CHAPTER I

INTRODUCTION

Rationale for the study

Trikatu is an Ayurvedic preparation consisting of dried fruits of black pepper (*Piper nigrum* Linn; Piperaceae), dried fruits of long pepper (*Piper longum* Linn; Piperaceae) and dried rhizomes of ginger (*Zingiber Officinale Rosce*; Zingiberaceae) in the ratio of 1:1:1; w/w (Atal, et al., 1981). Trikatu is an herbal compound that is considered as potent carminative as it provides a natural and safe support system for gastric function associated with gaseous distension (Johri, *et al.*, 1992). Trikatu is known in Thailand as the "rainy season medicine" that strongly thermoregulation and removes cold, congestion, and revives weak organic functions. Trikatu is reported to be not only an anti-mucus and digestive powder used to tone up gastric and respiratory function, but also to be useful in cases of obesity, high cholesterol, high triglycerides, hypothyroid, slow metabolism and various inflammatory conditions (Sivakumar, 2004). Nevertheless, little is known about the pathways underlying the effect.

The pharmacological actions in herbal ingredient of Trikatu, in particular, on lipid control have been reported. Ginger had a significant decrease in blood glucose, total serum triglyceride and cholesterol, but increased in high-density lipoprotein cholesterol (HDL-c) (Ahmed and Sharma, 1997). However, it failed to decrease the body weight (Mascolo, et al., 1989). Sharma, et al. (1996) studied hypolipidemic and anti-atherosclerotic effects of ginger in cholesterol-fed rabbits. The administration of ginger extract increased fecal excretion of cholesterol, thus suggesting a modulation of absorption. In addition, lower total serum cholesterol and low density lipoprotein cholesterol (LDL-c) level were observed after ginger extract treatment. However, a few reports showed the lipid control action of black pepper and long pepper. The ethanol extract of the long pepper fruit yields piperlonguminine, piperine, and pipernonaline as the main antihyperlipidemic constituents. Piperlonguminine has the effect of regulating lipid metabolism by reducing the serum total cholesterol, triglyceride, LDL-c. The mechanism is likely related to increasing mRNA expression

of LDL receptor and decreasing apolipoprotein B (ApoB) expression (Ma, et al., 2008). Methyl piperine has been reported to significantly inhibit the elevation of total serum cholesterol in rats fed with a high cholesterol diet (Wang, et al., 1993).

But to our knowledge, the process of lipid control of Trikatu remains unclear. Liver is a major site of metabolism, which it is keys characteristic of pharmacologic and metabolic respond of drug administration. The processes of lipid control may be regulated by many proteins in liver. The proteome-wide approach is then used to study the dynamic change of proteins in liver as a result of Trikatu. The results may provide an insight in many biochemical pathways associated with lipid control.

Purpose of the study

1. To investigate the morphological changes in Trikatu fed rat including body weight, vital organs and histopathology.
2. To monitor rat liver function and lipid profile during exposure to Trikatu.
3. To study the proteome profile of rat liver proteins after treated with Trikatu.

The importance of the study

This is the first proteomics study regarding the effect of Trikatu in rats. This can be used as a model to study the effect of herbal treatment in animals. The differential expressed proteins in rat liver after feeding with Trikatu may be used as potential biomarkers and/or therapeutic targets.

Scope of the study

The *in vivo* effects of Trikatu extracts on rat liver were investigated. The experiments were divided into 2 parts, the acute effect and the subacute effect. Male Wistar rats (200-250 g) were treated with each ethanolic extract of Trikatu by gavage for 7 days for acute effect and 30 days for sub-acute effect. Changes in daily body weight and vital organ weights (liver, kidney, lung, spleen and heart), serum lipid profile and AST/ALT enzyme, and liver proteome after exposure to Trikatu was monitored and compared to control.

Hypothesis

Trikatu was reported to reduce serum lipid in rat but had no effect on the body weight. Then, proteomic approach was used to seek for proteins respond to Trikatu treatment and associate with the regulation of lipid metabolism in the liver of Trikatu fed rat. These proteins may serve as potential therapeutic targets and or may be used to treat patients clinically.

Anticipated outcomes

1. This study may help understanding the effect of Trikatu on metabolic pathways, enzymes or transcription factors which are essential to control lipid metabolism in liver.

2. This study can support herbal drug information that is beneficial for traditional doctors and users.