intake by acting on the ARC neurons. However, further investigations are needed to identify which neuronal types in the ARC are activated by the rambutan rind extract and to investigate further physiological mechanisms of the rambutan rind extract on body weight control.

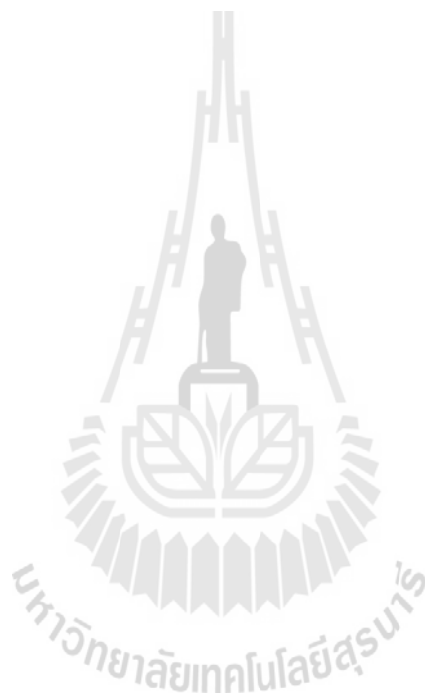
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CHAPTER VI

CONCLUSION

Acute and sub-chronic toxicity studies of the rambutan rind extract were demonstrated for the first time in male Wistar rats. The acute toxicity data indicated that the NOAEL for the oral dose of the rambutan rind extract was 3000 mg/kg and the LOAEL was 4000 mg/kg. The evaluated oral LD₅₀ of the rambutan rind extract was greater than 5000 mg/kg in male Wistar rats. In the sub-chronic toxicity study, the NOAEL of the rambutan rind extract was 1000 mg/kg and the LOAEL was 2000 mg/kg. Thus, these studies of acute and sub-chronic toxicity in rats will help for future clinical studies of the medicinal safety and *in vivo* experimental studies of the pharmacological potential of medicine plant.

The rambutan rind extract is a good source of phenolic compounds, including anthocyanin and tannin, which possess potent antioxidant activity. In the present study, the rambutan rind extract contained high total phenolic content (416.18 ± 0.01 mg gallic acid/g dry extract), and total anthocyanin content (14.90 ± 0.8 μ g/g dry extract), with IC₅₀ value of 0.288 ± 0.04 mg/ml extract. For the first time, in the present study, we have described the potent anti-obesity effect of the rambutan rind extract, both *in vitro* and *in vivo*. The rambutan rind extract and phenolic compound and tannin could inhibit pancreatic lipase, alpha-amylase, and alpha-glucosidase enzyme activities *in vitro* (Figure 6.1). The maximum percent inhibition of pancreatic lipase, alpha-amylase, and alpha-glucosidase activities of the rambutan rind extract were higher than that of the anti-obesity drug orlistat (40 mg/ml). Inhibitions of pancreatic lipase, alpha-amylase, and alpha-glucosidase of the rambutan rind extract

lead to a reduction in the intestinal absorption of lipids and carbohydrates. Therefore, inhibitions of these digestive enzymes are beneficial in prevention and treatment of obesity. The *in vivo* study provided the first evidence of the rambutan rind extract and tannin could suppress the increased plasma levels of TG, glucose, AST, and ALT in lipid emulsion-induced hyperlipidemia rats.

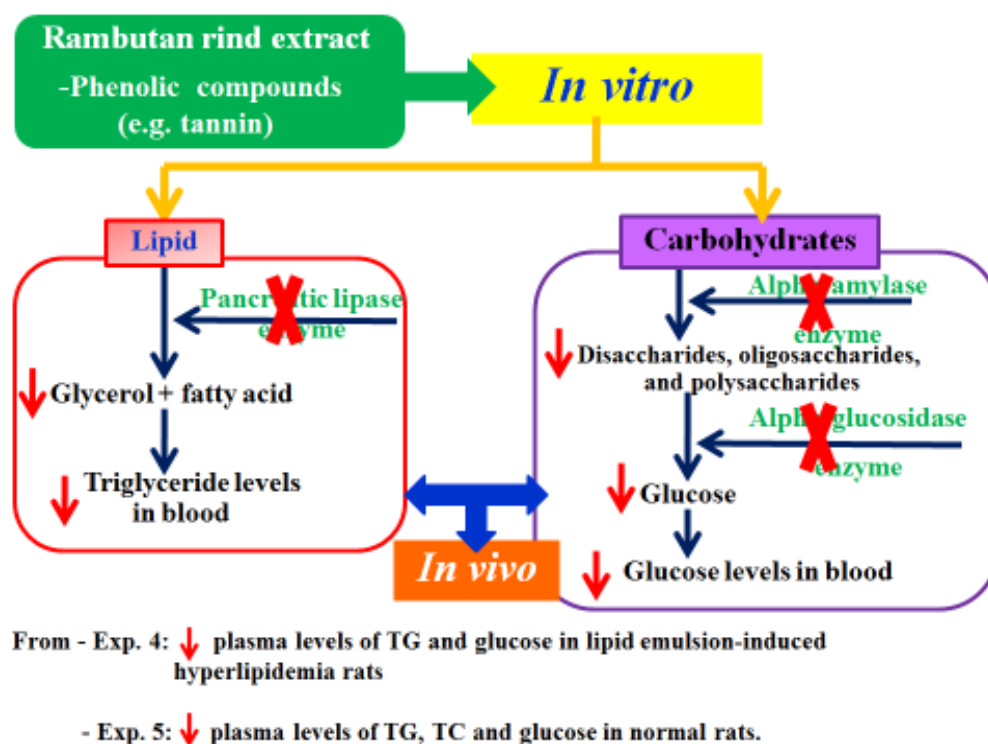


Figure 6.1 Mechanism of anti-obesity actions of the rambutan rind extract *in vitro* and *in vivo*. (TG: triglyceride; TC: total cholesterol).

Additionally, central effect of the rambutan rind extract was demonstrated (Figure 6.2). The rambutan rind extract had a modulatory effect on the expression of a neuronal activation marker Fos in both medialposterior and lateralposterior regions of the ARC. Thus, the rambutan rind extract may involve in the regulation of feeding.

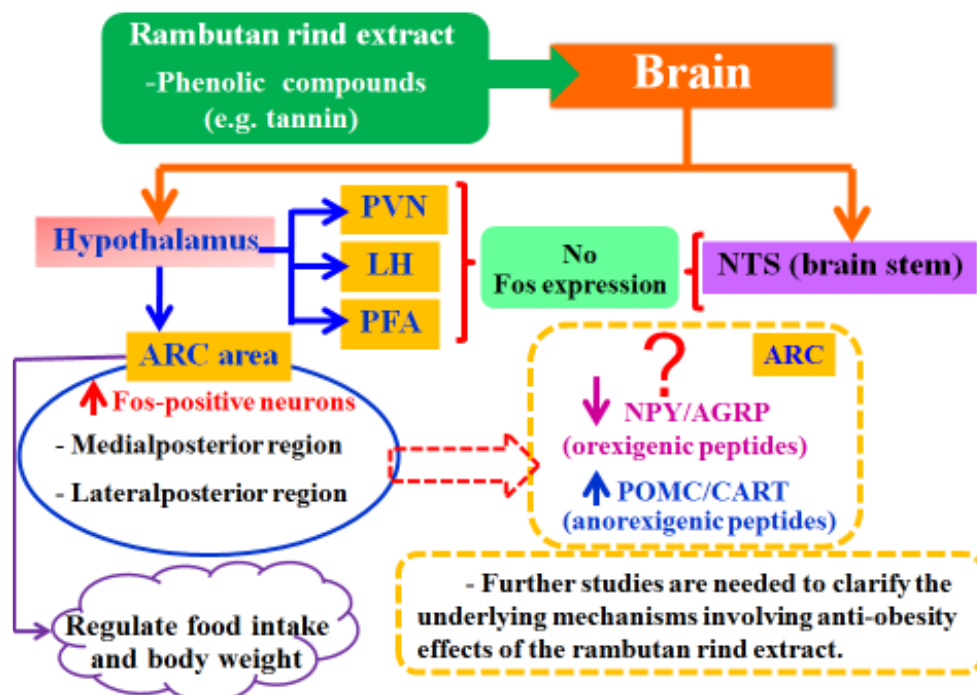


Figure 6.2 Central effect of the rambutan rind extract in male Wistar rats. (ARC: arcuate nucleus; NTS: nucleus of the solitary tract; PVN: paraventricular nucleus; LH: lateral hypothalamic area; PFA: perifornical area; NPY: neuropeptide Y; AGRP: Agouti-related peptide; POMC: pro-opiomelanocortin; CART: cocaine- and amphetamine-regulated transcript).

In conclusion, this study demonstrated potent anti-obesity of the rambutan rind extract *in vitro* and *in vivo*. Phenolic compounds, notably tannin, may be bioactive compounds responsible for the anti-obesity activity of the rambutan rind extract. Further studies are needed to clarify the underlying mechanisms involving anti-obesity effects of the rambutan rind extract.