

CHAPTER I

INTRODUCTION

1.1 Background and definition of research problems

Estrogen deficiency is considered to be an important factor resulting in the high risk of the cognitive degeneration diseases, such as Alzheimer's disease (AD) or dementia, in the postmenopausal women. Estrogen replacement therapy (ERT) has been widely used in clinical to facilitate these cognitive degeneration syndromes (Anderer *et al.*, 2005; Genazzani *et al.*, 2007; Grossman, 2000). However, the adverse effects of ERT (i.e. the high risk of carcinogenesis in reproductive systems) come with the benefits. The concerns led to the requirement in the new drug development. (Barnabei *et al.*, 2005; Collins, Blake & Crosignani, 2005; Grodstein *et al.*, 2000). The more selective substitutes of estrogen which could target to the central nervous system (CNS) are required to replace the current argumentative ERT. Phytoestrogens are natural compounds isolated from various plants with the estrogenic-like functions. Its natural probabilities give it the high potential to replace the estrogen in the ERT (Dixon, 2004; Glazier & Bowman, 2001).

Curcuma comosa Roxb. (Waan chak mot luk in Thai) is a Thai traditional herbal medicine, which is widely used in folk medicine for anti-inflammatory action, treatment of postpartum uterine bleeding, peri-menopausal bleeding and for relieving uterine inflammation and aromatic stomachic. Its hexane extract has been scientifically reported to have the estrogenic-like effects (Piyachaturawat, Ercharuporn & Suksamrarn, 1995a, 1995b). This unique characteristic provides us an option for developing an estrogen substitute which might mimic the positive effect of estrogen in the central nervous system selectively.

1.2 Objectives of the research

- a. To investigate the effects of CHE on the spatial memory of the ovariectomized rats.
- b. To investigate the effects of CHE on the biochemistry and morphological level in the rat brain and its relationship to the behaviors.

c. To provide the pharmacokinetic information of the CHE, of which is a key factor for the further development of the product.

1.3 Hypothesis of the research

The CHE exerts the effects similar to estrogen in improving the cognitive conditions in the ovariectomized rats. Some similar biochemical and morphological changes in the rat brain from the CHE and estrogen treatments could be observed.

1.4 The scope of the research

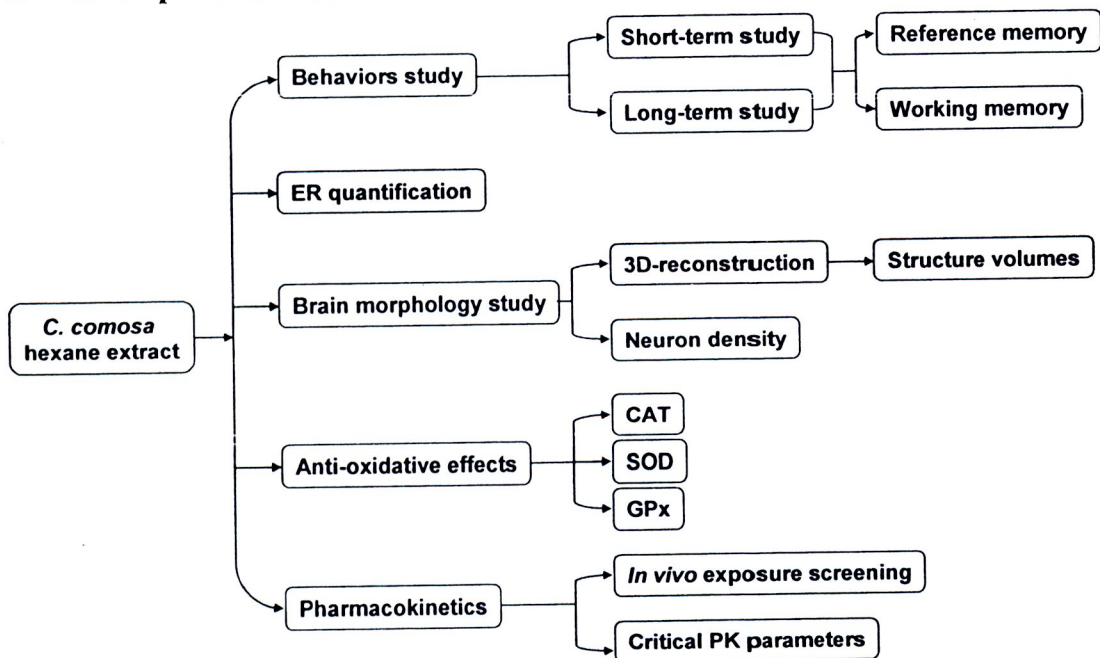


Figure 1 The research frame work.

Ovariectomized (OVX) rats that are widely used as the animal model to mimic the estrogen deficiency in the postmenopausal status have been reported to have the impairment in the memories. In the present study, the classic Morris water maze and radial arm maze test were conducted to evaluate the spatial reference memory and working memory on the OVX rats, which were received the administration of estradiol, CHE or the vehicle. Both short-term and long-term memories were evaluated, which could directly provide the information of the effects from the treatments.

Estrogen receptors (ER) (alpha and beta subtypes) have been reported to distribute in various brain regions and respond to the estrogen levels. The ER mRNA quantification in the present study provided the information whether the CHE regulate the ER, or more accurately, selectively regulate the ER in the brain, which could be the evidence that links the performance in the behaviors test and the estrogen effect at the cellular levels.

A three-dimensional (3D) reconstruction method was developed to reconstruct a brain and its regional structures from the AChE and Nissl staining brain sections, and the brain volume measurement was obtained. The 3D model of the brain regions could provide a better vision on evaluating the morphology changes than using the traditional 2-dimension images. The hippocampus which is believed to be the most important region in the brain was reconstructed to quantify the volumes for evaluating the morphology changes subsequent to the behaviors results.

The anti-oxidative abilities of CHE was also measured considering the probability of containing antioxidants in this plant extract, which might also help in keeping the healthy status in the brain. Ethanol (EtOH) was used to induce the oxidative stress in the normal rat brains with or without the CHE administration. The superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities were measured to evaluate whether the CHE could increase the antioxidative abilities in the brain. These studies in the non-estrogenic-like pathway would help to clarify the mechanism under the behaviors changes.

Finally, a pharmacokinetics study of the crude extract was conducted by using the high pressure liquid chromatography (HPLC) at the animal level. By this method, the compounds which could be absorbed from the gastro-intestinal tract were identified. The distributions of the compound in the brain, reproductive systems and some other organs were also examined. These results provided the information of the target compounds in the crude extract for purification. Having the purified active compounds, the more delicate further studies could be conducted (Figure 1)

1.5 The anticipated outcomes

The present study will prove whether CHE exerts similar effect to estradiol on the spatial memory on the OVX rats and the related effect on the biochemistry and

morphology levels in the rat brain. The information of the potential active compounds and their pharmacokinetic parameter will be provided. The result will contribute to the development of a new phytoestrogen substitute in the ERT.