CHARPTER II

LITERATURE REVIEWS

Menopause

Menopause is a status resulted from ovarian function decline in women followed by termination of menstrual cycle. It is determined by disappear for consecutive 12 months of menstrual periods without pathologic or physiologic causes (Notelovitz, 1989). Menopause can be divided into 4 stages: premenopause, perimenopause, menopause and postmenopause. Premenopause often referred to as perimenopause, is the phase before the beginning of menopause. The average age for a woman to begin experiencing premenopausal symptoms is 45 year old. Perimenopause is the time leading up to menopause, characterized by normal change which often lead to menopausal symptoms and usually occurs between 45 and 60 years of age. Menopause is the final menstrual bleed and it is generally considered to have occurred after 12 months of amenorrhea. This stage has a wide starting range, but can usually be expected in the age range of 45 to 55 year. In Thai women, the average age at menopause is about 50.13 years (Punyahotra, et al., 1997). The last stage of postmenopause is the time after the last episode of menstrual bleeding. During this stage, there are risks for numerous diseases such as osteoporosis and heart disease.

Menopausal signs and symptoms associated with physical and psychological alterations. Physical signs and symptoms may include hot flushes, vaginal dryness, and risk of osteoporosis and cardiovascular disease. Psychological signs and symptoms may include anxiety, difficulty concentrating, depression and forgetfulness (Miquel, et al., 2006). Chronic deficiency of estrogen in menopause has many effects in non-reproductive functions. There is the possibility that the estrogen deprivation could worsen the age-related impair in memory (Maki, et al., 2001; Henderson, et al., 2003).

Numerous evidences revealed that loss of gonadal function in aging females reduced metabolic function (Alonso, et al., 2008), increased oxidative stress and acetylcholinesterase activity in brain (Martins, et al., 2012) leading to alteration of brain homeostasis and cognitive function. Moreover, clinical studies have shown that

ovarian hormone deprivation after menopause can increase the risk of Alzheimer's disease (AD) (Gao, et al., 1998).

There were several studies had been used murine experimental models. For menopausal models, ovariectomy in rodent became a good model for mimicking human ovarian hormone loss. Additionally, there had been increase in the number of publication, which focuses on the obtaining results of ovariectomy in rats such as physiological difference of nervous system, cardiovascular function, hepatocytes, bone, skin (Castillo, et al., 2005; Tresguerres, et al., 2008) as well as immune system (De la Fuente, et al., 2004; Baeza, et al., 2009).

Cognition

Cognition is the mental process that may be described as an experience of knowing, including learning, memory, reasoning and performance of effective executive action. Learning is attainment of new information and refers to the behavior adaptation caused by experiences while memory is the retention of learning information. Memory formation is processed by three main stages; encoding (receiving, processing and combining of obtained information), storage (recording of the encoded information) and retrieval (recalling the stored information to responding some cue for use in process or activity) (Carlson, 2004; Bear, et al., 2007). Memory is classified into three different types based on the duration of memory retention. At first, sensory memory is the shortest-term element of memory and decays very quickly, typically in the region of 200 - 500 milliseconds after the perception of an item, and certainly less than a second, such as visual information is detected by photoreceptor cells in the eyes. The second type, short-term memory is an immediately memory for stimuli that have just been perceived and the temporary storing limited amount of information. It allows recall from many seconds to minute without rehearsal. Finally, long-term memory is storage of an unlimited amount of information for potentially unlimited duration. The maintaining in stable and permanent changes of neural connections throughout the brain is the critical function to processes the long-term memory (Carlson, 2004; Bear, et al., 2007).

Long-term memory can be divided into two classifications. First is declarative memory which involves events and facts about the people, places, and things. This

memory requires conscious recollection and association of information. Second is non-declarative memory which involves training reflexive motor and skill consequences from direct experience. This memory is formed tending to require repetition and practice over a longer period of time, but it is less likely to be forgotten (Kandel, et al., 2000).

Recognition memory is the ability to identify information after experiencing it again. This memory were documented that it depend on the hippocampus and prefrontal cortical input (Ennaceur, et al., 1997). Some study demonstrated that lesions 30–50% of the dorsal hippocampus could disturb spatial memory, while lesions 75–100% in these area could impair the performance in object recognition (Broadbent, et al., 2004). Additionally, lesion in ventromedial prefrontal cortex displayed recognition memory impairment (Bachevalier and Mishkin, 1986; Meunier, et al., 1997).

Amnesia

The resulting of memories loss prominent in cognitive function decline described in the term "Amnesia". There are two main types of amnesia. The first type is anterograde amnesia which is characterized as an impairment or loss of ability to learn new information. People who have this illness can remember events that have occurred in the past but cannot retain information received after the brain damage. The second type is retrograde amnesia that is characterized by an inability to remember events that happened before the brain damage occurred. Usually, pure anterograde amnesia is rare, there is also a retrograde amnesia for events that occurred for a period of time before the brain damage has occurred (Carlson, 2004; Bear, et al., 2007).

Hippocampus and medial prefrontal cortex associated with cognition

The hippocampus and cerebral cortex are the main structures of the brain that participate critical role to process learning and memory. There were several reports indicated that recognition memory impairment was occurred following the lesion appearing of hippocampus and ventromedial prefrontal cortex (Drachman and Ommaya, 1964; Correll and Scoville, 1965; Sidman, et al., 1968; Bachevalier and Mishkin, 1986; Meunier, et al., 1997).

The hippocampal formation plays an important role in the long-term memory consolidation. It mediates the initial steps of long-term storage and then slowly transforms into neocortical storage system which permits the new data to be stored without disrupting the existing information (Martin, 1991). The mPFC is suggested to mediate the decision making which involved in the retrieval of long-term memory that associates context, locations, events, and corresponding adaptive responses (Euston, et al., 2012).

Hippocampus is subdivided into four longitudinal zones, the CA1, CA2, CA3 and CA4 (CA stands for Cornu Ammonis, Latin for the Ammon's horn) (Brodal, 1998). The composition of hippocampus, dentate gyrus as well as subiculum is called the hippocampal formation (Martin, 1991). The perforant pathway which originates from entorhinal cortex is the major input to the hippocampus by the way of pyramidal cell axons and synapses on granule cells of the dentate gyrus. After that, the granule cell axons which are called "Mossy fibers" synapse on pyramidal cells of CA3 which send their axons in termed "Schaffer collaterals" to pyramidal cells of CA1. Output from the CA1 sends the information to subiculum which sends back to the entorhinal cortex (Martin, 1991) (Figure 2).

The association between hippocampus and prefrontal cortex was shown in the figure 3. The major cortical afferents to the hippocampus initiates from the lateral and medial entorhinal cortex. Input from perirhinal cortex directs to terminate the lateral entorhinal cortex where the information is sent to the hippocampus by the lateral perforant pathway which synapes the granule neurons in dentate gyrus (Witter, et al., 1989; Witter, et al., 2000; van Strien, et al., 2009). The major outputs from hippocampus are subiculum and CA1 where neuronal axons project to the prefrontal cortex (Jay and Witter, 1991; Verwer, et al., 1997). Additionally, there are also the directly projections from the sub-region in the mPFC, infralimbic and prelimbic region, to the hippocampus (Burwell and Amaral, 1998).

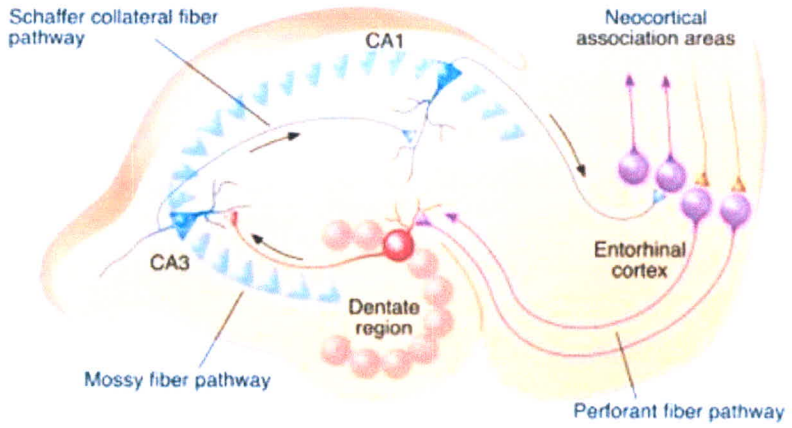

Figure 2 Connection and component of hippocampal formation

Source: http://www.helmholtz-muenchen.de/typo3temp/pics/9c415e7cd6.jpg

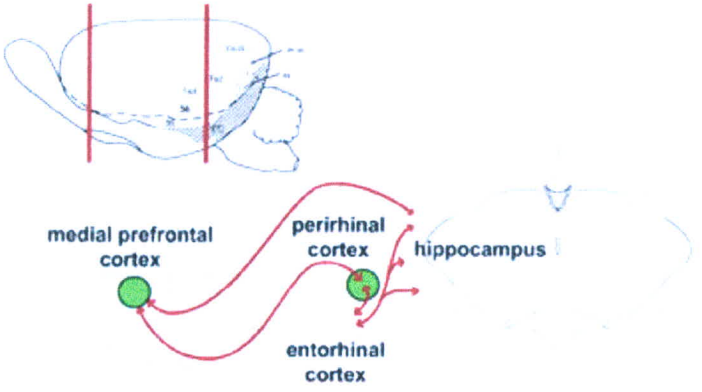

Figure 3 Association between the hippoacampus and prefrontal cortex

Source: http://www.bris.ac.uk/synaptic/research/projects/memory/recognition-memory/connections.jpg

Estrogen and Cognition

Estrogen is a steroid gonadal hormone that is mostly produced by ovarian follicles. Some is also produced in smaller amounts by other tissues such as liver, adrenal glands, adipose tissue and the breasts. Estrogen has not only critical impact on reproductive system and reflects sexual behavior in mammals but also has effects on non-reproductive behaviors such as anxiety and depressive-like behaviors along with cognitive function. It had been reported that OVX rats administrated with 17β-estradiol exhibited decreases in anxiety and depressive behaviors (Walf and Frye, 2006). However, estrogen affects on cognitive function which rely on type of task and area of brain. For instance, the administration of estrogen in female rats diminished the ability on a striatum-dependent task (Korol, 2004) while it enhanced the ability of prefrontal cortical-dependent learning (Luine, et al., 2003). Estrogen affects on several brain areas to mediate the cognitive function such as hippocampus, striatum, basal forebrain as well as prefrontal cortex (Spencer, et al., 2008). It had been suggested that estrogen fluctuation manipulates on the test of memory across estrus cycle (4-6 days), when serum estradiol (E2) peaks in proestrus, the rats that could solve a plus maze task (Korol, et al., 2004). Accordingly, the OVX rats treated with E2 it showed a similar result when using same task (Korol and Kolo, 2002). In women, some cognitive abilities are fluctuated through the menstrual cycle and related with the serum estrogen levels (Rosenberg and Park, 2002; Sherwin, 2003). It had also been accepted that estrogen fluctuation across of menstrual cycle associates with the activation of hippocampal formation (Dreher, et al., 2007). These studies suggested that ovarian steroid hormones affect on cognitive function requiring the hippocampal formation. Moreover, the suppression of ovarian hormones showed the impairment of hippocampal- and prefrontal-cortical-dependent memory but this effect was relieved by the exogenous E2 administration. For instance, OVX rats impaired ability on a task dependant on hippocampal and prefrontal cortical faculties, the object recognition memory task (Wallace, et al., 2006) was reversed by E2 administration (Korol, 2004).

Estrogen has been documented that it can mediate the brain function through cholinergic neurotransmission by reverse the decreasing in choline, the precursor of acetylcholine (ACh) synthesis, which induced by ovariectomy (Singh, et al., 1994). Furthermore, it was recognized that estrogen can process learning and memory by

mediate the morphology, physiology and chemical change of hippocampal neurons (Mukai, et al., 2010).

A current study has showed that the cognitive impairment in aging humans is the results from the change in neuronal morphology and neuronal chemistry such as the disturbance of neuron connections, loss of neurons and changes in neuronal transmission (Juraska and Lowry, 2012). Accordingly, these evidences were supported by prior investigation in rodents and human, which found cognition decline correlated with age and estrogen deficit by menopause, but it was improved by estrogen therapy (Sherwin and Tulandi, 1996; Markowska and Savonenko, 2002). Over all evidences suggest that estrogen has an important role for preventing cognitive abilities impairment induced by aging and ovarian hormone loss in women.

The effects of estrogen to be a neuroprotective and neurotropic abilities are recognized by improving and enhancing in cognitive function (Shughrue and Merchenthaler, 2000; Wise, et al., 2001). The previous investigation suggested that estrogen replacement therapy (ERT) can decrease risks and delay the onset of AD after menopause by increase cerebral blood flow, reduce neuronal inflammatory as well as enhance neuronal plasticity (Toran-Allerand, et al., 1999). In addition, estrogen is also crucial role to optimal brain function which were explained by exposure to estrogen *in vivo* and *in vitro* causes neurobiological changes in hippocampus that included dendritic spines increasing, neurotransmitter systems modulation, synaptic plasticity enhancing and cell signaling events controlling (Dohanich, 2002).

Notably, ERT is suggested to relive the menopausal symptoms and prevent cardiovascular disease and osteoporosis including reduce the risk for neurodegenerative disorder. However, there are several concern for using the ERT, because it produces serious side-effects such as increase the risks for endometrial cancer, breast cancer and venous thromboembolic events (Barrett-Connor and Grady, 1998).

Estrogen receptor

Estrogen receptors are divided into two classical subtypes, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). There are several distinct pathways

which estrogen and ERs regulate biological process. The first is the classical (genomic) pathway, estrogen binds to nuclear receptor and this complex binds to estrogen response element (EREs) to activate gene transcription. Second, it is known as "tethered pathway". This pathway involves protein-protein interaction with other transcription factor after ligand activation and thereby gene transcription is regulated by indirect DNA binding. The third pathway is also known "nongenomic" which produced by binding of estrogen to membrane receptors that leads to trigger the phosphorylation cAMP response element-binding protein (CREB), and consequently bind to DNA promoter. Finally, estrogen exerts it effects through the ligand-dependent pathway that involves activation through other signaling pathway such as growth factor signaling. In this pathway, the activated kinases phosphorylate ERs and thereby activate them to form dimerization and regulates gene following DNA binding (Heldring, et al., 2007; Cui, et al., 2013).

Both ERα and ERβ are distributed in many brain areas associating learning and memory process such as cerebral cortex and hippocampus (Shughrue, et al., 1997). ERα is the predominant subtype in hypothalamus and amygdale suggesting that involves the regulation of neuroendrocine system and emotional reactions. ERβ expression is highest in the hippocampus and cerebral cortex suggesting this subtype is importance for learning and memory process (Osterlund, et al., 2000). Several studies have suggested that ERs implicate in neuronal functions such as synaptic potentiation, synaptic depression and synapse formation (Day, et al., 2005; Szymczak, et al., 2006; Liu, et al., 2008). Some studied revealed that significant attenuation of both ERα and ERβ expression in spine synapse complex in hippocampal CA1 decreased ability of E2 in promoting spine density in hippocampus during aging (Adams, et al., 2001). Previous study has also shown that ERβ agonist, diarylpropionitrile (DPN), could attenuate neuronal damages in ischemic-induced mice, but not ERα agonist, propyl pyrazole triol (PPT). It was considered that a significant estrogen receptor-induced neuroprotective effect in a global ischemia involving ERβ (Carswell, et al., 2004). In the case of central nervous system (CNS) inflammation, it has been revealed that ERα could mediate to be a neuroprotection by signaling through astocytes (Spence, et al., 2013). These data suggest that ERα or ERβ can signal through distinct intracellular pathways to mediate the neuroprotective

activities, while the distinct molecular pathway by which ERα and ERβ affect neuronal functions are poorly understood.

Recent investigation revealed that subcellular distribution and activities of ERα and ERβ also change in aging and lead to differential response to estrogen in aging brain (Navarro, et al., 2012). The effects of low estrogen levels have been controversial for ERs expressions. Recent study had shown a decrement of the ERα in telencephalon and hippocampus of long-term OVX rats (6-24 months) which was reversed by E2 administration (Navarro, et al., 2012). Meanwhile, shot-term estrogen declined in OVX rats referred to up-regulation of ERα in the hippocampus and this effect would be absence when theses animals were immediately treated with estrogen after surgery (Cardoso, et al., 2010). Furthermore, there was some study showed a significant decrease in the expression of ERβ in the brains in three months OVX rats (Rose'Meyer, et al., 2003). These evidences have been demonstrated that estrogen might affect to regulate the expression of their receptor subtypes in the brain.

Brain-derived neutotrophic factor

Brain-derived neurotrophic factor (BDNF) is member in neurotrophins family which comprises nerve growth factor (NGF), neurotrophin-3 (NT-3) as well as neurotropin-4/5 (NT-4/5). BDNF exerts its effect by binding with its specific receptor, tyrosine kinase receptor B (TrkB), and few binding with p75 neurotrophin receptor (P75NTR). These binding lead to mediate the neuronal growth, survival, and plasticity in both central and peripheral nervous system (Chao, 2003).

BDNF was extensively presented throughout the brain with highest levels in the hippocampus followed by cerebral cortex (Hofer, et al., 1990). Previous reports explained that BDNF plays a critical role in learning and memory formation (Lindsay, et al., 1991) by enhancing the cholinergic system in the basal forebrain (Nonomura, et al., 1995). Furthermore, BDNF also produces LTP formation in the hippocampus (Patterson, et al., 1996), encourages activity-dependent dendritic growth (Ma, et al., 2002) as well as protects cell apoptosis (Courtney, et al., 1997).

Age-related decrease BDNF expression impacted on learning and memory ability by using the task of memory in animals (Croll, et al., 1998). In addition, aging could also diminish the expression of BDNF associating neurodegenerative disease,

such as AD. Numerous documents have demonstrated that AD patients showed decrease of BDNF in several brain areas such as hippocampus (Hock, et al., 2000), entorhinal cortex (Narisawa-Saito, et al., 1996) and cortex (Connor, et al., 1997; Michalski and Fahnestock, 2003).

Previous study has shown that hormonal status can greatly influence the expression of BDNF. It was reported that BDNF mRNA and protein levels were changed throughout the estrous cycle and the level of BDNF protein was highest during proestrus phase in female rats (Scharfman, et al., 2003). Moreover, ovarian hormones can regulate the BDNF synthesis. Spencer et al. evaluated effects of ovarian hormones on the hippocampus and found that fluctuation of these hormones could mediate the TrkB activation (Spencer, et al., 2008). These results are consistent with other studies that established the expression of BDNF was increased when administrated with exogenous estrogen in the olfactory bulb (Jezierski and Sohrabji, 2000), hippocampus (Allen and McCarson, 2005), cortex (Sohrabji, et al., 1995), amydala (Zhou, et al., 2005) and septum (Gibbs, 1998) in OVX rats.

Furthermore, extensive literature gathered evidences to decide the exactly role of ERs on BDNF expression. Previous study considered that estrogen exerts its effects on BDNF expression via binding nuclear ERs and brings to estrogen response element (ERE) formation followed by stimulating BDNF gene transcription in the pyramidal neurons of hippocampus (Sohrabji, et al., 1995; Luine and Frankfurt, 2013). In addition, other studies have also proposed that binding of estrogen to extranuclear ERs activate the CREB which trigger the CRE in DNA followed by promoting the BDNF gene transcription (Tao, et al., 1998; Luine and Frankfurt, 2013). Previous reports have considered that ERβ co-localize with GABAergic neuron and plays an indirect role to up-regulate BDNF in rats suggesting ERβ is an importance on BDNF regulation (Blurton-Jones and Tuszynski, 2002). The role of ERs to regulate the BDNF expression has been observed by the recently study, ERβ knockout mice (BERKO) showed a significant decreasing of BDNF mRNA, not ERα knockout mice (AERKO), suggesting ERβ affect on the regulation of BDNF transcription (Spencer-Segal, et al., 2012). Although ERα had no affect on BDNF transcription, it has been proposed that ERα involved the regulation of posttranscription and releasing of BDNF (Jezierski and Sohrabji, 2003; Sato, et al., 2007). These data have indicated that both

ERα and ERβ subtypes have a crucial role on the BDNF regulation. Moreover, some study has also concluded that both ERα and ERβ are associated with hippocampal neurogenesis by enhancing cell proliferation in the dentate gyrus (Mazzucco, et al., 2006). Theses findings suggested that ERs may modulate brain function in partial regions of the brain which associated learning and memory.

Phytoestrogens

Phytoestrogens are non-steroid compounds obtained from plants and have estrogenic-like effects (Patisaul, et al., 2001), which has the proven ability to attach to ERs in humans. It were referred to in literature as "plant estrogens" (Glazier and Bowman, 2001).

Phytoestrogens are divided into three main classes: isoflavones, comestans, and lignans (Thompson, et al., 2006). Among various phytoestrogens, isoflavones had been extensively studied while few had been studied for lignans and comestans. Isoflavones was found predominately in soybeans and soybean products. It was interested to useful prevent several diseases such as cancers, cardiovascular disease and oseteoporosis in menopausal women (Duncan, et al., 2003). Recently, phytoestrogens attracted interest as a potential alternative to the estrogen supplements which produce side-effects when use it for long time (Cornwell, et al., 2004).

Since several literatures indicate that ERT can improve the cognitive abilities decline and reduce the risk for neurodegenerative disorder in post-menopausal women, phytoestrogens may play a positive role in these conditions. Several studies have determined beneficial effects of soy phytoestogen on brain function. They showed that soy phytoestrogens regulated choline acetyltransferase, NGF and BDNF in brain areas such as the frontal cortex and hippocampus of female rats (Pan, et al., 2010). Moreover, OVX rats treated with phytoestrogens had been shown a dose-dependent improvement of visual spatial memory (Pan, et al., 1999). Additionally, study of Kim et al. found that dietary phytoestogens attenuated tau protein phosphorylations. They established conclusively that phytoestrogen may be effective to improve AD (Kim, et al., 2000). Previous study have considered that isoflavones was estrogen mimics which bound to ERβ with higher affinity than ERα. The estrogenic potency of phytoestrogens might trigger many biological responses on

brain function, which were evoked by the physiological estrogens (Kuiper, et al., 1998).

Phytoestrogens might able to substitute of estrogen that followed increase BNDF levels and bring to improve cognitive function. Pan et al. had shown that administration with soy germ phytoestrogen in OVX rats could increase BDNF and improve spatial memory without side-effects on reproductive organs in OVX rats. They have considered that phytoestrogen effects on hippocampal function by increase BDNF expression which bring to activate the synaptic formation and results to enhance learning and memory in circulating estrogen deficit (Pan, et al., 2010).

***Asparagus racemosus* Willd.**

Asparagus racemosus (AR) or Willd is known as Asparagaceae family. It is well known as "Shatavari" in the Ayurveda drug of India. AR is herbaceous plant which distinguished by has numerous roots (Figure 4). Its roots are used as herbal medicine and called as "Rak Sam Sip" in Thai (Boonsom, et al., 2012). The major active compounds (Figure 5) which presented in the root of AR are steroidal saponins or shatavarin and other constituents such as sarsasapogenin, racemosol and asparagamine (Bopana and Saxena, 2007).

AR was recognized for its neuroprotective properties in the animal models. Parihar and Hemnani found that the AR root methanolic extract could reverse the neuronal injury in the mice hippocampus and striatum by kainic acid (KA) injection. The neuroprotective effects of the AR root methanolic extract were revealed by increasing of glutathione peroxidase (GPx) activity and glutathione (GSH) content and lead to improve memory. They have demonstrated that AR root methanolic extract has potential effects to reduce oxidative stress by its anti-oxidant activity (Parihar and Hemnani, 2004). Numerous studies have been reviewed on the potential benefits of AR in cerebroprotective effects. It has been suggested that administration of AR methanolic extract 400 mg/kg B.W. protected the rats from ischemic-induced brain injury may be due to reduction of oxidative stress which occurs by alternation in levels of antioxidants, neurotransmitters and the AR extract had the potential to use in treatment of ischemia (Nandagopal, et al., 2011). It has been found that administration of AR methanolic root extract 150 mg/kg B.W. for 7 days could reverse learning and

memory deficits in mice (Ashwlayan and Singh, 2011). AR had also been documented for its nootropic and anti-amnesic activities by Ojha and co-workers. They have considered that AR root extract could mediate the augmentation of cholinergic system which brought to improve memory impairment induced by scopolamine (Ojha, et al., 2010). Furthermore, Mentat™ is an herbal psychotropic AR preparation that could improve the withdrawal symptoms caused by alcohol abstinence such as tremors, convulsions, hallucinations and anxiety in rats (Kulkarni and Verma, 1993).

Beside the saponin-rich fraction is extracted form AR, there are another active compounds that derived form AR. Wiboonpun and colleagues have found DPPH (α, α –diphenyl- β-picrylhydrazyl) which was decided to be a new antioxidant namely 'racemofuran' (Wiboonpun, et al., 2004). AR was also used in traditional ayurvedic formulations and known as "Shatavari kalpa" (Unnikrishnan, 1998). Furthermore, there were the several herbal medicine which were prepared from AR such as Abana® (containing 10 mg Stavari root extract per table) and Diabecon® (containing 20 mg Stavari root extract per table) by Himalaya Herbal Healthcare, India (Bopana and Saxena, 2007). There was report found the active constituent namely "Asparagamine", which was isolated form AR, showed anti-oxytocic properties (Sekine, et al., 1994). Moreover, isolation of AR root extract established the new compound called 'Racemosol' (Sekine, 1997).

For pharmacological applications of AR, it has been reported that AR was used for galactagogue, diuretic, anti-spasmodic as well as nerve tonic (Sharma, 2000). This herbal is one significant remedial plant in rasayana drugs which was report that it could increase cellular vitality and resistance to verity insults (Goyal, et al., 2003) which was recognized in Indian and British Pharmacopoeias (Bopana and Saxena, 2007).

The phytoestrogenic properties of AR was widely known and used to be a hormonal modulator to stimulant health tonic for women (Mayo, 1998), which has effects and structure similar to estrogen (Saxena, et al., 2010) (Figure 6). In vivo studies revealed that AR could inhibit the mammary carcinogenesis induced by DMBA (Rao, 1981) and showed inhibitory effect on uterine contractions induced oxytocin (Gaitonde and Jetmalani, 1969). It was suggested that "U-3107" or EveCare®, the herbal preparation containing 32 mg AR per 5 ml syrup, was used to

improve several menstrual disorders and defense abortion as well as increased wet and dry uterine weights accompanied by increasing of estrogen levels (Mitra, 1999). Furthermore, previous investigation of Neverkar had provided that EveCar capsules could improve uterine breeding and menstrual cycle in the 63 volunteer women (Nevrekar, 2002). In another study had provided that EveCare also showed its effect to correct the suffering from dysmenorrheal and pre-menstrual syndrome in 40 patients (Swarup, 1998).

(A) **(B)**

Figure 4 The characteristic of *Asparagus racemosus* (AR) Willd
The leaves and fruits of AR (A) and the root of AR (B)

Source: http://www.twinlotus.com/asp-bin/pic_HL/000095_HL.jpg
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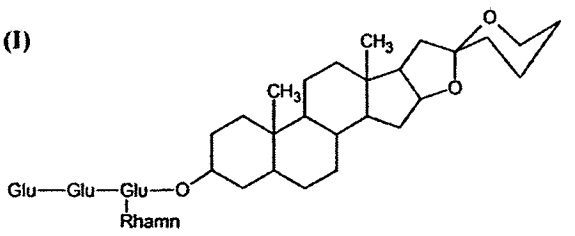
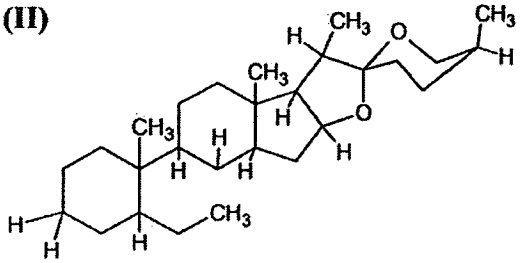
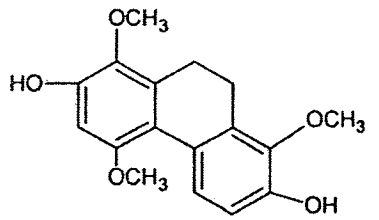
(I) **Shatavarin**



(II) **Sarsasapogenin**



(III) **Racemosol**



(IV) **Asparagamine**

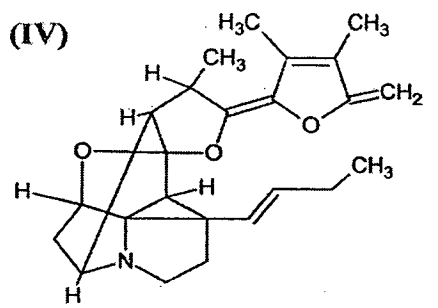
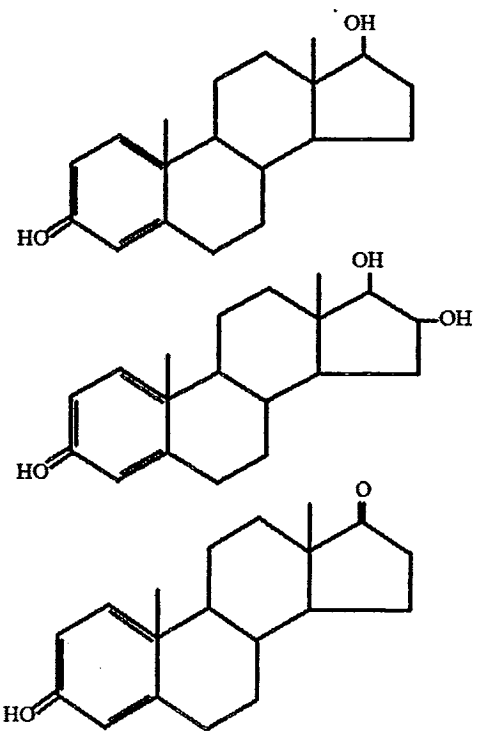


Figure 5 Active principles of AR (I) Shatavarin, (II) Sarsasapogenin, (III) Racemosol and (IV) Asparagamine

Source: Bopana and Saxena, 2007



Estradiol

Estriol

Estrone

Figure 6 Chemical structure of estrogens

Source: http://www.the-hormonal -nightmare.com/images/ Human_ Estrogen_ and_ Bioidentical_Estrogen.gif