CHAPTER I
INTRODUCTION

Anxiety is one of the most common psychological symptoms found in people, approximately 28.8 % of the populations are anxiety disorders and the incidence of clinical anxiety is increasing around the world (Kessler et al., 2005; Wittchen et al., 2011). Interestingly, women were more suffering from anxiety disorder than men (Seeman, 1997; Lim et al., 2005) especially during low estrogen levels i.e. following oophorectomy or during postmenopausal period (Avis et al., 2001; Schmidt, 2005). Such women required estrogen replacement therapy to improve these psychological symptoms (Ditkoff et al., 1991; Schmidt et al., 2000; Zeidan et al., 2011). These findings indicated that the ovarian steroid hormones especially estrogen may be partially responsible for anxiety disorder (Weiser et al., 2008; Hiroi and Neumaier, 2011). However, the molecular mechanism of estrogen to modulate anxiety is not as simple and possibly involved the different effects of estrogen on enzyme and neurotransmitter functions in brain related to anxiety.

The neural circuits that underlie anxiety were organized at different levels. Electrical and pharmacological studies indicated that the midbrain plays a key role in regulating anxiety (Schenberg et al., 2001; Zanoveli et al., 2005). In human and animal studies, anxiety-like responses such as unpleasant and fear-like sensations or freezing behavior were found when midbrain was stimulated (Amano et al., 1978; Schenberg et al., 2001; Brandao et al., 2003). The midbrain is known to contain a population of serotonergic neuron and project axon to innervate the limbic system, which is critically involved in regulation of anxiety (Beitz et al., 1986; Clement and Chapouthier, 1998; Oliveira et al., 2004). Activation of the serotonergic neuronal function could facilitate the generation of anxiety, whereas reduction of serotonergic function could induce the anxiolytic effects (Matos et al., 1996; Clement and Chapouthier, 1998). Moreover, Pandaranandaka and co-workers (2006; 2009) have shown that the alteration of serotonergic activity was related to anxiety disorder. In addition, the first-line prescribed drugs for treating anxiety is likely to modulate serotonergic system at some points; for example, selective serotonin reuptake

inhibitor (SSRI), 5-HT_{1A} agonist and monoamine oxidase inhibitor (Nash and Nutt, 2005; Koen and Stein, 2011).

Although various neurotransmitter systems are involved in the pathogenesis of anxiety disorder, the GABAergic system may be of major concerned as indicated by previous evidences (Carey et al., 1992; Malizia et al., 1998; Reddy and Kulkarni, 1999). Firstly, the most widely prescribed anxiolytic drugs are benzodiazepine groups which are acting on the $GABA_A$ receptor (Lopez-Munoz et al., 2011). Secondly, several anatomical studies found that serotonergic neurons in midbrain were under GABAergic system control (Gervasoni et al., 2000; Tao and Auerbach, 2000; Castilho et al., 2002). Local applications of $GABA_A$ receptor agonist, muscimol, into the midbrain inhibited serotonergic activity in the midbrain and forebrain, which could be blocked by $GABA_A$ receptor antagonist, bicuculline (Tao and Auerbach, 1994; Tao et al., 1996; Li et al., 2005). These results indicated that $GABA_A$ receptors in the midbrain have an important role in the regulation of serotonergic activity and thus dysfunction of $GABA_A$ receptor may contribute to the alteration of serotonergic system and lead to anxiety disorder. Finally, previous studies showed that the decrease in $GABA_A$ receptor sensitivity was found in anxiety disorder patient as demonstrated from [^{14}C] flumazenil positon emission tomography (Malizia et al., 1998; Abadie et al., 1999; Hasler et al., 2009). These altogether indicated that alteration of $GABA_A$ receptor function probably affected anxiety disorder.

There are number of studies indicated that ovarian steroid hormone can modulate $GABA_A$ receptor function (Henderson and Jorge, 2004; Picazo et al., 2006). For example, the sensitivity to benzodiazepine or GABA had been reported to be changed across the estrous cycle in rodents (Carey et al., 1992; Reddy and Kulkarni, 1999). Female mice were more responsive to the anxiolytic effects of diazepam during estrus and diestrus, but no response during proestrus or metestrus phase (Carey et al., 1992). In ovariectomized rat, the sensitivity of benzodiazepine was varied with the length of ovariectomy (Picazo et al., 2006). Interestingly, *in vitro* and *in vivo* studies had shown that estrogen can alter the mRNA expression of $GABA_A$ receptor subunit (Herbison and Fenelon, 1995; Gulinello et al., 2003; Pierson et al., 2005; Byrnes et al., 2007; Henderson, 2007). Herein, the deficiency of estrogen may affect

gene expression of GABA receptor subunits in brain and lead to the functional alteration of $GABA_A$ receptor, which has been postulated to the changing of behavior and emotion. However, few studies investigated the estrogenic effect on the plasticity of the $GABA_A$ receptor subunit gene expression in brain areas related to anxiety in animal models.

Therefore, the objectives of present study were as follows:

1) To investigate the effects of time of estrogen deprivation on anxiety-like behaviors, $GABA_A$ receptor subunit gene expression and serotonergic activity in brain associated with anxiety in ovariectomized rats

2) To investigate whether lacking of estrogen causes alterations of $GABA_A$ receptor function and whether these alterations affect serotonergic activity in brain associated with anxiety

3) To investigate whether estrogen can alleviate anxiety-like behavior in ovariectomized-induced anxiety rat, in relation to the recovery of $GABA_A$ receptor function and the serotonergic activity in brain areas related to anxiety

This study would provide more information concerning the roles of estrogen in causing, preventing and treating anxiety which can be used as supportive information for the therapeutic effect of estrogen on anxiety disorders.