CAPTER I
INTRODUCTION

Methamphetamine (METH) is an illicit recreational drug that commonly abused in Thailand and worldwide. It has been known for many years that it can cause neurotoxicity, which cause long-lasting changes in the central dopaminergic pathway. METH-induced neurotoxicity has been associated with the formation of toxic reactive oxygen species (ROS), it induced excessive release of dopamine (DA) from vesicles to cytosolic and extracellular space. DA undergo degradation either via enzymatic or non-enzymatic pathway; ROS and/or DA quininone products are produced (Wu, Ping et al. 2007). Recent studies have suggested that humans who abuse METH have an increased risk for Parkinson's disease later in life as the loss of dopaminergic neuron in nigro-striatal pathway which is vital to control of motor functions (Guilarte 2001).

Caffeine, white crystalline xanthine alkaloid that acts as a stimulant drug was concocted to amphetamine-type stimulants (ATSs) tablets such as Ya-Ba to increase their weight and volume and to enhance the stimulating effect of METH (Puthaviriyakorn, Siriviriyasomboon et al. 2002). Combination treatment of METH and caffeine at individually non-toxic concentrations significantly decreased viability *in vivo* and *in vitro* (Sinchai, Plasen et al. 2011). The study showed that caffeine potentiates the toxic effects of methamphetamine possibly via a mechanism involving an increase in dopamine release and excess ROS generation. However, more studies regarding the mechanism of caffeine potentiating effect of METH is needed to be done.

Autophagy or self-eating process is an intracellular degradation process responsible for the clearance of most long-lived or damaged proteins and organelles in which cytoplasmic components are delivered into lysosomes for degradation. Autophagy is critical to life. It contributes to cell survival under stressful conditions and also contributes to cell death when it is either impaired or over activated. There

are many studies showed relation of up regulation of autophagy with either METH or caffeine treatment (Foukas, Daniele et al. 2002; Larsen, Fon et al. 2002; Kudchodkar, Yu et al. 2006; Castino, Lazzeri et al. 2008; Pasquali, Lazzeri et al. 2008; Kongsuphol, Mukda et al. 2009; Saiki, Sasazawa et al. 2011). According to critical role of autophagy to degrade oxidatively damaged proteins, it is believed to play a role in potentiating effect of caffeine on METH toxicity.

In addition to autophagy, apoptosis is another pathway believed to relate to either METH or caffeine toxicity. Apoptosis is a form of cell death in which individual cell committed suicide for the good of the whole organism. There are a variety of stimuli and conditions that can trigger apoptosis such as oxidative stress, radiation, DNA damaged, or chemical-induced cell death. According to that both autophagy and apoptosis can be triggered by ROS and to its critical role to cellular homeostasis. In this study, we aim to study the involvement of autophagy and apoptosis on caffeine potentiated METH-induced neurotoxicity in SH-SY5Y cells by examining cell viability and proteins that involve in these two pathways by using western blot analysis.

CHAPTER II
OBJECTIVES

1. To examine the potentiating effect of caffeine on METH-induced neurotoxicity of SH-SY5Y cells using cell viability assay.
2. To examine the involvement of autophagy on METH-induced neurotoxicity.
    a. To assess the effect of METH treatment on the expression of LC3II using western blot analysis.
    b. To assess the role of autophagy in METH-induced neurotoxicity using 3-methyladenine (3-MA), an autophagy inhibitor.
3. To determine pathways involving in caffeine potentiating effect of METH-induced neurotoxicity through western blot analysis.
    a. To examine the modulation of autophagy pathway by measuring the expression of LC3II, p-mTOR, and p-4Ebp1.
    b. To examine the modulation of apoptosis pathway by measuring the expression of cleaved caspase-3.
    c. To assess the role of autophagy in caffeine potentiates effect of METH-induced cell death using 3-methyladenine, an autophagy inhibitor.