CHAPTER II
LITERATURE REVIEW

2.1 Estrogen Replacement Therapy (ERT) and Cognition

One of the most exciting areas of research in women's health over the past 10 years involves our growing appreciation that estrogens play important neurotrophic and neuroprotective roles during adulthood. This brings new meaning to the potential impact of the prolonged post-menopausal hypoestrogenic state on learning and memory and the potential increased vulnerability of ageing women to brain injury and neurodegenerative diseases. The increase in female life expectancy during the past century has implied that women now live one-third of their lives beyond cessation of their ovarian function. This evolution in demography has increased the need for the development of new therapeutical strategies to promote successful ageing, defined as low probability of disease, high cognitive and physical capacity and active engagement in life. Because changes in the aging nervous system are subtle, it may be possible to reverse them and to improve cognitive performance by pharmacological treatments. The administration of steroids may be particularly promising in this regard: (i) they play an important role in the functioning of the central and peripheral nervous system (CNS and PNS); (ii) some steroids have neuroprotective effects; (iii) the levels of some neuroactive steroids markedly decrease with age; and (iv) unconjugated steroids easily cross the blood–brain barrier and rapidly accumulate throughout the brain.

Epidemiological data provide the notion that ERT will reduce the likelihood of two common and debilitating conditions linked with menopause and aging, namely depression and dementia. Although it is not commonly appreciated, dementia is typically accompanied by mood dysregulation. Both depression and anxiety can affect cognitive performance in older persons, mood dysregulation and stress mimic and enhance the likelihood of dementia (Grossman, 2000). This clinically recognized relationship between mood and cognition is another example of the brain as a target of sex steroids. Convergent evidence for effects of estrogen on cognitive function comes from studies that have examined cognition in relation to menstrual cycle phase,

biomarkers of lifelong estrogen exposure, menopausal symptoms, ER polymorphisms, neuroimaging studies and circulating hormone levels (Berman *et al.*, 1997; Carlson & Sherwin, 1998; Jacobs *et al.*, 1998; Maki, Rich & Rosenbaum, 2002; Phillips & Sherwin, 1992; Yaffe *et al.*, 2000) . Several observational and longitudinal studies of healthy community-dwelling women suggest that women who use ERT may perform better on a broad spectrum of cognitive skills or may outperform non-users on more discrete memory measures. Estrogen users showed significantly higher scores on verbal memory, verbal fluency and visual memory and higher scores on the Modified Mini-Mental State Examination (3MS) compared with age-matched non-users (Maki, Zonderman & Resnick, 2001; Miller, Conney & Small, 2002; Resnick, Metter & Zonderman, 1997). A well-characterized cohort study in Baltimore, women receiving estrogen performed significantly better than women who had never used estrogen on the Benton Visual Retention Test, a task that measures short-term non-verbal memory and drawing skills (Kawas *et al.*, 1997). In this study, estrogen users seemed resistant to age-associated declines in the test scores. More recent randomized controlled trials (RCTs) of estrogens and cognition have better characterized post-menopausal subjects. Several of the RCT studies imply that women given estrogen outperformed placebo-treated women on various psychometric measures, including choice reaction time, attention and concentration, distract ability, verbal memory and abstract reasoning (Henderson *et al.*, 2003). Sherwin (1997) has argued persuasively that estrogen-induced improvement is most apparent on tasks assessing the recall of verbal information, such as recalling details from a paragraph-length narrative. In general, the magnitude of an estrogen effect in healthy women appears to be modest, but in some circumstances, differences appear to be large enough to be clinically meaningful. Compared with observational and longitudinal studies, RCTs provide stronger evidence of an estrogen effect on cognition: although the preponderance of findings shows that estrogen users performed better on cognitive tests and experienced less deterioration in aspects of cognition with increasing age than the non-users, findings from longitudinal and observational studies are much more inconsistent than those from the RCTs. The epidemiological data on the neuroprotective effects of estrogen-based therapy were reviewed by LeBlanc *et al.* (LeBlanc, Janowsky & Nelson, 2001): women who were symptomatic from the menopause had improvement in verbal

memory, vigilance, reasoning and motor speed when given ERT. The same meta-analysis of observational studies examining ERT and cognitive function also suggests a significant reduction in the risk of Alzheimer's disease (AD) among women who have ever used ERT. In particular, the strongest evidence for an association between ERT and AD comes from two cohort studies: the Manhattan Study of Aging (Tang *et al.*, 1996) and the Baltimore Longitudinal Study of Aging (Kawas *et al.*, 1997). These two prospective cohort studies that reported a significantly reduced risk of AD in estrogen users are particularly compelling because they are avoid both recall and prescribing-practice bias. In an Italian Longitudinal Study on Aging, ERT was associated with a reduced prevalence of AD in 2816 women [odds ratio (OR), 0.24; 95% CI, 0.07–0.77]. Analysis of observational data from the Cache County Study suggested a reduction in the risk of AD for past ERT users for 3–10 years. In the same study, the 'excess' risk of AD when compared with age-equivalent men disappeared among women who received ERT for >10 years. However, like the longitudinal studies on estrogen use and cognition, these studies on ERT and the risk for AD show possible biases that suggest caution in their interpretation. Nevertheless, their findings should be considered consistent in suggesting a protective effect of ERT with regard to the development of AD.

Evidence to support the idea of a window of effectiveness for the initiation of ERT to protect against cognitive aging is also raised in the studies of Henderson *et al.* (Henderson, Guthrie & Dennerstein, 2003; Matthews, Cauley & Zmuda, 1999). Similarly, evidence from animal studies indicates that the neuroprotective effects of estrogen and neuroprotection are based on a model of early therapy initiation after the menopause. Ageing may affect the expression of ERs and ERs' coactivators such as growth factors, neuromodulators and neurostrasmitters. For instance, young adult rats responded to estrogen with an increased expression of brain-derived neurotrophic factor (BDNF), which is important for the maintenance of plasticity in the ageing brain, whereas estrogen administered to senescent rats decreased BDNF expression in the olfactory bulb and basal forebrain, suggesting that there is a general decline in hormonal responsiveness of trophin receptors in older, reproductively senescent animals compared with younger animals. (Adams *et al.*, 2002; Cardona-Gomez, Mendez & DonCarlos 2001; Jezierski & Sohrabji, 2001) Thus, the effects of estrogen

in the brain of young animals might be not predictive of the effects of the same molecules in an aged brain. To test this hypothesis in women, MacLennan *et al.* (2006) presented the results of a pilot study examining the timing of initiation of ERT on later cognitive function in a population-based study of 428 women. Early initiators of ERT performed better than late initiators on the 3MS and were faster than non-users on the Trail Making Test Part A (MacLennan *et al.*, 2006). However, only the results from a more representative population-based study will address more consistent data on the 'critical window hypotheses' for ERT.

2.2 Estrogen Receptor in the central nervous systems

Despite the long history of estrogen effects in the brain, the knowledge of estrogen action at the cellular and subcellular level is still scanty. It is beyond any question that steroids modulate gene transcription by interacting with nuclear receptors. The ligand dependent regulation of gene expression is generally sensitive to transcriptional and translational inhibitors as well as to inhibitors of nuclear receptors. Two types of nuclear receptors are known: ERα and ERβ which are similar in their structural organization into domains but which have distinct differences in their binding affinities for different ligands and selective ER modulators (Gruber, Tschugguel & Schneeberger 2002). Regarding the CNS, specific receptors for estrogen have been localized in the amygdala, hippocampus, cortex basal forebrain, cerebellum, locus coeruleus, midbrain, raphe nuclei, glial cells and central grey matter, confirming an involvement of estrogen in controlling well-being, cognitive functions and memory processes in physiological as well as in pathological conditions (Sherwin, 1997) . In these genomic mechanisms, steroids induce relatively long-term actions on neurons modulating the synthesis, release and metabolism of many neuropeptides and neuroactive transmitters and the expression of their receptors (Alonso-Solis *et al.*, 1996). ERα and ERβ are differently expressed throughout the rat brain, and there is anatomical evidence of distinct roles of each subtype. Hybridization and histochemical studies have shown that both receptors are present in the rat cortex, pituitary and hypothalamus (ERα mostly in the arcuate and ventromedial nuclei, whereas ERβ is mostly present in the paraventricular and ventromedial nuclei), whereas the cerebellum expresses only ERα and the hippocampus expresses mostly

ERβ (Couse *et al.*, 1997). Moreover, sex steroids exert very rapid effects in the brain that cannot be attributed to genomic (i.e. transcriptional) mechanisms (Gruber *et al.*, 2002). These 'non-genomic or non-classical' effects are probably to be mediated by receptors integrated or associated with the plasma membrane and by an activation of distinct intracellular signalling cascades (Falkenstein & Wehling, 2000; Kuppers *et al.*, 2001). Various non-genomic estrogen effects includes (i) rapid actions on excitability of neuronal and pituitary cells, (ii) activation by estrogens of cyclic adenosine monophophate (cAMP) and mitogen-activated protein (MAP) kinase pathways that affect the activity of such targets as kinate and insulin-like growth factor-1 (IGF-1) receptors, (iii) modulation of G-protein coupling and effects on calcium currents, (iv) effects on calcium channels and calcium ion entry and (v) protection of neurons from damage by excitotoxins and free radicals (Brinton, 2001; Kelly & Levin, 2001; Kelly & Wagner, 1999; Lee & McEwen, 2001; McEwen & Alves, 1999; McEwen *et al.*, 2001). Regarding the subcellular localization of ER, experimental data show that, in addition to the nuclear ERs, there is a predominant localization of ERs in proximity to the plasma membrane, including that of neurites and of the soma, dendritic spines and axon terminals (Clarke *et al.*, 2000; McEwen & Alves, 1999; McEwen *et al.*, 2001). These findings also imply that classical ERs may act within a cell in a dynamic way and suggest that ERs can be found in various subcellular structures. This is supported by the demonstrations that estrogen is capable of binding and interacting with proteins in the mitochondrial membranes and that ERs are associated with pre-synaptic structures, thereby controlling synaptic transmission (Kelly *et al.*, 1999; Levin, 1999; McEwen & Alves, 1999; Wong, Thompson & Moss, 1996). In conclusion, estrogen effects in the brain include complex cellular mechanisms ranging from classical nuclear to non-classical membrane-mediated actions. Both ways of cell signalling may be activated separately, although there is good evidence that they are intertwined at several cellular instances and can affect each other reciprocally, producing synergistic effects (McEwen & Alves, 1999). Regarding the cellular and subcellular location of ERs, Beyer and colleagues proposed a highly dynamic intracellular mobility with classical ERs being located at nuclear sites but also extra-nuclear sites in different cell compartments including the plasma membrane (Adams *et al.*, 2002; Behl, 2002; Beyer, Pawlak & Karolczak, 2003; Ramirez, Kipp & Joe, 2001).

2.3 Phytoestrogen and the Brain

Phytoestrogens are a diverse group of nonsteroidal plant compounds that can behave as estrogens and occur naturally in most plants, fruits, and vegetables (Thompson *et al.*, 1991). They were first noted in 1926 to have estrogenic activity (Murkies, 1998). Because they possess a phenolic ring, this enables them to bind to estrogen receptors in humans. They bind to both types of estrogen receptor, ERα receptors and the more recently discovered ERβ receptors (Setchell, 1998). Many phytoestrogens seem to have a higher affinity for the ERβ receptor than steroidal estrogens, which suggests that they may exert their actions through distinctly different pathways. However, despite their ability to bind to the estrogen receptor, they are much weaker than human estrogens, with 10^2 to 10^5 times less activity (Price & Fenwick, 1985). They seem to possess both estrogenic and antiestrogenic behavior, but whether they act primarily as an estrogen or as an antiestrogen seems to depend on an individual's amount of circulating endogenous estrogens and the number and type of estrogen receptors (Cassidy, Bingham & Setchell, 1994). Even though they have low estrogenic activity, they are frequently present in the body in much higher quantities than endogenously produced estrogens (Adlercreutz, Markkanen & Watanabe, 1993). Some have also demonstrated progesterone receptor activity (Zava, Dollbaum & Blen, 1998).

There are 3 main types of phytoestrogens—the isoflavones (the most potent), coumestans, and lignans. There are more than 1000 types of isoflavones, but the most commonly investigated compounds are genistein and daidzein, which are also thought to have the highest estrogenic properties. They are found in legumes such as soy, chickpeas, clover, lentils, and beans (Price & Fenwick, 1985). The amount of phytoestrogen found in each soy protein depends on the processing techniques used and its relative abundance in the specific soy product of interest. The secondary soy products (milk or flour) contain lower amounts of isoflavones than the primary products (Zava *et al.*, 1998). The isoflavones are bound to glucose, and when ingested by humans, are enzymatically cleaved in the gut to the active forms (Setchell, 1998). The metabolism of the phytoestrogens varies from person to person, and there is also a sex difference, with women appearing to metabolize them more efficiently (Lu &

Anderson, 1998). The estrogenic activity of the various isoflavones varies greatly. We do not yet know which the most biologically active form is.

The lignans (enterolactone or enterodiol) are found in flaxseed (in huge quantities), lentils, whole grains, beans, fruits, and vegetables (Price & Fenwick, 1985). Other classes, which are much more rarely ingested, are the coumestans (found in sprouting plants), flavones, flavanones, chalcones, terpenoids, and saponins.

The neurobehavioral effects of phytoestrogens have been reviewed, however, limited data exists regarding the influence of soy-derived dietary isoflavones on brain structure and function (Halbreich & Kahn, 2000; Lephart *et al.*, 2003; Linford & Dorsa, 2002; Lund *et al.*, 2001; Pan *et al.*, 2000). Of the available data, (Pan, Anthony & Clarkson, 1999a, 1999b) showed that soy phytoestrogens regulate choline acetyltransferase, nerve growth factor and brain-derived neurotrophic factor in brain areas such as the frontal cortex and hippocampus of female rats. Subsequently, (Kim *et al.*, 2000) found that dietary phytoestrogens attenuates tau protein phosphorylations associated with Alzheimer's disease, suggesting a neuroprotective effect of phytoestrogens. Moreover, (Linford & Dorsa, 2002) or (Zhao, Chen & Diaz, 2002) showed evidence that isoflavones displayed neuroprotective effects in primary cortical or hippocampal cultures, respectively. Finally, other studies, utilizing the distribution and/or relative levels of ER- alpha and ER-beta expression in brain (Shughrue, Lane & Merchenthaler, 1997; Shughrue & Merchenthaler, 2000; Zhang *et al.*, 2002) examined over the counter nutritional supplements containing soy-derived phytoestrogens (i.e., isoflavones). These latter findings revealed that dietary isoflavones antagonized brain ER-alpha in female rats and altered reproductive behavior but had negligible influences via brain ER-beta. The altered reproductive behavior observed was most likely due to the antiestrogenic effects (via ER-beta) of the isoflavone supplement in brain (Patisaul *et al.*, 2001; Patisaul *et al.*, 2002).

2.4 Oxidative stress and the brain

2.4.1 Oxidative stress in the brain

There are several observations suggesting that the brain may be particularly vulnerable to oxidative stress. (Halliwell, 2001; Muller, 1997; Olanow, 1993; Reiter, 1995). Some of the most important of these are summarized below:

(i) The brain, which accounts for only 2% of body weight, consumes a fifth of the total oxygen inspired and carries out the turnover of large quantities of ATP at a high rate. Since approximately 5% of oxygen consumed by cells is estimated to be reduced to ROS, relatively higher amounts of reactive oxygen species (ROS) may be generated in the brain as compared to other tissues that use less oxygen.

(ii) Brain is rich in polyunsaturated fatty acids (PUFAs), which are particularly susceptible to ROS damage.

(iii) The cerebrospinal fluid (CSF) contains small molecular weight iron and copper complexes, which catalyze the formation of the highly reactive hydroxyl radicals. It is poor in the antioxidants, transferrin, and ceruloplasmin, which normally bind to and segregate these transition metals.

(iv) The release of excitatory neurotransmitters, such as glutamate, induces a cascade of reactions in the postsynaptic neuron, resulting in the formation of ROS. Depending on the density of such neurons, this could cause localized lesions in the nervous system. Glutamate toxicity, enhanced by amyloid-h-peptide (AhP)-mediated Ca^{2+} signals, may involve the participation of ROS and oxidative stress. Activation of the glutamate N-methyl-D-aspartic acid (NMDA) receptors, for example, stimulates the release and metabolism of arachidonic acid (AA) via lipoxygenase pathways. AA hydroperoxides, such as 12-hydroxyeicosatetraenoic acid (12-HETE) and 12-hydroperoxyeicosatetraenoic acid (12-HPETE), produced during the AA cascade–lipoxygenase pathways not only act as second messengers but are also a recognized source of ROS.

(v) The liberation of ROS during the oxidation of dopamine by monoamine oxidase in the nerve terminals of dopaminergic neurons may produce increased oxidative stress in brain regions, such as substantia nigra. This is suggested to have a causative role in the etiology of Parkinson's disease (PD).

(vi) Nitric oxide (NO), also called endothelium-derived relaxing factor because of its production in the endothelial cells, is also formed in the neurons by the enzyme, nitric oxide synthase (NOS), which is widely distributed in the brain and is activated by calmodulin. The interaction of NO with the superoxide radical is believed to be implicated not only in the normal metabolism of the neuron but also in its degeneration.

(vii) Ascorbic acid, which can act as an antioxidant as well as a prooxidant, is present at elevated levels in both the white and grey matters of the brain. There exist transport systems in the choroid plexus and neurons, which serve to concentrate ascorbic acid into brain cells and in the CSF. It acts as a prooxidant when the free iron in brain regions increases due to intracerebral hemorrhage.

(viii)The brain contains almost no catalase and less glutathione peroxidase (GPx) and vitamin E, as compared to liver.

(ix) An endogenous antioxidant produced in the pineal gland, called melatonin, is found to be a good scavenger of ROS, but its concentration decreases markedly with age.

(x) Neurons are nonreplicating cells and any damage to brain tissues by the ROS tends to be cumulative over time.

2.4.2 Oxidative stress induced by the ethanol

Ethanol consumption has been reported to generate ROS and related neurodegenaration disease. In the human or the animal body, ethanol is generally oxidized by the enzyme alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1) (Figure 2). ADH, present in the fluid of the cell (i.e., cytosol), converts alcohol to acetaldehyde. This reaction involves an intermediate carrier of electrons, nicotinamide adenine dinucleotide (NAD^+), which is reduced by two electrons to form NADH. Catalase, located in cell bodies called peroxisomes, requires hydrogen peroxide (H_2O_2) to oxidize alcohol. CYP2E1, present predominantly in the cell's microsomes, assumes an important role in metabolizing ethanol to acetaldehyde at elevated ethanol concentrations. Acetaldehyde is metabolized mainly by aldehyde dehydrogenase 2 (ALDH2) in the mitochondria to form acetate, and NADH and ROS. (Zakhari, 2006).

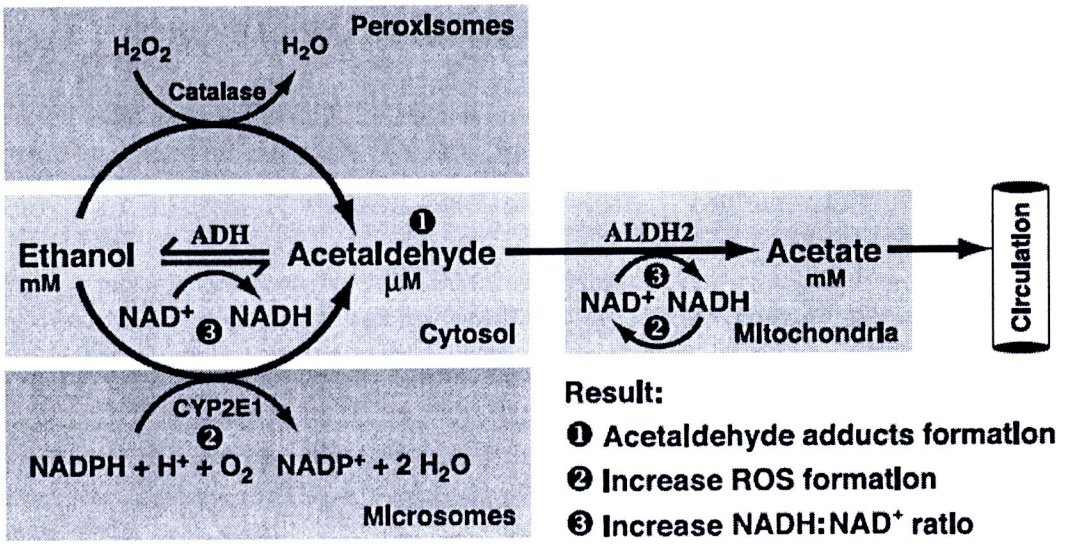

Figure 2 The pathway of ethanol metabolism (Zakhari, 2006).

Many processes and factors are involved in causing alcohol-induced oxidative stress, including (Wu & Cederbaum, 2003)

a. Changes in the NAD^+/NADH ratio in the cell as a result of alcohol metabolism. Alcohol is metabolized in two steps. First, the enzyme alcohol dehydrogenase converts alcohol to acetaldehyde, a toxic and reactive molecule. Next, the enzyme aldehyde dehydrogenase converts the acetaldehyde to acetate. Each of these reactions leads to formation of one molecule of NADH, thereby providing more starting material and thus enhanced activity of the respiratory chain, including heightened O_2 use and ROS formation.

b. Production of acetaldehyde during alcohol metabolism, which through its interactions with proteins and lipids also can lead to radical formation and cell damage.

c. Damage to the mitochondria resulting in decreased ATP production.

d. Effects on cell structure and function caused by alcohol's interactions with either membrane components or enzymes and other protein components of the cells.

e. Alcohol-induced oxygen deficiency in tissues, especially in certain areas of the liver lobules, where extra oxygen is required to metabolize the alcohol.

f. Alcohol's effects on the immune system, which lead to altered production of certain signaling molecules called cytokines, which in turn lead to the activation of an array of biochemical processes.

g. Alcohol-induced increase in the ability of the bacterial molecule endotoxin to enter the bloodstream and liver, where it can activate certain immune cells.

h. Alcohol-induced increases in the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which metabolizes alcohol and other molecules and generates ROS in the process.

i. Alcohol-induced increases in the levels of free iron in the cell, which can promote ROS generation.

j. Effects on antioxidant enzymes and chemicals, particularly a molecule called glutathione (GSH)

k. Biochemical reactions generating an alcohol-derived radical.

l. Conversion of the enzyme xanthine dehydrogenase into a form called xanthine oxidase, which can generate ROS.

2.4.3 Enzymatic mechanism of ethanol oxidation in the brain

Similar to the liver, catalase, CYP2E1, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the major enzymes involving in the oxidation of ethanol in the brain, but their role are different. The activities to oxidize the ethanol by these enzymes in the brain were investigated by using the inhibitors of these 4 enzymes in the brain homogenates of mice with genetic deficiency in ethanol-metabolic enzymes and the brain subcellular fractions known to have differential activity of these enzymes, and followed the measurement of the ethanol-derived acetaldehyde and acetate. The catalase was found to have the key role, up to 60%, in the ethanol metabolism in the brain. CYP2E1 plays an important role in ethanol oxidation in the rodent brains. Alcohol dehydrogenase plays a minor role. Aldehyde dehydrogenase plays the crucial role in the further oxidation of acetaldehyde in the brain homogenates. (Zimatkin *et al.*, 2006) In another report, ethanol in 0.9% NaCl (85-90 mM) was perfused into the rat brain in a speed of 6-43 ul/min from the lateral ventricle and the perfusate was collected from the Cisterna magna to measure the ethanol and the ethanol metabolite—acetaldehyde. The passage of the brain

significantly eliminated the ethanol in the perfusate in which the acetaldehyde was found increasing. This metabolic process was not observed when the catalase inhibitor--3-amino-1, 2, 4-triazole (aminotriazole) (10 mM) was added to the perfusate, indicated the catalase was the metabolic enzyme involved in the ethanol oxidation in the brain. (Zimatkin & Buben, 2007)

2.4.4 The effect of ethanol in antioxidant enzyme in the brain

By feeding the rat with ethanol (15% in supplied drinking water) for 2 months, the significant decrease in the blood and brain total antioxidant enzyme activity was reported (Kurban, 2008). Ethanol (20%) (1.6 g/kg body weight) administered to the normal rats intragastricly significantly increased MDA level in the cerebral cortex. Ethanol also significantly increased superoxide dismutase (SOD) activity in the cortex and catalase (CAT), GSH-Px, and GR activities in the corpus striatum. Ethanol significantly augmented CAT activity in the medulla and GSH-Px activity in the hypothalamus. However, CAT activity significantly decreased in the hypothalamus after ethanol ingestion (Somani et al., 1996).

2.4.5 Superoxide dismutase (SOD) (EC 1.15.1.1)

All living cells, from the smallest bacterium to the longest nerve cell or the biggest ovum, must carry on a variety of cellular processes to be able to stay alive. Some of these activities are proactive in nature, such as gaining nourishment, and reproducing, whereas other actions are defensive in nature. Every cell is susceptible to harm from a variety of sources, both activities within and outside the cell. One of the activities that happen as a normal part of living for any cell can also be something that causes damage- generation of ROS. In fact, these ROS are used by many types of cells as part of their ability to defend themselves, stored in the lysosomes of cells.

The ROS is superoxide, and the defense mechanism is the enzyme superoxide dismutase (SOD) followed either by the enzyme catalase or glutathione peroxidase. The superoxide radical ($O_2^{\cdot-}$) is formed in the mitochondria as a byproduct of electron transport. They are very dangerous to the cell because they steal electrons from neighboring molecules, starting a cascade of electron stealing, the end result being damaging of the cell. The superoxide dismutase enzyme is the first step that cells use to stop this process.

SOD converts superoxide radicals to a less toxic ROS, hydrogen peroxide (H_2O_2), as follows:

$$Cu^{2+} + O_2^{\cdot-} \rightarrow Cu^{+} + O_2$$

$$Cu^{+} + O_2^{\cdot-} + 2H^{+} \rightarrow Cu^{2+} + H_2O_2$$

Net rate: $2O_2^{\cdot-} + 2H^{+} \rightarrow H_2O_2 + O_2$

The hydrogen peroxide molecule, which is still a danger to cells, is then further processed to nontoxic by-products. In an aqueous environment, the enzyme catalase degrades the hydrogen peroxide as follows:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The oxygen gas created in this reaction accounts for the bubbles seen when hydrogen peroxide is poured on a skin injury. Many bacteria do not have catalase, and the cells are destroyed, which is why hydrogen peroxide is effective against bacteria. In a lipid environment, the body uses glutathione peroxidase to degrade the H_2O_2 as follows:

$$H_2O_2 + 2H^{+} \rightarrow 2H_2O$$

2.4.6 Glutathione peroxidase (GPx)

Cellular glutathione peroxidase (c-GPx, EC 1.11.1.9) is a member of a family of GPx enzymes whose function is to detoxify peroxides in the cell. Because peroxides can decompose to form highly reactive radicals, the GPx enzymes play a critical role in protecting the cell from free radical damage, particularly lipid peroxidation. The GPx enzymes catalyze the reduction of H_2O_2 to water and organic peroxides (R-O-O-H) to the corresponding stable alcohols (R-OH) using glutathione (GSH) as a source of reducing equivalents:

$$ROOH+2GSH \xrightarrow{GPx} ROH+GSSG+H_2O$$

With the exception of phospholipid-hydroperoxide GPx, all of the GPx enzymes are comprised of 4 identical subunits (monomer Mr 22-23 kDa). Each subunit contains a molecule of selenocysteine in the enzyme active site. The selenocysteine is thought to participate directly in electron donation to the peroxide substrate and become oxidized in the process. The enzyme then uses glutathione as an electron donor to regenerate the reduced form of the selenocysteine. The GPx enzymes accept a wide variety of organic peroxides as substrates. However, with the exception of phospholipid hydroperoxide GPx and perhaps pl·GPx, the enzymes

exhibit a strong preference for glutathione as a source of reducing equivalents. Phospholipid-hydroperoxide GPx (Mr 19 kDa) is the only enzyme with significant activity on esterified phospholipids and cholesterol in membranes.

2.4.7 Catalase

Catalase (EC 1.11.1.6), present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. The mechanism of catalysis is not fully elucidated, but the overall reaction is as follows:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

The protein exists as a dumbbell-shaped tetramer of four identical subunits (220,000 to 350,000 kD). Each monomer contains a heme prosthetic group at the catalytic center. Catalase monomers from certain species (e.g. cow) also contain one tightly bound NADP per subunit. This NADP may serve to protect the enzyme from oxidation by its H_2O_2 substrate.

Catalase is one of the first enzymes to be purified to homogeneity, and has been the subject of intense study. The enzyme is among the most efficient known, with rates approaching 200,000 catalytic events/second/subunit (near the diffusion-controlled limit). Catalase structure from many different species has been studied by X-ray diffraction. Although it is clear that all catalases share a general structure, some differ in the number and identity of domains. In this display, beef liver catalase is used as a model for catalase structure. It is compared to catalase structure from a fungus, Penicillium vitale.

Proposed Mechanism of Catalase: The chemistry of catalase catalysis has not been precisely solved yet, but the following, which is similar to the mechanism of cytochrome c peroxidase, has been proposed. The catalytic process is thought to occur in two stages:

$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E$ (1)

$H_2O_2 + O{=}Fe(IV)\text{-}E \rightarrow H_2O + Fe(III)\text{-}E$ (2)

where Fe-E represents the iron center of the heme attached to the rest of the enzyme (E).

Peroxide, upon entering the heme cavity, is severely sterically hindered and must interact with His74 and Asn147. It is in this position that the first stage of catalysis takes place. Transfer of a proton from one oxygen of the peroxide to the other, via His74, elongates and polarizes the O-O bond, which eventually breaks heterolytically as a peroxide oxygen is coordinated to the iron center. This coordination displaces water and forms Fe(IV)=O plus a heme radical. The radical quickly degrades in another one electron transfer to get rid of the radical electron, leaving the heme ring unaltered. During the second stage, in a similar two electron transfer reaction, Fe(IV)=O reacts with a second hydrogen peroxide to produce the original Fe(III)-E, another water, and a mole of molecular oxygen.

The heme reactivity is enhanced by the phenolate ligand of Tyr357 in the 5th iron ligand position, which may aid in the oxidation of Fe(III) to Fe(IV) and the removal of an electron from the heme ring. The efficiency of catalase may, in part, be due to the interaction of His74 and Asn147 with reaction intermediates. This mechanism is supported by experimental evidence indicating modification of His74 with 3-amino-1,2,4-triazole inhibits the enzyme by hindering substrate binding.

2.5 Estrogen

2.5.1 Synthesis of estrogens

a. Theca cells are the major sources of 17-β-hydroxyprogesterone and of androstenedione (the principle androgen produced by the ovary). Granulosa cells are the major source of estradiol (E2). Leutinizing hormone (LH) stimulates synthesis of progesterone from pregnenolone.

b. Significant amounts of estrogens are produced by the peripheral aromatization of androgens. In human males, the peripheral aromatization of testosterone to E2 accounts for 80% of the production rate of estrogens.

c. In females, as much as 50 % of the E2 produced during pregnancy comes from the aromatization of the adrenal androgen dehydroepiandrosterone (DHEA) sulfate.

d. The conversion of androstenedione to estrone (E1), is the major source of estrogens in postmenopausal women. Aromatase activity is present in adipose cells and in liver, skin, and other tissues.

2.5.2 Ovarian cycle: hormonal regulation of oogenesis

a. The female reproductive system has cyclic variation, whereas the male system has tonic and constant production of hormones.

b. Cyclic activity of the hypothalamic-pituitary-gonadal axis is reflected by sloughing off of the endometrial lining approximately every 28 days. Menses (month in Latin) is due to ovarian activity and, therefore, is named the ovarian cycle.

c. The cycle begins with 15–30 follicles developing due to follicle-stimulating hormone (FSH) stimulation.

d. On day 6, one follicle begins to produce antifollicular compounds, which lead to atresia of other follicles.

e. The dominant follicle produces estrogen, which decreases FSH and LH by negative feedback.

f. Near midcycle, about 48 hours prior to ovulation, a huge increase in estrogen production results in a positive feedback that causes a surge of gonadotropins (primarily LH), leading to rupture of the follicle and ovulation 16–24 hours later.

g. After ovulation, the follicular cavity fills with yellowish luteal cells due to luteinization of the theca and granulosa cells (corpus luteum) resulting from LH stimulation. The corpus luteum produces progesterone and E2 for 8–9 days.

h. The increased plasma levels of E2 and progesterone negatively feed back to keep FSH and LH levels low.

i. If fertilization does not occur, the corpus luteum then regresses (luteolysis).

2.6 The *Curcuma comosa*

Curcuma comosa Roxb is belonged to Zingiberaceae family, and is commonly known as Waan chak mod look in Thailand. It's 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. In the same species, plants such as *Curcuma longa* L., *Curcuma aromatica* Salisb., *Curcuma zedoaria* Roxb. and *Curcuma xanthorrhiza* Roxb. along with *Curcuma comosa* have been reported to have anti-cancer and anti-tumor, emmenagogue and abortifacient activities. Although *C. comosa*'s long-term and wide use, there was few research

report of its biological activities, especially the effects in the reproductive system until 1995, Piyachaturawat and colleagues launched a series of research of its bio-activities.

The first studies were conducted to probe *C. comosa*'s estrogenic-like effects, in which the uterotrophic effect and estrogenic activities were studied (Piyachaturawat *et al.*, 1995a) by analyzing the uterine weight and glycogen content of bilaterally ovariectomized immature rats, which were found increase significantly after intragastrically receiving the *C. comosa* extract when compared to the control group. These effects were similar to the effects of estradiols, indicating the *C. comosa* has uterotrophic effect. The estrogenic effect was also supported by the histology study of the vaginal mucosa in the *C. comosa*-treated rats, which showed a remarkable proliferation and keratinization; concurrent with the uterine mucosa exhibited a dramatic proliferation and hypertrophy in dose-dependence. Moreover, the extract effectively induced and increase of specific estrodiol binding site in the uterine nuclei (Piyachaturawat *et al.*, 1995b). These results suggest that *C. comosa* exerts an estrogenic-like action by acting at specific estrodiol binding sites in the cells. Different solution-extracts were tested and the hexane-extract was found to be the most active fraction when compared to ethyl acetate, butanol and aqueous extract.

C. comosa hexane extract (CHE) was also effective on the reproductive system of male rats including the growth suppressing effect of reproductive organs and fertility effects (Piyachaturawat *et al.*, 1998), which were similar to the effects of estradiol. CHE was administered intragastrically at a dose of 500mg/kg BW to male rats for 7 consecutive days and it significantly suppressed weights of testes, epididymis, ventral prostate, seminal vesicle and *levator ani* muscle. The suppression of the growth of the organs by the hexane extract was dose-dependent. Histological examination revealed the regression of the spermatogonium in the seminiferous tubules and necrosis of epithelial cells in the epididymis. All these effects were similar to the effects of estradiol. Concurrent treatment with either human-chorionic gonadotrophin (hCG) or testosterone did not fully abolish the suppressing effects of the extract. However, this 7-days treatment did not significantly affect the fertility of the animals, it is possible to explain that the time required for a rat spermatogonium to yield spermatozoa in the ejaculate is approximately 60 days and thus 7-day treatment may not have been long

enough to affect fertility (Piyachaturawat *et al.*, 1999). But sperm concentration and motility in the cauda epididymides were significantly suppressed.

Another experiment investigated the choleretic properties of various extracts of *C. comosa* in rats. Intraduodenal administration of the ethyl acetate extract caused a dose-dependent stimulation of bile secretion. A low dose of the extract (20 to 200 mg/kg BW) increased bile flow rate without altering bile salt concentration whereas high doses (1000 mg/kg BW) increased the bile flow rate with decreased bile salt concentration (Piyachaturawat, Gansar & Suksamrarn, 1996). With regard to the total output of biliary constituents, high dose of extract did not alter secretary bile salt output, but markedly increased biliary cholesterol output. Concurrent with increased biliary cholesterol secretion, the plasma cholesterol level decreased, indicating the influence of the extract of *C. comosa* on lipid metabolism. The decrease of plasma cholesterol level may be related to the previous estrogenic-like function studies in which the *C. comosa* extract enhanced the estradiol effects in reproductive tract when treated the animals with *C. comosa* extract and estradiol together (Piyachaturawat *et al.*, 1999). *C. comosa* maybe increase the estradiol consumption and then result in the cholesterol, precursor of estrogen, decreased.

2.7 Brain morphology study

2.7.1 Function and morphology of brain

The intact of brain is very important for exerting the cognitive functions appropriately. Brain volumetric plays a relevant role in clarifying some related aspects of the pathology of neurodegenerative dementias, changes in brain volume, either global or localized to specific structures, are observed in a range of neurological conditions. Normal aging or the pathological processes were considered to be the relevant conditions underlying the brain atrophy. Although the pathological processes result in atrophy may be variable due to the different conditions, its detection and localization are of clinical interest, as they can support the diagnosis, help in monitoring the condition and give insight into some clinical features of the disease.

2.7.2 Alzheimer's disease (AD) and brain atrophy

Autopsy pathology and neuroimaging studies have shown that the neurodegeneration processes occurring in AD eventually result in a widespread and

faster brain atrophy in patients with AD than in healthy elderly subjects (Adak *et al.*, 2004; Braak *et al.*, 1999; Fama *et al.*, 1997; Foundas *et al.*, 1997; Fox, Freeborough & Rossor, 1996) , The atrophy processes, which normally begin from the medial temporal lobe structures, are thought to due to the extracellular deposition of β-amyloid (Aβ) containing plaques and the intracellular accumulation of neurofibrillary tangles (Small & McLean, 1999; Small, Mok & Bornstein, 2001) , which eventually result in cellular damage and apoptosis and the principal pathway of neuronal death in AD (Bamberger & Landreth, 2002). Localised atrophy is also likely to be clinically eloquent: Symmetric or asymmetric reduction was also reported in the volume of hippocampus, enthorinal cortex and in other medial temporal structures (Busatto *et al.*, 2003; Head *et al.*, 2005; Lehtovirta *et al.*, 1995) and the hippocampus volume is associated with memory impairment, while executive functions appear to be related to multiple brain components (Mungas *et al.*, 2005) . The atrophy in selected limbic structures was shown to be able to distinguish patients with AD from normal elderly individuals with up to 90% accuracy (Barnes *et al.*, 2004; Baron *et al.*, 2001; Callen *et al.*, 2001; Gao *et al.*, 2004; Hirata *et al.*, 2005) . Other consistent findings indicate an involvement of the whole association cortex (Boxer *et al.*, 2003; Bozzali *et al.*, 2006; Janke *et al.*, 2001) , with a relative preservation of the sensory–motor cortex, the occipital cortex and the cerebellum. The involvement of subcortical grey matter is more controversial (Karas *et al.*, 2003; Pennanen *et al.*, 2004). Mild cognitive impairment is a transition stage between the cognitive decline of normal aging and the more serious problems caused by Alzheimer's disease. The disorder can affect many areas of thought and action — such as language, attention, reasoning, judgment, reading and writing. However, the most common variety of mild cognitive impairment causes memory problems (Petersen *et al.*, 2001). And in the recent years, efforts have been concentrated in developing tools, including Magnetic resonance imaging (MRI)-based volumetric, able to discriminate the subjects with amnestic mild cognitive impairment (MCI) who are more likely to convert to AD. Longitudinal voxel-based optimized morphometry (VBM) studies, thanks to the unbiased analysis of the whole brain, demonstrated that a pattern of grey matter atrophy similar to that typical of AD can be found in those patients with MCI who are more likely to convert to AD in a short time (Bozzali *et al.*, 2006; Chetelat *et al.*, 2002) . Conversely, MCI

patients who do not convert to AD in a short time present with a pattern of grey matter density which is similar to that observed in normal elderly people. Some authors also suggested that patients with MCI and a selective impairment of memory functions present with a pattern of grey matter atrophy which can be differentiated from that of MCI patients with diffuse cognitive dysfunction (Bell-McGinty *et al.*, 2005) . In a 18-month follow-up longitudinal study on patients with mild cognitive impairment (MCI), hippocampus atrophy was found more obvious in the rapid converters to AD when compared to age-matched controls (Chetelat *et al.*, 2008). Similar result was found in another study on MCI patients, in which the areas of lower baseline gray matter value in AD converters mainly included the hippocampus, parahippocampal cortex, and lingual and fusiform gyri. Regions of significant gray matter loss include both converters and non-converters are the temporal neocortex, parahippocampal cortex, orbitofrontal and inferior parietal areas, and the left thalamus. There was significantly greater gray matter loss in AD converters relative to non-converters in the hippocampal area, inferior and middle temporal gyrus, posterior cingulate, and precuneus (Chetelat *et al.*, 2005). Posterior cigulate cortex and hippocampus atrophy in MCI patients were also reported from Japan (Shiino *et al.*, 2006)

2.7.3 Hippocampus

The hippocampus is recognized anatomically as a medial bulge in the temporal horn of the lateral ventricle. The bulge is caused by the invagination (turning in) of the ventricular wall. This invagination is the result of the infolding called the hippocampal fissure. The dentate gyrus is a narrow band along the medial aspect of the hippocampus. The dentate gyrus and hippocampus are part of the allocortex, which has a laminar (layered) structure similar to the neocortex, although with less layers and somewhat more simplified. The allocortex is phylogenetically older than the neocortex, but is still nevertheless highly complex. The hippocampus has a clearly defined layer of large pyramidal cells, which communicate extensively through GABAergic basket cell interneurons. The pyramidal cells communicate with layers above and below the pyramidal layer through extensive axonal and dendritic processes. The hippocampus has been subdivided into three areas termed CA1, CA2, and CA3. The dentate gyrus consists mainly of smaller neurons termed granule cells,

which synapse with the dendrites of the pyramidal cells. The axons of the granule cells are termed mossy fibers and terminate mainly on the apical dendrites of the pyramidal cells of the hippocampal area CA3. Cells of CA3 project Schaffer collaterals to apical dendrites of pyramidal cells in area CA1. From CA1, there is a major efferent input to the subiculum, and thence to the entorhinal area (Greenstein & Greenstein, 2000).

2.7.4 CA1 and CA3

From a behavioral perspective, the CA3 subregion of the hippocampus plays an important role in the encoding of new spatial information within short-term memory with a duration of seconds and minutes. This can easily be observed in tasks that require rapid encoding, novelty detection, one-trial short-term or working memory, and one-trial cued recall primarily for spatial information. These are tasks that have been assumed to reflect the operations of episodic memory and require interactions between CA3 and the dentate gyrus via mossy fiber inputs into the CA3. The CA3 is also important for encoding of spatial information requiring multiple trials including the acquisition of arbitrary and relational associations. These tasks tend to be non-episodic and can be mediated by arbitrary and conjunctive operations. All these tasks are assumed to operate within an autoassociative network function of the CA3 region. The output from CA3 via the fimbria and the medial and lateral perforant path inputs play a supporting role in the neural circuit that supports the operation of these tasks. The CA3 also plays a role in sequential processing of information in cooperation with CA1 based on the Schaffer collateral output from CA3 to CA1. The CA3 also supports retrieval of short-term memory information based on a spatial pattern completion process. Finally, CA3 may, in cooperation with the dentate gyrus, serve an important role in processing the geometry of the environment (Kesner, 2007).

The CA1 subregion of the hippocampus receives inputs from two major sources: the Schaffer collateral inputs from CA3, and the perforant path inputs from the entorhinal cortex. CA1 outputs are directed towards the subiculum, entorhinal cortex, prefrontal cortex and many other neural regions including the lateral septum, anterior thalamus and the mammillary bodies. The anatomical and physiological characteristics suggest that the CA1 can operate as a competitive network and have triggered the development of computational models of CA1 in which for example the

CA1 system is involved in the recall process by which backprojections to the cerebral neocortex allow neuronal activity during recall to reflect that present during the original learning. In this terminology, cortico-cortical back projections originate from deep (layer 5) pyramidal cells, and terminate on the apical dendrites of pyramidal cells in layer one of the preceding cortical area. In contrast, corticocortical forward projections originate from superficial (layers 2 and 3) pyramidal cells, and terminate in the deep layers of the hierarchically next cortical area. Within this context, the projections from the entorhinal cortex to DG, CA3 and CA1 can be thought of as forward projections; and from CA1 to entorhinal cortex, subiculum etc. as the start of the backprojection system (Rolls & Kesner, 2006).