The Effects of Oral Combined Nutraceuticals on Menopausal Symptoms and Hormonal Changes in Post-menopausal Women

Teerapong Rattanatantikul

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Department of Anti-Aging and Regenerative Medicine, College of Integrative Medicine, Dhurakij Pundit University

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ใบรับรองวิทยานิพนธ์

วิทยาลัยการแพทย์บูรณาการ มหาวิทยาลัยธุรกิจบัณฑิตย์

ปริญญาวิทยาศาสตรมหาบัณฑิต

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(ผู้ช่วยศาสตราจารย์ นายแพทย์มาศ ไม้ประเสริฐ)

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Author	Teerapong Rattanatantikul
Thesis Advisor	Asst. Prof. Pansak Sugkraroek, M.D.
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ABSTRACT

Menopausal symptoms severely reduce the quality of life of post-menopausal women worldwide, mainly due to estrogen deficiency. Hormone therapy containing estrogen is the current gold standard treatment for menopausal symptoms. However, research indicates these synthetic hormones vary clinically in safety and efficacy. Although bioidentical hormones doesn't have the evidence risk of cancer, people is still thinking that using hormones can affect that. Therefore, nutraceuticals that have estrogenic activity may be the alternative therapy for menopausal symptoms. The aim of this study was to evaluate the results of oral combined nutraceuticals on menopausal symptoms and hormonal changes in post-menopausal women. The study design was randomized, double-blinded, placebo-controlled clinical trial. 110 post-menopausal women aged 45-60 years old were enrolled and randomly assigned into treatment group and placebo group, equally. The oral combined nutraceuticals containing 100 mg of soy isoflavones, 80 mg of black cohosh, 40 mg of chasteberry, and 500 mg of evening primrose oil, were administered 1 capsule per day for 12 weeks in the treatment group, while the other group received a placebo. Menopausal symptoms and hormone levels including estradiol, luteinizing hormone, follicle-stimulating hormone, sex hormone binding globulin and blood chemistry levels were evaluated at baseline, week 6^{th}, and week 12^{th} of the study. For the evaluation of menopausal symptoms, there were significant differences of proportions in severity levels between the treatment group and the control group of hot flushes and sweating, sleep problems, depressed mood and irritability symptoms in receiving nutraceuticals group for 12 weeks found minimal severe symptoms and consecutively for 6 weeks group. For the evaluation of hormone levels, there were not significant differences between two groups. Additionally, there were found significantly improved of low-density lipoprotein cholesterol (LDL-C), triglyceride and high-sensitivity C-reactive protein (hs-CRP) within the treatment group at week 6^{th} and week 12^{th}. As well as only hs-CRP was significant lower in the treatment group than the control at week 12^{th}. Conclusion : Our study suggests that the intake of these oral

combined nutraceuticals can improve several menopausal symptoms, as well as some blood chemistry including LDL-C, Triglycerides, and hs-CRP in post-menopausal women.

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Teerapong Rattanatantikul

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Chapter 1

Introduction

1.1 Background and Signification of the Research Problem

Menopause symptoms severely reduce the quality of life of women worldwide, up to 80% of women may experience symptoms and it is estimated that in the year of 2030 the at risk groups of peri- and post-menopausal women will reach 1.2 billion globally (Gold et al., 2006). The main symptoms are hot flushes and night sweats, collectively referred to vasomotor symptoms; sleep disturbance, and other secondary symptoms often present (Palacios et al., 2010). Menopause is further associated with increased risk of osteoporosis, cardiovascular disease (CVD), and negative changes to lipid profile (Alexander and Clearfield, 2006 and Finkelstein et al., 2008). Hormone therapy (HT) containing estrogen is the current gold standard treatment for menopausal symptoms. Estradiol acts both on the estrogen receptors subtypes alpha (ERα) and beta (ERβ) (Jordan, 2001). Currently, hormonal therapy used in a form known as "bioidentical hormones" that doesn't have a precise medical definition. The endocrine society defines bioidentical hormones as compounds that have exactly the same chemical and molecular structure as hormones that are produced in the human body. Clinicians usually use the word to describe preparations containing either progesterone or one or more of three estrogens: estradiol (the predominant estrogen in premenopausal women), estrone, and estriol (the main estrogen produced during pregnancy) (Harvard Health Publishing, 2018). The majority of these hormone preparations, commonly referred to as hormone replacement therapy (HRT), should perhaps be more aptly referred to as hormone substitution therapy, as most of the therapies utilized do not exactly match those produced in the body. Research indicates these synthetic hormones vary clinically in safety and efficacy (Moskowitz, 2006). However, people is still thinking that using hormones can affect the risk of cancer. And some researches provide results that considerable evidences support that therapy increases cancer risk in estrogen receptor (ER) α rich tissues (e.g. uterus and breast) (Zhou et al., 2008 and Ross-Innes et al., 2012).

An ideal selective estrogen receptor modulators (SERMs) would act as antagonist-activity in the mammary gland and uterus, and the same as agonist activity in other target tissues that benefit from an estrogen-like action, such as the cardiovascular, skeletal, and central nervous systems (Jordan, 2001). Some nutraceuticals that have estrogenic activity are promising alternative medicines which have been used for treatment of menopausal symptoms and they are not prone to cancer.

Soy are widely used for diet. It is one of the richest natural sources of isoflavones, in particular the phytoestrogens. Isoflavones selectively bind to estrogen receptors (ERs) as they retain a strong binding affinity to ERβ (Kuiper, 1997). As a result, it is used to relieve menopausal symptoms.

Black cohosh is a perennial member of the buttercup family, native to North America. It is used to treat women's hormone-related symptoms, including menopausal symptoms. It contains phytochemicals that have an effect on the endocrine system (Seidlova-Wuttke, 2003).

Chasteberry is native to Mediterranean regions has been used for centuries to treat several menstrual problems. Chasteberry contains a wide variety of synergistic active constituents which include flavonoids, diterpenes. In the UK, chasteberry is the most common treatment prescribed for menopausal symptoms amongst herbalists (van Die et al. 2009).

Evening primrose oil (EPO) is extracted from the seeds of evening primrose (*Oenothera biennis*). It is native to North and South America and grown in Europe and Asia. Extracts have been used traditionally for women's health issues. It contains a high content of essential fatty acids, including gamma-linolenic acid and various phytosterols. In a previous study, supplementation with evening primrose oil significantly reduced the severity of menopausal symptoms in menopausal women, compared to placebo (Farzaneh et al., 2013).

Therefore, the combination of these natural compounds might have potential to improve menopausal symptoms and hormone levels in menopausal women. It could be an alternative nutraceutical for menopausal symptoms relief. To date, the clinical studies of these mixed natural supplementation in post-menopausal women have not been conducted. The objective of this study is to investigate the effects of the nutraceutical on menopause symptoms and hormone levels in post-menopausal women.

1.2 Research Hypothesis

The menopausal symptoms of post-menopausal women improve after taking nutraceuticals.

1.3 Objectives of Research

1. To study the results of oral combined nutraceuticals on menopausal symptoms in post-menopausal women.

2. To study the effects of oral combined nutraceuticals on level of estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone binding globulin (SHBG) in post-menopausal women.

3. To study the adverse effect of oral combined nutraceuticals in post-menopausal women.

1.4 Expected Benefits

1. Understood of the result of the oral combined nutraceuticals on menopausal symptoms in post-menopausal women.

2. Understood of the result of the oral combined nutraceuticals on hormonal changes in post-menopausal women.

3. Understood of the knowledge of alternative treatment on menopausal symptoms in post-menopausal women.

1.5 Definition of Terms

1. Menopausal symptom is the symptoms that assessed by using the Menopausal Rating Scale (MRS).

2. Post-menopausal women in this research is women age between 45-60 years who have menopausal symptoms and is amenorrhea for at least 12 consecutive months.

3. Nutraceuticals in this research is the product that contain 4 specific ingredients as follows.

Nutraceuticals Ingredients	
Soy Isoflavones	100 mg
Black cohosh extract	80 mg
Chasteberry extract	40 mg
Evening primrose oil	500 mg

Table 1.1: Constituents of nutraceutical

1.6 Research framework

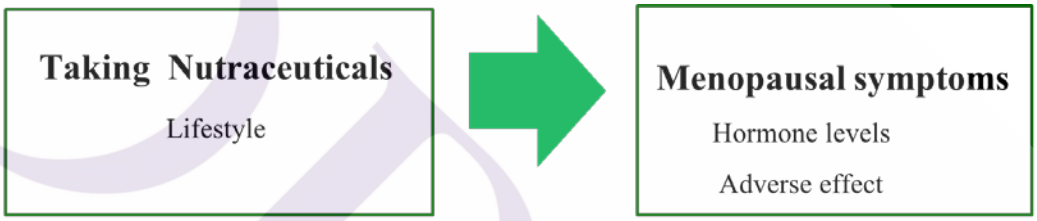


Figure 1.1 Research framework

Chapter 2

Review of Related Literature

2.1 Overview of Reproductive Endocrinology

2.1.1 Oogenesis

Oogenesis is the development of a mature oocyte. It is a prolonged process. Immature oocyte form in the female embryo, but do not complete their development until the beginning of puberty. The end number of follicles and oocytes is established in human ovaries during the fetal period of life and does not increase after birth. (Reece et al., 2011)

As a result of this, the number of oocytes are limited and will be ovulated during a woman's reproductive lifetime.

2.1.2 Ovulation

Ovulation is the release of mature oocyte at the midpoint of a female cycle from the follicle in the ovaries. Follicular cells produce estradiol prior to ovulation. The remaining follicular tissue grows within the ovary, forming a mass called the corpus luteum. The corpus luteum secretes progesterone that helps to maintain pregnancy. If the egg is not fertilized, the corpus luteum degenerates. (Reece et al., 2011)

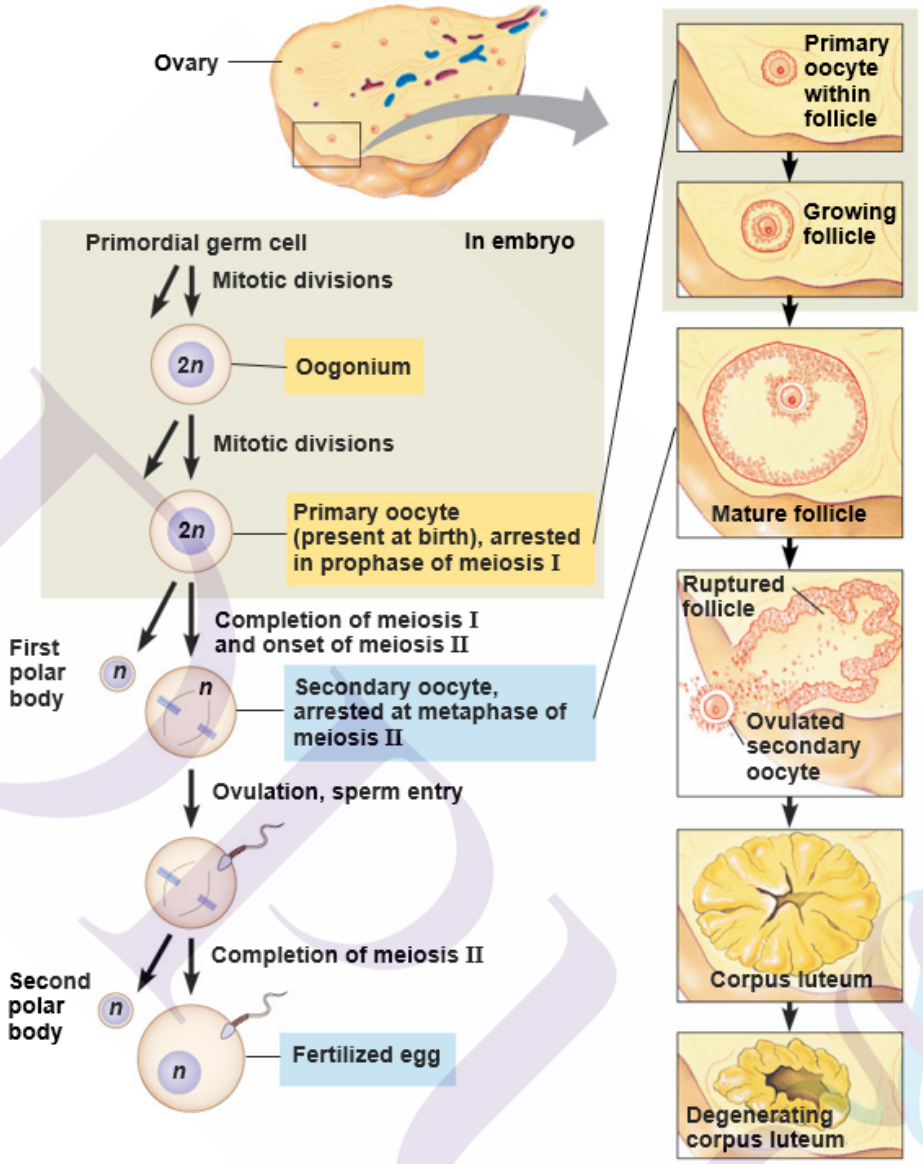


Figure 2.1 Human Oogenesis

Source: Reece et al., 2011

2.1.3 Menstruation

Prior to ovulation, the endometrium thickens with blood vessels in preparation for embryo implantation. If an embryo does not implant in the endometrium, the endometrium is shed in a process called menstruation.

Menstruation is the cyclic shedding of the blood-rich uterine lining, in response to the interactions of hormones produced by the hypothalamus, pituitary, and ovaries. A process occurs in flow through the cervix and vagina.

2.1.4 The Ovarian Cycle

The ovarian cycle is defined as changes in the ovaries. This cycle is divided into two phases.

1. Follicular phase

Follicle growth and an increase in the hormone estradiol characterize the follicular phase of the ovarian cycle. The follicular phase ends at ovulation, and the secondary oocyte is released.

2. Luteal phase

Following ovulation, the follicular tissue left behind transforms into the corpus luteum. The corpus luteum disintegrates, and ovarian steroid hormones decrease (Reece et al., 2011).

2.1.5 The Menstrual (Uterine) Cycle

The menstrual cycle (also called the uterine cycle) is defined as changes in the uterus. This cycle is divided into two phases.

1. Proliferative phase

Thickening of the endometrium during the proliferative phase coordinates with the follicular phase.

2. Secretory phase

Secretion of nutrients during the secretory phase coordinates with the luteal phase.

Shedding of the endometrium during the menstrual flow phase coordinates with the growth of new ovarian follicle. A new cycle begins if no embryo implants in the endometrium

2.1.6 Hormones

Human reproduction is coordinated by hormones from the hypothalamus, anterior pituitary and gonads.

2.1.6.1 Hypothalamus

Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus and directs the release of FSH and LH from the anterior pituitary gland.

2.1.6.2 Pituitary Gland

There are two hormones secreted from the anterior pituitary gland and regulate processes in the ovaries to develop follicles, ovulation and the production of sex hormones.

1. Follicle stimulating hormone (FSH)

In female, FSH stimulates the growth of follicular cells in ovaries that release estrogen. As a result of this, it causes growth and increased estrogen production.

2. Luteinizing hormone (LH)

In female, LH stimulates ovulation and development of the corpus luteum.

2.1.6.3 Ovary

There are two hormones secreted from the ovaries and act on the uterus to prepare for pregnancy.

1. Estrogen

It is secreted by growing follicle. This is low at the beginning of the menstrual cycle and peaks at the middle (follicular or proliferative phase) and then once again towards the end. Estrogens (e.g. estradiol, E2) are the primary hormones that provide negative feedback to the hypothalamus and anterior pituitary to inhibit FSH and LH secretion.

2. Progesterone

It is secreted by corpus luteum that transformed from follicular tissue following ovulation. There is a significant increase in the second half of menstruation (luteal or secretory phase).

2.1.7 Sex hormone binding globulin (SHBG)

SHBG is a glycoprotein possessing high affinity binding for 17 beta-hydroxysteroid hormones such as testosterone and estradiol. It is mainly produced in the liver. It is involved in transport of sex hormone in plasma (Selby, 1990). Estradiol stimulates the production of sex hormone-binding globulin (Kalme, 1999). Only unbound estrogen appears to be biologically active.

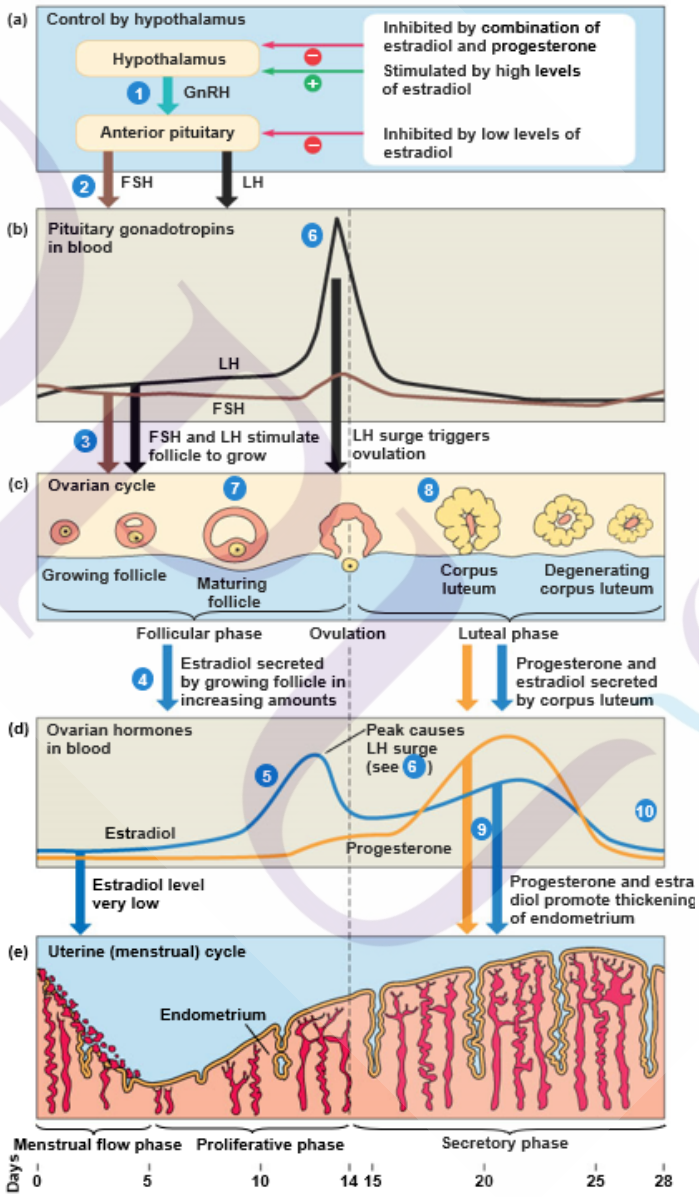


Figure 2.2 The reproductive cycle and hormonal change in human female.

Source: Reece et al., 2011

2.2 Review of menopause

2.2.1 Menopause

Menopause is defined as the absence of menstrual periods for 12 consecutive months and is the permanent cessation of menstruation and ovulation that resulting in the loss of ovarian follicle development (Sherwin, 2001; Spinelli, 2004)

Menopause is prompted by gradually decline in estrogen and progesterone production by ovaries until stop producing permanently, and rising FSH and LH levels. Their monthly periods become less regular and eventually stop completely.

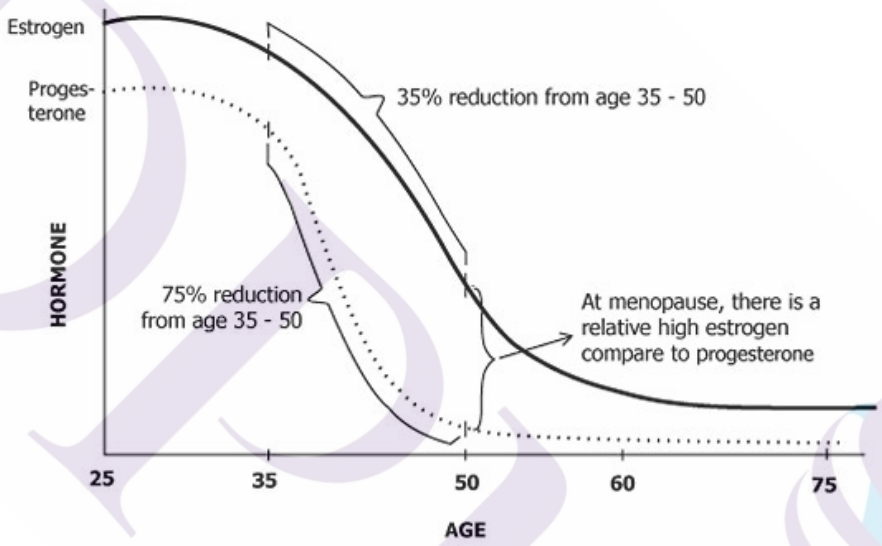


Figure 2.3 Reduction in estrogen and progesterone levels with age.

Sources: http://shodhganga.inflibnet.ac.in/jspui/bitstream/10603/176114/4/chapter%201.pdf

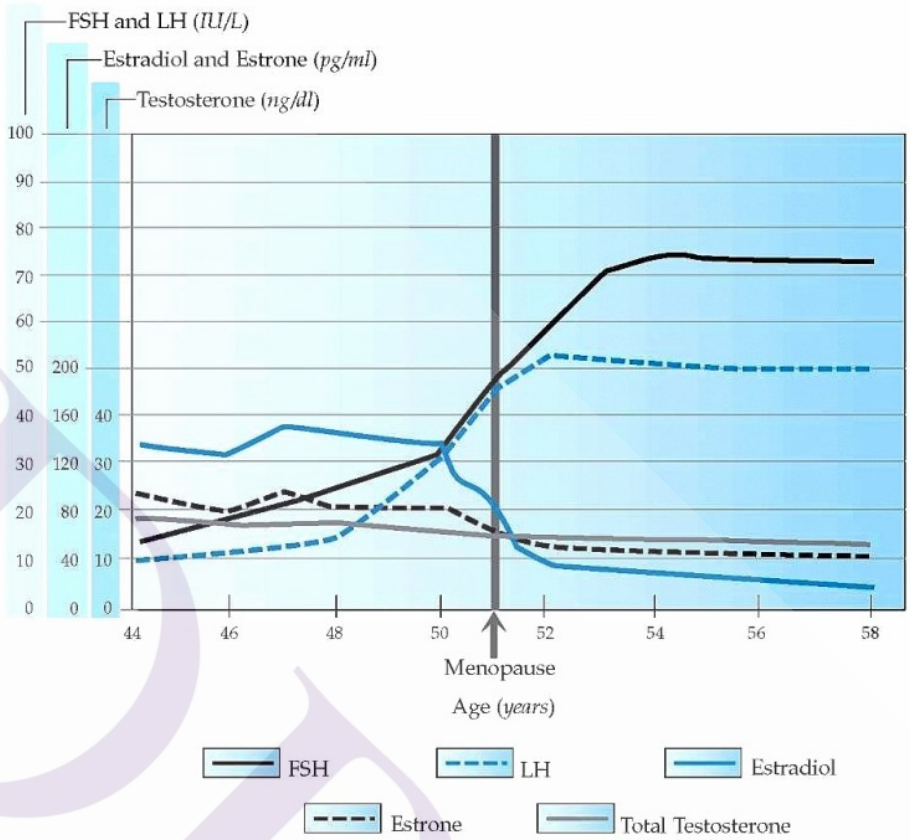


Figure 2.4 Hormonal changes during menopausal transition. A subtle rise in FSH occurs first, followed by a rise in LH and a decline in estradiol and estrone.

Sources: http://what-when-how.com/acp-medicine/menopause-part-1/

2.2.2 The age of menopause

In one study, the average age of natural menopause has been reported as 54 in Europe, 51.4 in North America, 48.6 in Latin America and 51.1 in Asia (Palacios et al., 2010). The age at menopause is unaffected by race, socioeconomic status, age at menarche, or number of prior ovulations (Dalal and Agarwal, 2015). Factors that are toxic to the ovary often result in an earlier age of menopause such as smoking (Adena, 1982).

2.2.3 Types of menopause

1. Natural menopause

It occurs when the ovaries naturally decrease their production of the sex hormones estrogen and progesterone. It usually begins between the ages of 45 and 55, but can develop before or after this age range.

2. Surgical menopause

This occurs when the both side of the ovary are surgically removed (bilateral oophorectomy).

2.2.4 Perimenopause

Perimenopause is defined as the time when the first clinical signs of physiological changes occur that begin the transition to menopause. In average, it usually begins 2 to 8 years prior to menopause (around mid forties) and includes the first year after menopause. (Gynecology 3rd edition)

2.2.5 Premature menopause

Premature menopause is defined as menopause before the age of 40.

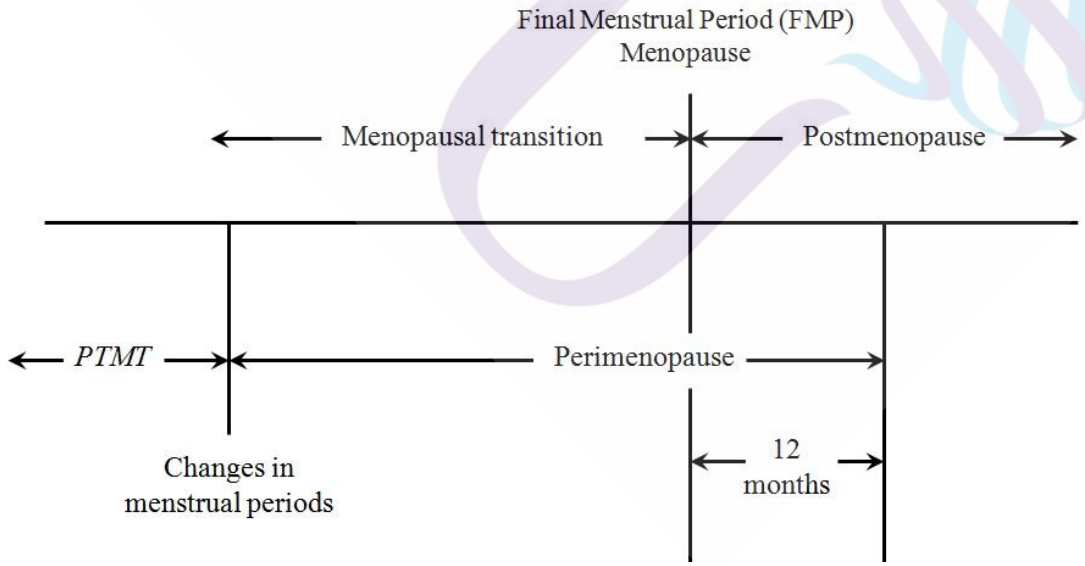


Figure 2.5 Diagrammatic representation of different phases of menopause. PTMT - Prior To Menopausal Transition.

Sources: http://www.academia.edu/Documents/in/Shodhganga

2.2.6 The climacteric

Climacteric is the period of life starting from the decline in ovarian activity until after the end of ovarian function. According to the definition, the period includes peri-menopause, menopause and post-menopause (Taechakraichana et al., 2002).

2.3 Menopausal symptom

The decline in estrogen that occur during the menopausal transition can cause menopausal symptoms. There are many symptoms of menopausal women. These include 4 cores symptom : vasomotor, vaginal, insomnia and mood. The fluctuations in estrogen that occur during the menopausal transition can cause menopausal symptom (Santoro et al., 2015).

Vasomotor symptoms (VMS)

VMS typically refer to hot flushes, dizziness, headache, night sweat and palpitation. The hot flushes are the most common and bothersome symptoms reported by women during the menopausal transition (perimenopause and post menopause) (Freeman et al., 2007). The onset of hot flushes are present before menopause 1-2 years. The average duration of hot flushes is about 5 years after menopause. However, VMS may improve for a longer period (Gynecology 3rd edition). The prevalence of hot flushes is high in diverse population of menopausal and premenopausal women (Reed et al., 2014 and ACOG 2014). From recent study, there are no relationships between these symptoms and plasma Estradiol (Freedman, 2014) However, the exact cause of hot flushes still has not been clarified (Santoro et al., 2015).

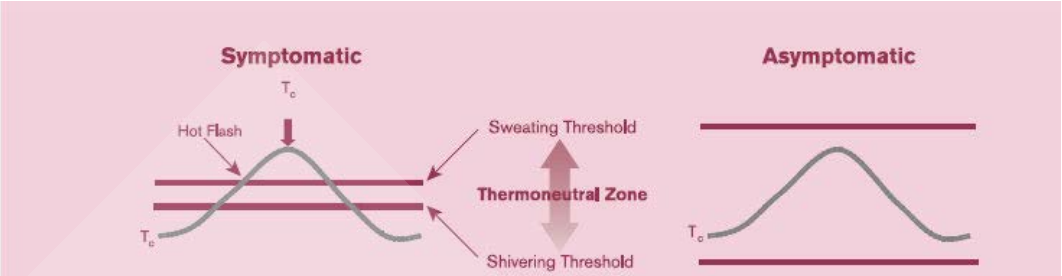


Figure 1A. Small core body temperature (T_c) elevations acting within a reduced thermoneutral zone trigger hot flashes in symptomatic postmenopausal women

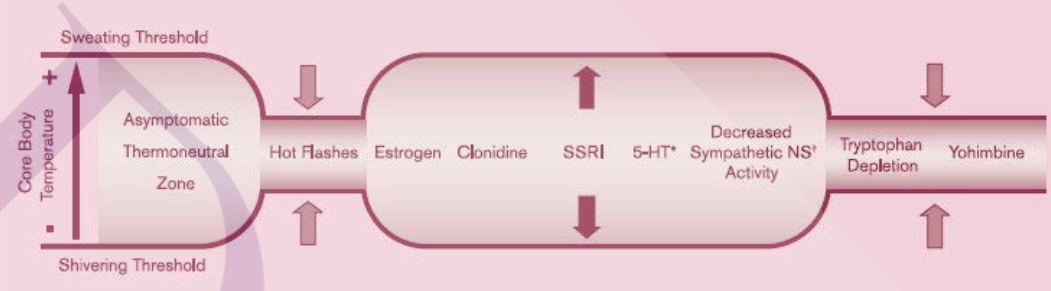


Figure 1B. Factors that influence the thermoneutral zone

*5-HT = serotonin (5-hydroxytryptophan)
†NS = nervous system

Figure 2.6 Mechanism of hot flush and Thermoneutral zone

Sources: https://www.menopausemgmt.com/current-best-treatments-for-hot-flashes/

Vulvovaginal atrophy

Urogenital tissues are intensely sensitive to estrogen, and the decrease of estrogen during the menopausal transition can cause these tissue thinner and troubling symptoms. Genital symptoms include vaginal dryness, dyspareunia and decline in sex drive (Gynecology 3rd edition).

Sleep disturbances and insomnia

Insomnia is one of the typical menopausal symptoms. During menopausal transition, vasomotor symptoms are typically experienced and cause sleep disturbances (Jehan, 2017).

Mood Changes

Other independent risk factors for the development of depressed mood during the menopause transition including poor sleep, stressful or negative life events, lack of employment, higher body mass index, smoking, younger age, and race (African Americans twice as likely to have depressive symptoms). In addition, there is evidence that hormonal changes occurring during menopause play a role, as evidenced by increased risk for depression in association with variability in estradiol levels, increasing FSH levels, surgical menopause, the presence of hot

flushes, and a history of premenstrual syndrome. Contrary to prior belief, hot flushes are not necessary to the development of depression. Some have proposed the cascade theory, in which hot flushes lead to sleep disturbance and then to daytime fatigue, poor quality of life, and then depressive symptoms. Research instead shows 33 that depressive symptoms more often precede hot flushes when they co-occur.

2.4 Estrogen receptors subtypes alpha (ERα) and beta (ERβ)

The physiological functions of estrogenic compounds are modulated largely by the estrogen receptors subtypes alpha (ERα) and beta (ERβ). In humans, both receptor subtypes are expressed in many cells and tissue.

ERα is present mainly in mammary gland, uterus, ovary (thecal cells), bone, male reproductive organs (testes and epididymis), prostate (stroma), liver, and adipose tissue. The alpha subtype has a more prominent role on the mammary gland and uterus, as well as on the preservation of skeletal homeostasis and the regulation of metabolism.

ERβ is found mainly in the prostate (epithelium), bladder, ovary (granulosa cells), colon, adipose tissue, and immune system. The beta subtype seems to have a more profound effect on the central nervous and immune systems, and it generally counteracts the ERα-promoted cell hyperproliferation in tissues such as breast and uterus (Dahlman-Wright et al., 2006 and Heldring et al., 2007).

In many breast cancers, ERα activation is generally considered responsible for enhanced proliferation, whereas this is counteracted by the presence of ERβ, which exerts an antiproliferative effect (Chang EC et al., 2006).

Nowadays, a great deal of attention is being devoted to the administration of selective ERβ activators, since they are considered likely to be safer than non-specific estrogens, due to the lack of stimulation of ERα (Mauvais-Jarvis et al., 2013).

2.5 Hormonal replacement therapy of menopausal symptoms and cancer risk

Hormone replacement therapy (HRT) is the current gold standard treatment for menopausal symptoms. HT that contains estrogen binds strongly to both ERα and ERβ acting as a potent agonist (Brzozowski et al., 1997). Treating menopausal symptoms with estrogen and

progestin (synthetic progesterone) together is called estrogen-progestin therapy (EPT) or combined hormone therapy. Treating menopausal symptoms with estrogen alone is called estrogen therapy (ET).

ET improves the menopausal symptoms but it increases the risk of endometrial cancer. In contrast, ET is not linked to a higher risk of breast cancer.

EPT does not increase the risk of endometrial cancer. In contrast, EPT is linked to a higher risk of breast cancer. The longer EPT is used, the higher the risk (The American Cancer Society medical and editorial content team, 2015).

According to National Collaborating Centre for Women's and Children's Health (UK) Guideline, they offer women HRT for vasomotor symptoms after discussing with them the short-term (up to 5 years) and longer-term benefits and risks. Offer a choice of preparations as follows:

1) Estrogen and progestogen to women with a uterus.

2) Estrogen alone to women without a uterus. (National Collaborating Centre for Women's and Children's Health (UK), 2015).

2.6 Phytoestrogen

Phytoestrogen are plant compounds with estrogen-like activity (Price and Fenwick, 1985). Estrogenic activity has been reported among compounds produced by animals, plants, and microorganisms, as well as in industrially manufactured chemicals, including pesticides and insecticides, and their breakdown products (Duax and Griffin, 1985). Pesticides and insecticides also contain estrogen-like compounds and are now classified as xenoestrogens (Davis and Bradlow, 1995).

There are three main classes of phytoestrogens: isoflavones, coumestans, and lignans, which occur in either plants or their seeds. A single plant often contains more than one class of phytoestrogen. The major isoflavones, genistein which has the greatest affinity to estrogen receptors with a 7-fold greater preference towards ERβ than ERα, and daidzein, commonly exist as inactive glucosides (Axelson et al., 1984). Daidzein is further partially metabolized to form the isoflavone, Equol which also has potent ERβ affinity.

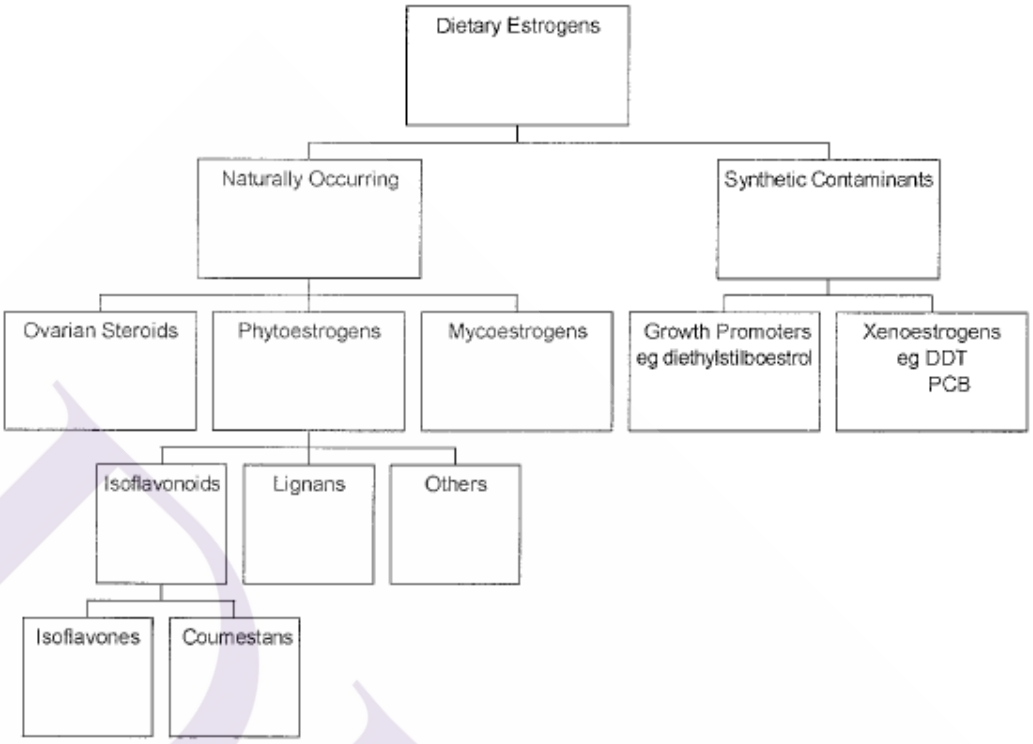


Figure 2.7 Sources and classification of dietary estrogens

Sources: Murkies et al., 1998

In humans, after consumption of plant lignans and isoflavones, complex enzymatic metabolic conversions occur in the gastrointestinal tract, resulting in the formation of heterocyclic phenols with a close similarity in structure to estrogens (Setchell et al., 1984) deconjugated by intestinal flora, reabsorbed, reconjugated by the liver, and excreted in the urine (Adlercreutz, 1986).

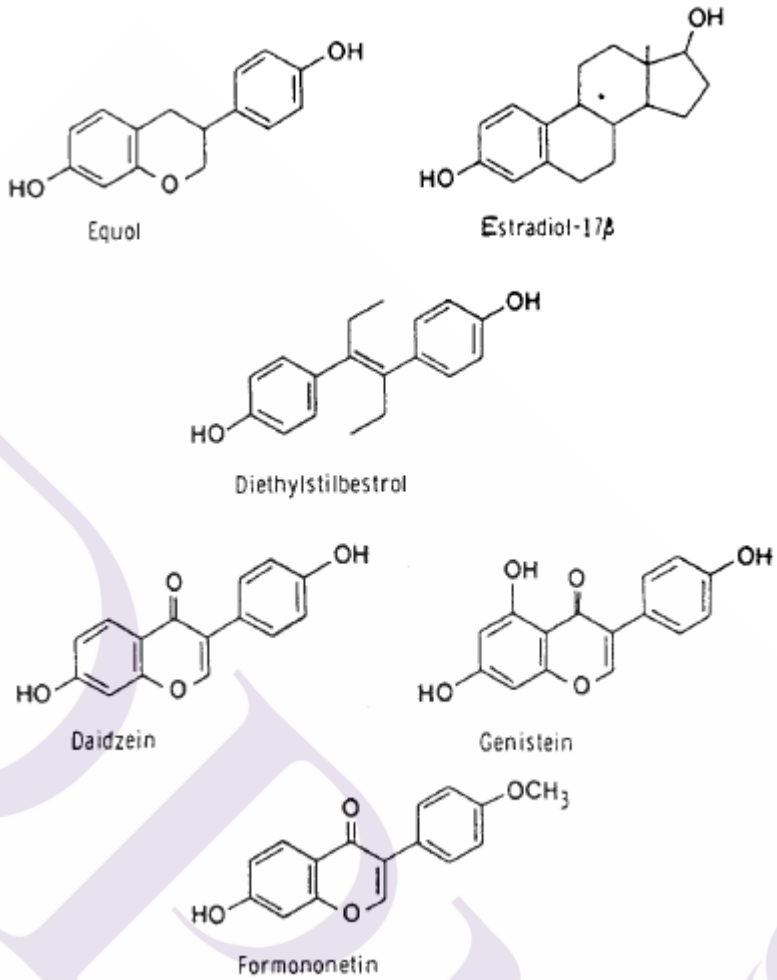


Figure 2.8 A comparison of the chemical structure of the phytoestrogen equol, formed in the gastrointestinal tract of man and animals, estradiol-17β, diethylstilbestrol and several phytoestrogens of plant origin.

Sources: Setchell et al., 1984

2.6.1 Soy Isoflavone

Soy is one of the richest natural sources of isoflavones with steroid-like effects, in particular the phytoestrogens genistein, daidzein and glycitein, which are structurally similar to estradiol. Isoflavones selectively modulate ERs as they retain a strong binding affinity to ERβ and weak affinity to ERα (Kuiper, 1997). Isoflavones have 1000 fold weaker binding affinity than estradiol to ERα (Escande et al., 2006).

Meta-analyses have demonstrated that soy isoflavone supplementation above 50 mg/d can significantly reduce hot flush frequency and severity greater than placebo in menopausal

women (Clarkson et al., 2011 and Taku et al., 2012). Soy isoflavones can also reduce night sweats (Upmalis, 2000), improve lipid balance (Han et al., 2002), quality of life (sexual, physical and psychological) (Basaria et al., 2009) and improve general menopause symptoms (Nahas et al., 2007) greater than placebo. Similarly, in target groups such as 18-35 years old women suffering from premenstrual syndrome (PMS), soy isoflavone supplementation can reduce symptoms such as headaches, mastalgia, cramps and swelling more efficiently than placebo (Bryant et al., 2005).

The side effects of soy isoflavone includes stomach upset constipation, bloating and nausea. It can cause allergic reaction such as rash and itching. But a very serious allergic reaction such as anaphylaxis to this product is rare (Soy Isoflavones, 2019).

2.6.2 Black cohosh (*Actaea racemose*)

Black cohosh, medicinal root, is native to North America. It is used to treat women's hormone-related symptoms, including PMS, menstrual cramps, and menopausal symptoms. It contains potent phytochemicals that have an mild effect on estrogen receptor (Seidlova, 2003). Several studies have demonstrated that black cohosh can significantly improve all symptoms of menopause (reducing Kupperman Index and Menopause Rating Scale scores) including vasomotor effects, anxiety, depression, vaginal atrophy as well as other physical and psychological symptoms (Bai et al., 2007; Mohammad et al., 2013; Nappi et al., 2005; Osmers et al., 2005 and Wuttke and Gorkow, 2003). Further benefits include improvement of sleep quality in post-menopausal women with disordered sleep patterns (Jiang et al., 2015), as well as improvement of vaginal maturity and increased osteoblast activity (Wuttke et al., 2006) trials.

A review of 2 randomized controlled studies revealed that black cohosh significantly improved menopause symptom scores greater than control or placebo (Beer et al., 2013).

A review of 1 study, black cohosh was able to improve peri-menopause symptom as effectively as Tibolone and does not have an obvious estrogen-like effect. It could be safer choice for patients who are concerned about the cancer risk of estrogen (Chen et al., 2014).

From American Botanical Council recommendation, the dose of black cohosh crude extract is 40–80 mg (or oral dose equivalent) per day. The adverse effects of black cohosh include occasional gastrointestinal discomfort, vertigo, headache, nausea, vomiting, impaired vision and

impaired circulation have been reported in cases of overdose (American Botanical Council, 2002).

However, the mechanism by which black cohosh relieves symptoms is unclear. The alleviation of menopausal symptoms by black cohosh suggests an estrogenic mechanism, but menopausal symptoms can also be alleviated by selective serotonin reuptake inhibitors (SSRIs), suggesting that black cohosh may work through a serotonergic mechanism. Many menopausal symptoms including hot flushes, mood swings and anxiety, insomnia are mediated through the central nervous system (CNS) and may be relieved through a variety of mechanisms. It is possible that black cohosh can act via multiple tissue-dependent mechanisms, including estrogenic (or antiestrogenic), serotonergic, antioxidative, and inflammatory or anti-inflammatory (Ruhlen et al., 2008).

2.8 Chasteberry (*Vitex agnus-castus*)

Chasteberry has been used for centuries to treat several menstrual problems. It contains many phytochemicals including flavonoids that are thought to have many positive effects on health. A review of 8 randomized-controlled clinical studies (van Die et al.,2013) revealed that chasteberry was effective in normalizing irregular cycles and relieving premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) in 7 of the 8 trials, compared to their respective controls. On average, around 67% of irregular cycles returned to normal and symptoms were reduced by 25-50%. Chasteberry can improve a large variety of symptoms of moderate to severe PMS (Ma et al., 2010). In the UK, chasteberry is the most common treatment prescribed for menopausal symptoms among herbalists (van Die et al. 2009).

Chasteberry extract is likely safe for most people taking orally. The side effects include upset stomach, nausea, itching, rash, headaches, acne, trouble sleeping, and weight gain. A change in menstrual flow when they taking chasteberry is probable noticed by some women. The dose of chasteberry is indicated 20-40 mg of taken daily for 8 weeks has been used (Vitex agnus-castus, 2019).

2.9 Evening primrose oil (EPO)

EPO is extracted from the seeds of evening primrose (*Oenothera biennis*). It contains a high content of essential fatty acids, including gamma-linolenic acid and various phytosterols. In previous study, it was reported that supplementation with evening primrose oil (500 mg) significantly reduced the severity of hot flushes in menopausal women, compared to placebo (Farzaneh et al., 2013), with additional improvements in quality-of-life indicators of social and sexual activity. For premenstrual syndrome, there is some evidence that gamma-linolenic acid can regulate the severity of symptoms. This was demonstrated in one trial of an essential oil extract containing more than 60% purified linolenic/gamma linolenic acids in 120 PMS sufferers, aged in their 30's (Rocha et al., 2011). After 6 months of daily consumption, an approximately 3-fold reduction in self-reported indices of symptom severity was observed during both the follicular and luteal phases, when compared to placebo.

In one study about the efficacy of Black cohosh in comparison with evening primrose oil (EPO) on menopausal symptoms. The result showed that it seems that black cohosh is more effective than evening primrose oil because of reducing of hot flushes too (Mehrpooya et al., 2018).

Evening primrose oil is likely safe for most people when used for up to a year. It can sometimes cause mild side effects including upset stomach, nausea, diarrhea, and headache (Evening primrose oil, 2019).

Table 2.1 Data safety

Ingredients	The dose study	The maximum dose in previous study	Data Safety
Soy Isoflavones	100 mg	160 mg	This systematic review showed that maximum dose of using isoflavone was 160 mg per day. (Perna et al., 2016)
Black Cohosh Extract	80 mg	160 mg	The study was 8 to 54 weeks in length. Doses of 160 mg per day by mouth do not appear to have any adverse effect on the user. (Wobser and Takov, 2019)
Chasteberry Extract	40 mg	40 mg	The dosage in this study is 40 mg per day for PMS for 3 months period. No patients reported remarkable side effects. (Ambrosini et al., 2013)
Evening Primrose Oil	500 mg	6,000 mg	The maximum doses in the study was 6,000 mg for 4 weeks by oral administration. (Sherwin, 2001)

2.10 The Menopause Rating Scale (MRS) scale

The Menopause Rating Scale was initially designed to measure the severity of aging symptoms. It is accepted internationally. The scale was designed and standardized as a self-administered scale to assess symptoms/complaints of aging women under different conditions, to evaluate the severity of symptoms over time, and to measure changes pre- and post-menopause therapy (available 10 languages). In a previous study, the currently available methodological evidence points towards a high quality of the MRS scale to measure and to compare health-

related quality of life (HRQoL) of aging women in different regions and over time, it suggests a high reliability and high validity as far as the process of construct validation could be completed yet (Heinemann et al., 2004).

Menopause Rating Scale (MRS)

Which of the following symptoms apply to you at this time? Please, mark the appropriate box for each symptom. For symptoms that do not apply, please mark 'none'.

Symptoms:	none	mild	moderate	severe	very severe
Score =	0	1	2	3	4
1. Hot flushes, sweating (episodes of sweating)	☐	☐	☐	☐	☐
2. Heart discomfort (unusual awareness of heart beat, heart skipping, heart racing, tightness)	☐	☐	☐	☐	☐
3. Sleep problems (difficulty in falling asleep, difficulty in sleeping through, waking up early)	☐	☐	☐	☐	☐
4. Depressive mood (feeling down, sad, on the verge of tears, lack of drive, mood swings)	☐	☐	☐	☐	☐
5. Irritability (feeling nervous, inner tension, feeling aggressive)	☐	☐	☐	☐	☐
6. Anxiety (inner restlessness, feeling panicky)	☐	☐	☐	☐	☐
7. Physical and mental exhaustion (general decrease in performance, impaired memory, decrease in concentration, forgetfulness)	☐	☐	☐	☐	☐
8. Sexual problems (change in sexual desire, in sexual activity and satisfaction)	☐	☐	☐	☐	☐
9. Bladder problems (difficulty in urinating, increased need to urinate, bladder incontinence)	☐	☐	☐	☐	☐
10. Dryness of vagina (sensation of dryness or burning in the vagina, difficulty with sexual intercourse)	☐	☐	☐	☐	☐
11. Joint and muscular discomfort (pain in the joints, rheumatoid complaints)	☐	☐	☐	☐	☐

Figure 2.9 The English version of the Menopause Rating Scale (MRS)

Source: Heinemann et al., 2003

Chapter 3

Research Methodology

3.1 Target Sample Size

110 subjects were selected by using a questionnaire and physical examination by a medical doctor. This study was proceeded at department of nutrition, faculty of public health, Mahidol university, Thailand.

The sample size of this clinical study was referred to previous soy isoflavone and menopause symptoms studies at least 58 subjects (Maturitas,2005;51:127-34., Menopause 2004;11:400-404., Obstet Gynecol 2003;101:1213-1220.).

This study was a randomized, double-blinded, placebo-controlled trial. A total of 110 post-menopausal women were randomly allocated to treatment or control groups based on a sequence provided by an independent researcher and computer generated using a randomization plan from www.randomization.com. The treatment group (n = 55) was given a commercially available nutraceuticals, composition shown in table 3.1 (Estrosalus®, Max Biocare Pty Ltd, Victoria, Australia). The control group (n = 55) was provided with a placebo for 12 weeks.

Subjects were selected based on the following criteria

Inclusion criteria:

1. Women 45-60 years of age
2. Absence of menstrual periods for 12 consecutive months
3. Have menopausal symptoms
4. Willing to attend the project

Exclusion criteria:

1. Women who had been taking any herbs, dietary supplements, medicine, or hormonal replacement therapy that effected on hormone in 1 month prior to the study.

2. History of tumor or cyst or any surgical operation that might interfere to the hormone testing in this study.

3. Currently smoking

4. Patients with underlying disease or health condition which might interfere with the treatment or might cause higher risk to develop side effects such as cardiovascular disease, liver disease, kidney disease, hematologic diseases, thyroid diseases, immunologic diseases, and cancer

5. History of food allergy

6. Volunteers who developed any adverse reaction or request to quit during study.

7. The non-compliance subjects who missed oral combined nutraceuticals capsules more than 20 percents.

3.2 Research Instrument

1. Nutraceuticals

Table 3.1 Composition of Nutraceutical

Composition (per tablet)	Content
SoyLife® isoflavones (mainly as daidzin/ein, genistin/ein, glycitin/ein)	100 mg
from Glycine max (Soya Bean) seed germ ext. dry conc. std. equiv. to fresh	*7.5 g*
Actaea racemosa (Black Cohosh) root & rhizome ext. dry conc.	80mg
equiv. Triterpene glycosides calc. 27-desoxyactein	*2 mg*
equiv. dry	*520 mg*
Vitex agnus-castus (Chasteberry) fruit ext. dry conc.	40 mg
equiv. dry	*400 mg*
Evening Primrose Oil	500 mg
Containing: gamma-Linolenic acid 50mg & Linoleic acid 325 mg	

Placebo capsules were soybean oil that manufactured to match the nutraceutical capsules in size, excipients, color and appearance.

2. The Menopause Rating Scale (MRS) questionnaire

3. Blood biochemistry test:

Hormone; Estradiol, LH, FSH, and SHBG

Basic lab; Lipid Profile (TC, LDL-C, HDL-C, TAG), FPG, hs-CRP, AST, ALT, BUN, and Cr

4. Satisfaction self-evaluation form

3.3 Data Collection Methods

Screening Day:

Physical examination was assessed by physicians.

A menopausal symptoms questionnaire was assessed by research assistants.

Study Day:

1st Visit : week 0

110 subjects was randomly divided into 2 groups (55 subjects/each)

a. Treatment group

b. Placebo group

1. Height/weight was measured by nurses.

2. Blood pressure/pulse was measured by nurses.

3. Menopause symptoms was assessed by research assistants using the menopause rating scale (MRS) questionnaire.

4. Diet consumption was assessed by nutritionists using 24-hour diet recall.

5. Fasting blood sample was collected by nurses (blood sample volume: 15 ml)

Blood biochemistry tests:

- Estradiol, LH, FSH, and SHBG
- Lipid Profile; TC, LDL-C, HDL-C, and TAG
- FPG
- hs-CRP
- AST, ALT
- BUN, Cr

6. Satisfaction self-evaluation form was assessed by subjects.

7. Subjects took nutraceutical or placebo 1 capsule/day after breakfast for 12 weeks.

2nd Visit : week 6

1. Height/weight was measured by nurses.

2. Blood pressure/pulse was measured by nurses.

3. Menopause symptoms was assessed by research assistants using the menopause rating scale (MRS) questionnaire.

4. Diet consumption was assessed by nutritionists using 24-hour diet recall.

5. Fasting blood sample was collected by nurses (blood sample volume: 15 ml).

Blood biochemistry tests:

- Estradiol, LH, FSH, and SHBG
- Lipid Profile; TC, LDL-C, HDL-C, and TG
- FPG
- hs-CRP
- AST, ALT
- BUN, Cr

6. Satisfaction self-evaluation form was assessed by subjects.

3rd Visit : week 12

1. Height/weight was measured by nurses.

2. Blood pressure/pulse was measured by nurses.

3. Menopause symptoms was assessed by research assistants using the menopause rating scale (MRS) questionnaire.

4. Diet consumption was assessed by nutritionists using 24-hour diet recall.

5. Fasting blood sample was collected by nurses (blood sample volume: 15 ml).

Blood biochemistry tests:

- Estradiol, LH, FSH, and SHBG
- Lipid Profile; TC, LDL-C, HDL-C, and TAG
- FPG

- hs-CRP

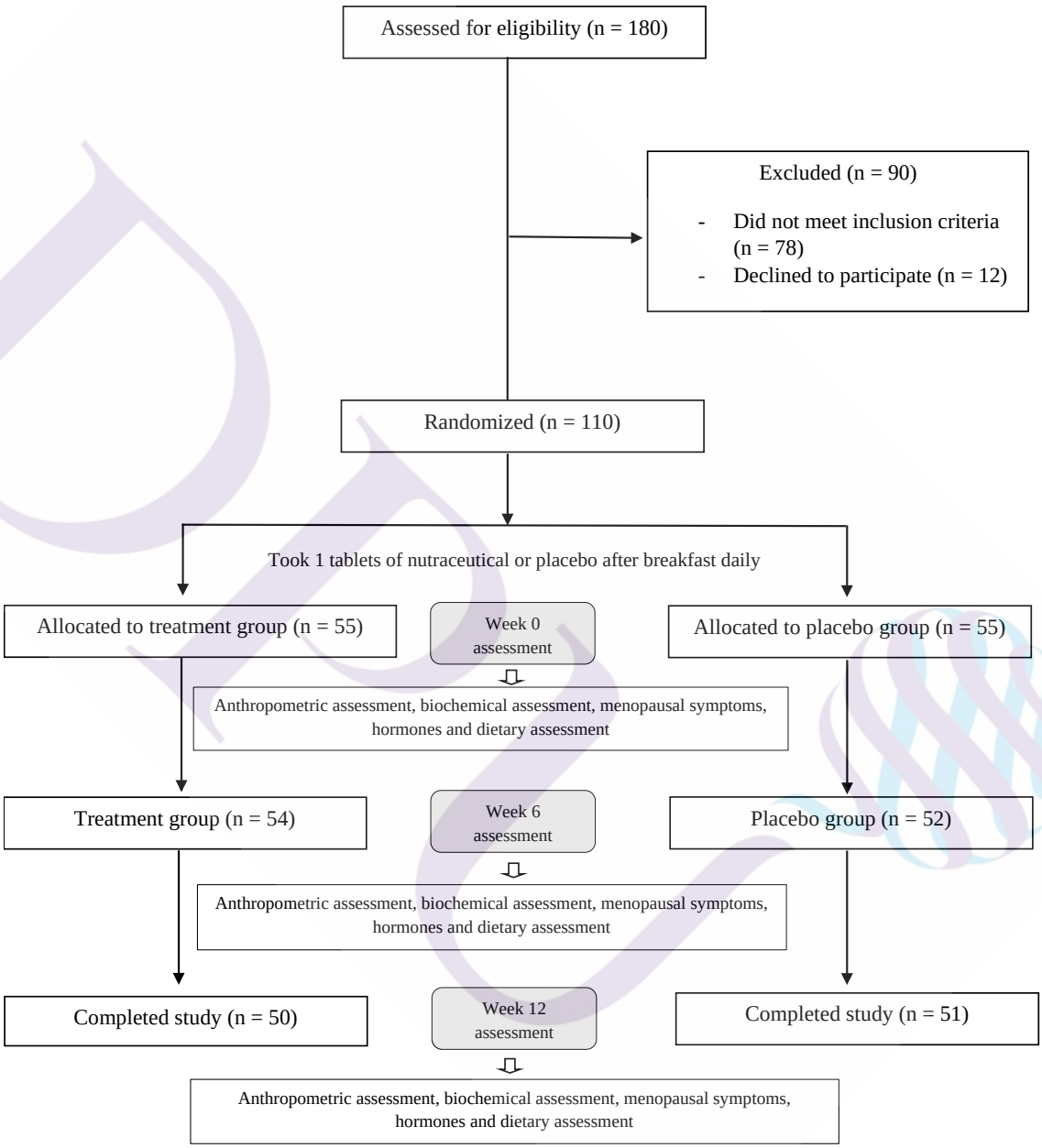
- AST, ALT

- BUN, Cr

6. Satisfaction self-evaluation form was assessed by subjects.

7. Compliance was assessed at the last visit. We calculated how many capsules of the medicine that the subjects had taken in order to be fully compliant. The "Compliance Index" was calculated as the number of taken tablets/the number of capsules that should have been taken multiply by 100 to be a percentage of compliance.

Safety parameters comprise liver (AST, ALT) and kidney function tests (BUN, Cr), FPG, lipid profiles, hs-CRP, sex hormone levels including estradiol, FSH, LH, and SHBG. These were assessed at before (week 0), during the intervention (week 6^{th}) and after intervention (week 12^{th}). The adverse reaction events of the subjects during the intervention was recorded. They were included kidney and liver function tests.



The lost subjects during study were not able to follow visit. Dropout due to inconvenience and loss of follow up

Figure 3.1 Study design and flow diagram

3.4 Data Analysis and Statistics

SPSS version 18.0 for Windows was used for statistical analyses. Data was expressed as means standard deviations.

1. General analysis data by using descriptive statistic statistics, which were mean and standard deviation of the samples.

2. The comparison test between the treatment group and placebo group to evaluate the average of the general characteristic and blood chemistry of subjects, the average hormone parameters at the baseline at the 6 weeks and at the 12 weeks, and the average of total score on energy and nutrient intake at the baseline at the 12 weeks by using t-test and set the statistical significance at P-value <0.05.

3. The comparison test of menopausal symptoms parameters of the samples between the treatment group and placebo group. The assessment in the proportions of relationship between the severe (rating scale 3-4) to not severe (rating scale 0-2) in each symptoms was used. As well as comparative study of satisfaction scores perceived improvement in menopausal symptoms parameters in order to assess the relationship between the satisfied group and unsatisfied group affecting in each syndrome by using chi-squared statistics and set the statistical significance at P-value <0.05.

4. Test the comparative studies within the treatment group at the baseline at the 6 weeks and at the 12 weeks to assess the average of general characteristic and blood chemistry of subjects score by using one-way ANOVA statistics in case of normal distribution or using Friedman's test statistics in case of abnormal distribution and set the statistical significance at P-value <0.05.

Chapter 4

Results

This study was an experimental, randomized, double-blinded, placebo controlled, clinical trial) to study the effectiveness of nutraceuticals supplementation on menopausal symptoms and hormone levels in post- menopausal women.

In this chapter, the following information will be reported:

1. Characteristics of the subjects
2. Dietary assessment
3. Evaluation of clinical improvement in hormone parameters
4. Menopausal symptoms parameters
5. Satisfaction assessment

4.1 Characteristics of Subjects

Table 4.1 General characteristic and blood chemistry

General Characteristics and Biochemical Parameters	Treatment			Placebo			P1	P2	P3
	Baseline	Week 6	Week 12	Baseline	Week 6	Week 12			
Age (year)	53.3 (47-57)	-	-	52.6 (45-58)	-	-	0.055	-	-
Weight (kg)	59.79 ± 10.18	59.41 ± 10.16	59.20 ± 10.17	62.13 ± 11.19	61.79 ± 11.17	60.45 ± 13.67	>0.999	>0.999	>0.999
BMI (kg/m^2)	23.94 ± 4.03	23.82 ± 4.01	23.08 ± 5.14	24.72 ± 4.28	24.58 ± 4.21	24.47 ± 4.33	>0.999	>0.999	>0.999
Body fat (%)	34.42 ± 6.27	34.18 ± 6.41	33.86 ± 6.52	36.21 ± 6.49	36.08 ± 6.54	36.00 ± 6.71	>0.999	>0.999	>0.999
Blood pressure (mmHg)									
- Systolic	123.1 ± 19.32	122.0 ± 18.45	120.8 ± 16.30	126.5 ± 13.82	124.1 ± 11.63	124.7 ± 15.54	>0.999	>0.999	>0.999
- Diastolic	78.92 ± 11.27	78.78 ± 9.360	77.08 ± 12.15	82.36 ± 9.209	80.36 ± 10.07	78.62 ± 10.55	0.662	>0.999	>0.999
Pulse rate (bpm)	72.60 ± 9.52	72.92 ± 10.57	72.58 ± 9.47	73.96 ± 11.00	72.73 ± 10.18	75.94 ± 10.75	>0.999	>0.999	>0.999
FBG (mg/dL)	86.33 ± 8.66	85.35 ± 8.33	84.33 ± 8.92	84.80 ± 10.96	85.98 ± 11.71	84.00 ± 10.51	>0.999	>0.999	>0.999
Total Cholesterol (mg/dL)	209.2 ± 36.30	199.1 ± 37.99	200.6 ± 36.50	202.4 ± 34.52	202.4 ± 33.63	198.1 ± 31.35	>0.999	>0.999	>0.999
LDL-C (mg/dL)	143.5 ± 27.29	125.4 ± 22.84[a]	122.1 ± 23.68[b]	134.3 ± 22.68	133.2 ± 23.21	133.9 ± 23.67	>0.999	>0.999	0.2832
HDL-C (mg/dL)	62.26 ± 13.35	64.56 ± 13.64	63.36 ± 12.54	60.84 ± 15.93	62.63 ± 15.95	57.88 ± 13.87	>0.999	>0.999	0.49
Triglycerides (mg/dL)	108.6 ± 38.03	99.88 ± 34.03[c]	96.57 ± 33.53[d]	112.60 ± 32.10	112.80 ± 33.24	110.80 ± 34.52	>0.999	0.5397	0.4777
BUN (mg/dL)	12.58 ± 2.41	12.54 ± 2.44	12.94 ± 2.46	12.58 ± 1.73	13.79 ± 2.56	13.73 ± 2.59	>0.999	0.3576	>0.999
Cr (mg/dL)	0.71 ± 0.16	0.87 ± 0.17	0.66 ± 0.17	0.72 ± 0.22	0.82 ± 0.22	0.67 ± 0.17	>0.999	0.98	>0.999
AST (U/L)	21.89 ± 4.80	23.17 ± 4.71	21.76 ± 4.55	23.29 ± 5.44	23.84 ± 8.11	23.55 ± 6.37	>0.999	>0.999	>0.999
ALT (U/L)	20.59 ± 8.61	20.14 ± 6.98	21.80 ± 7.64	18.33 ± 5.37	18.96 ± 7.04	21.92 ± 9.59	>0.999	>0.999	>0.999
hs-CRP (mg/L)	1.67 ± 1.20	1.45 ± 0.97	1.25 ± 1.07[e,f]	1.66 ± 1.02	1.87 ± 1.26	1.94 ± 1.16	>0.999	0.6054	0.0002

Values are means ± SD. Means in a row with superscript letters without a common letter differ within group; Significant differences at $p < 0.05$. P1 = Comparison of mean between the two groups at baseline; P2 = Comparison of mean between the two groups at 6 wk; P3 = Comparison of mean between the two groups at 12 wk; Significant differences at $p < 0.05$; [a]P=0.024 (Baseline vs Week 6); [b]P=0.0018 (Baseline vs Week 12) based on ANOVA; [c]P=0.046 (Baseline vs Week 6); [d]P=0.0015 (Baseline vs Week 12) based on Friedman's test within treatment group; [e]P=0.0001 (Baseline vs Week 6) and [f]P=0.0011 (Week 6 vs Week 12) based on Freidman's test within treatment group. BMI, body mass index; FBG, fasting blood glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; BUN, blood urea nitrogen; Cr, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

The results of average age in general health information are 53.3 years old in treatment group and 52.6 years old in placebo group. The average score of weight, BMI, body fat, blood pressure indicators both systolic and diastolic , pulse rate at the baseline, 6 weeks and 12 weeks are compared. Comparative study between treatment group and placebo group found no significant difference of the mean score at the level of statistical significance of p-value <0.05.

The average score for evaluating health risks regarding glucose level and blood cholesterol by FBG, total cholesterol, LDL-C, HDL-C and triglycerides at baseline, 6 weeks and 12 weeks found no significant difference of mean score between treatment group and placebo group at the statistical significance of p-value <0.05. However, the average scores of LDL-C indicators within the treatment group showed significant different results in average scores of triglycerides indicator at the baseline, 6 weeks and 12 weeks at statistical significance of p-value <0.05 with the baseline having a higher mean when compare with 6 weeks and at 12 weeks. Average scores of triglycerides indicators within treatment group compared with the baseline, 6 weeks and 12 weeks are statistical significance with p-value <0.05 with the baseline having a higher mean than at 6 weeks and 12 weeks.

The average score on assessment of health risks regarding side effects on liver and kidneys using BUN, creatinine, AST and ALT indicators at the baseline, 6 weeks and 12 weeks, when compare between treatment group and placebo group found no difference of the mean score in those indicators at the level of statistical significance p-value <0.05.

The average score on assess health risks regarding inflammation by hs-CRP indicator, compare between treatment group and placebo group found no significant difference of the mean score at the level of statistical p-value <0.05. The average results at 12 weeks show difference in average score between treatment group and placebo group with statistical significant at level of p value 0.0002 The mean score of hs-CRP in placebo group is higher than treatment group. The average score of hs-CRP in treatment group at the baseline, 6 weeks and 12 weeks had p-value at 0.0001 and 0.0011 consecutively. This average score of hs-CRP show difference at the level of statistical significance p-value <0.05 with the baseline having a higher mean when compare with 6 weeks and 12 weeks.

Table 4.2 Total energy and nutrient intake of subjects

Dietary Assessment	Treatment		Placebo		P1	P2
	Baseline	Week 12	Baseline	Week 12		
Energy (kcal/d)	1583 ± 448.10	1569 ± 477.40	1508 ± 455.90	1536 ± 409.10	>0.999	>0.999
Carbohydrate (% of energy)	57.17 ± 9.89	53.89 ± 8.14	57.90 ± 10.36	55.94 ± 12.47	>0.999	0.452
Protein (% of energy)	15.09 ± 4.31	17.79 ± 6.63	15.10 ± 4.76	15.67 ± 4.00	>0.999	>0.999
Fat (% of energy)	27.74 ± 8.56	28.32 ± 7.27	27.39 ± 8.83	28.39 ±10.31	>0.999	>0.999
Total cholesterol (mg/d)	295.30 ± 95.92	289.70 ± 83.81	292.4 ± 90.29	282.5 ± 62.09	>0.999	>0.999
Fiber (g/d)	11.21 ± 4.87	11.56 ± 5.85	12.26 ± 7.03	12.29 ± 6.26	>0.999	>0.999

Values are means ± SD. P1 = Comparison of mean between the two groups at baseline; P2 = Comparison of mean between the two groups at 12 wk; Significant differences at $p< 0.05$

The average score of energy, carbohydrate, protein, fat, total cholesterol and fiber indicators, comparing treatment group and placebo group at the baseline and 12 weeks found no difference of the mean score at the level of statistical significance p-value <0.05.

Table 4.3 Hormone Parameters of Subjects

Parameters	Treatment			Placebo			P1	P2	P3
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks			
FSH (mIU/mL)	60.27 ± 20.01	58.90 ± 19.07	57.98 ± 19.97	60.95 ± 19.32	61.12 ± 19.46	59.03 ± 18.71	>0.999	>0.999	>0.999
LH (mIU/mL)	20.50 ± 6.66	20.21 ± 5.75	19.56 ± 6.28	21.48 ± 8.69	22.49 ± 8.59	21.69 ± 8.55	>0.999	>0.999	>0.999
Estradiol (mIU/mL)	6.14 ± 2.42	5.18 ± 1.27	5.24 ± 1.70	7.52 ± 4.75	5.00 ± 4.72	5.00 ± 2.76	>0.999	>0.999	>0.999
SHBG (mIU/mL)	75.77 ± 37.26	74.76 ± 35.20	76.30 ± 34.76	69.96 ± 31.39	70.21 ± 33.73	72.76 ± 32.93	>0.999	>0.999	>0.999

FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; SHBG: Sex Hormone Binding Globulin

P1: Comparison of mean between the two groups at baseline

P2: Comparison of mean between the two groups at week 6

P3: Comparison of mean between the two groups at week 12

P-value < 0.05, determined as significant value

The average score of FSH, LH, Estradiol and SHBG indicators compared between Treatment group and Placebo group at the Baseline, 6 weeks and 12 weeks had p-value at >0.999 with no significant difference of the mean score at the level of statistical significance p-value <0.05.

Table 4.4 Menopausal symptoms parameters of subjects

Table 4.4 Menopausal symptoms Parameters of Subjects

Parameters	Treatment			Placebo			P
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks	
Hot flushes and sweating							<0.0001
Not severe	30	42	48	28	31	30	
Severe	23*	10*	3*	24	21	22	
Heart discomfort							0.9694
Not severe	48	48	47	47	47	48	
Severe	5	4	4	5	5	4	
Sleep problems							<0.0005
Not severe	31	43	45	29	32	33	
Severe	22	9*	6*	23	20	19	
Joint or Muscular discomfort							0.8808
Not severe	29	33	34	32	32	33	
Severe	24	19	17	20	20	19	
Depressed mood							0.0004
Not severe	35	44	49	37	39	40	
Severe	18	8	2*	15	13	12	
Irritability							0.0003
Not severe	37	48	51	39	41	41	
Severe	16	4*	0*	13	11	11	
Anxiety							0.8368
Not severe	36	38	39	38	41	40	
Severe	17	14	12	14	11	12	
Physical and mental exhaustion							0.3139
Not severe	37	40	41	32	33	37	
Severe	16	14	10	20	19	15	
Sexual problems							0.7661
Not severe	46	50	50	47	48	48	
Severe	7	2	1	5	4	4	
Bladder problems							0.6264
Not severe	34	38	38	38	40	41	
Severe	19	14	13	14	12	11	
Dryness of vagina							0.0510
Not severe	38	47	47	40	42	41	
Severe	15	5	4	12	10	11	

P: Comparison of proportions between the two groups

P-value assigned to group, with < 0.05, determined as significant value

*values responsible for the significant changes

For relationship in severity of the menopausal symptoms parameters between treatment group and placebo group the result showed difference in severity level of hot flushes and sweating, sleep problems, depressed mood and irritability symptoms at the level of statistical significance p-value <0.05. In treatment group after receiving nutraceuticals supplements for 12 weeks, the result showed less severe symptoms than 6 weeks group. The result of relationship in severity of heart discomfort, joint or muscular discomfort, anxiety, physical and mental exhaustion, sexual problems, bladder problems and dryness of vagina symptoms found no difference at the level of statistical significance p-value <0.05.

Table 4.5 Satisfaction scores improvement in menopausal symptoms parameters of all subjects

Parameter	**Treatment**		**Placebo**		**P**
	6 weeks	**Week 12**	**6 weeks**	**Week 12**	
Hot flushes/sweats					0.0001
Satisfied	36	45	15	19	
Not Satisfied	16	7	37	33	
Sleep problems					0.0001
Satisfied	41*	48*	12	17	
Not Satisfied	11	4	40	35	
Joint or muscular discomfort					0.0031
Satisfied	16*	24*	8	11	
Not Satisfied	36	28	44	41	
Feeling irritability, tension or aggression					0.0001
Satisfied	32*	40*	16	15	

Parameter	Treatment		Placebo		P
	6 weeks	Week 12	6 weeks	Week 12	
Not Satisfied	20	12	36	37	
Feeling happy					0.0001
Satisfied	44*	49*	21	24	
Not Satisfied	8	3	31	28	
Urination problems					0.0001
Satisfied	22*	27*	7	6	
Not Satisfied	30	25	45	46	
Sexual problems					0.8036
Satisfied	10	12	8	10	
Not Satisfied	42	40	44	42	
Quality of life					0.0001
Satisfied	45*	48*	22	25	
Not Satisfied	7	4	30	27	

P: Comparison of means between the two groups

P-value assigned to group, with < 0.05, determined as significant value

*values responsible for the significant changes

Result of relationship in satisfaction scores perceived improvement in menopausal symptoms parameters level between treatment group and placebo group showed satisfaction improvement on almost all menopausal symptoms including hot flushes, sleep problems, joint and muscular discomfort, irritability and anxiety, feeling better, urinating problems and quality of life at statistical significance p-value <0.05. For nutraceuticals group with 12 weeks supplements the results showed improvement in minimal severe symptoms when compare with 6 weeks group. Satisfaction scores perceived improvement in menopausal symptoms parameters level showed no difference in sexual happiness improvement symptom at the level of statistical significance p-value <0.05.

Chapter 5

Discussion Suggestion and Conclusion

5.1 Discussion

Nutraceuticals for treatment of menopausal symptoms in this study composed of 4 ingredients included soy isoflavones , black cohosh extract, chasteberry extract and evening primrose oil. The results of treatment towards reducing menopausal symptoms shown improvement in hot flushes , sweating, sleep problems, depressed mood and irritability after receiving treatment for 6 weeks and 12 weeks consecutively. These results go along well with previous studies that found soy isoflavone supplementation above 50 mg/d can significantly reduce hot flushes frequency (Clarkson et al., 2011 and Taku et al., 2012), reduce night sweats (Upmalis, 2000) and improve quality of life (physical and psychological) (Basaria et al., 2009). Black cohosh reduced vasomotor effects, anxiety, depression as well as other physical and psychological symptoms (Bai et al., 2007; Mohammad et al., 2013; Nappi et al., 2005; Osmers et al., 2005 and Wuttke and Gorkow, 2003) and improve sleep quality in post-menopausal women with sleep disordered (Jiang et al., 2015). Chasteberry can improve a large variety of symptoms of moderate to severe PMS (Ma et al., 2010). Evening primrose oil significantly reduced the severity of hot flushes in menopausal women (Farzaneh et al., 2013).

Menopausal symptoms including hot flushes , sweating, sleep problem, depressed mood and irritability were likely to find in post-menopausal women though the mechanism remains unclear. . Estrogen and progesterone are both involved in the regulation of two neurotransmitters: serotonin and gamma-aminobutyric acid (GABA). Serotonin helps to regulate mood and behavior, while GABA tends to promote calmness and ease anxiety (Premenstrual syndrome, 2015). Especially, the beta subtype of estrogen receptor seems to have a more profound effect on the central nervous system (Dahlman-Wright et al., 2006 and Heldring et al., 2007). Previous research suggests that women who suffer from menopausal symptoms may have

abnormal serotonin neurotransmission, leading to symptoms such as irritability and depressed mood (PMS and PMDD, 2015).

Soy isoflavones is one of phytoestrogens which has the greatest affinity to ERβ (Axelson et al., 1984) as well as with steroid-like effects, in particular the phytoestrogens which are structurally similar to estradiol. Isoflavones selectively modulate ERs as they retain a strong binding affinity to ERβ (Kuiper, 1997). Black cohosh contains potent phytochemicals that have mild effect on estrogen receptor (Seidlova, 2003). It was able to improve peri-menopause symptom as effectively as Tibolone and does not have an obvious estrogen-like effect (Chen et al., 2014). The mechanism by which black cohosh relieves symptoms is unclear. The alleviation of menopausal symptoms by black cohosh suggests an estrogenic mechanism, but menopausal symptoms can also be alleviated by selective serotonin reuptake inhibitors (SSRIs), suggesting that black cohosh may work through a serotonergic mechanism. Many menopausal symptoms including hot flushes, mood swings and anxiety, insomnia are mediated through the central nervous system (CNS) and may be relieved through a variety of mechanisms. It is possible that black cohosh can act via multiple tissue-dependent mechanisms, including estrogenic (or antiestrogenic), serotonergic, antioxidative, and inflammatory or anti-inflammatory (Ruhlen et al., 2008). Evening primrose oil might slow blood clotting. The therapeutic activity of evening primrose oil is attributed to the direct action of its essential fatty acids on immune cells as well as to an indirect effect on the synthesis of eicosanoids. How evening primrose oil might treat premenstrual or menopausal symptoms is not clear. Some studies have shown that women with premenstrual syndrome tend to have lower than normal levels of GLA. Epidemiological studies have shown a connection between low dietary levels of GLA and a number of illnesses. However, a precise mechanism of action for evening primrose oil is not known. (Hardy, 2000).

Combined nutraceuticals had no effect on hormone parameters which consistent with the Freeman's studied in 2014 that found no relation between menopausal symptoms and plasma estradiol (Freedman, 2014). Meanwhile, the chemical structure of phytoestrogens are similar to estradiol that have mild effect on estrogen receptor (Seidlova, 2003). Therefore, it may improve menopausal symptoms via mechanism without hormone levels changes. Contrary to prior belief, hot flushes are not necessary relate to depression. Some investigatos have proposed the cascade

theory, in which hot flushes can lead to sleep disturbance and daytime fatigue, poor quality of life, and depressive symptoms.

The results of this combined nutraceuticals showed effect on the reduction of hs-CRP when taken continuously for at least 12 weeks. hs-CRP was an useful indicator of inflammation at the cellular level for assessing the risk of heart disease in an anti-aging protocol. The effectiveness in reduction of inflammation in the body may come from phytonutrients that contain flavonoid, monoterpenoid in chasteberry, isoflavones from soy isoflavones which had an antioxidant effects, thereby reducing antioxidant, NF-kB functions and also reduce hs-CRP level in the body (Efstathia and Andriana, 2019). Gamma-linoleic acid in evening primrose oil reduce inflammation by activate peroxisome proliferator-activated receptor (PPARs) which reduce metabolism in cell and also reduce the inflammatory response (Sandeep et al., 2011). Evening primrose oil contains a high proportion of essential fatty acids. The two most common types present in evening primrose oil are linoleic acid (about 65%) and gamma-linolenic acid (GLA, 8-10%). Evening primrose oil is valued primarily for its GLA. It is one of the richest plant sources of GLA. GLA is converted into a number of anti-inflammatory prostaglandins in the body.

The effectiveness of this combined nutraceuticals for reducing the level of LDL-C and triglycerides in blood, lead to reduction in the risk of heart disease when receive continuously for at least 6-12 weeks. Saponins in soy bean, Triterpenoid glycosidase and Actein in black cohosh has ability to reduce cholesterol and triglycerides in blood by alters the expression of cholesterol biosynthetic genes, but does not inhibit HMG-CoA reductase activity. (Vinarova et al., 2015 and Einbond et al., 2018).

Regarding the side effects of treatment with nutraceuticals compared with placebo, all parameters including BUN, creatinine, AST and ALT which represented liver and kidney function had no significantly difference. No negative side effects include nausea, vomiting, dizziness, or any symptoms in treatment group. The results of the previous studies using soy isoflavone, black cohosh, chasteberry extract and evening primrose oil separately found side effects of soy isoflavone included stomach upset constipation, bloating, nausea and allergic reaction such as rash and itching (Soy Isoflavones, 2019). The adverse effects of black cohosh included occasional gastrointestinal discomfort, vertigo, headache, nausea, vomiting, impaired vision and impaired circulation have been reported (American botanical council, 2002). The side

effects of chasteberry extract included upset stomach, nausea, itching, rash, headaches, acne, trouble sleeping, and weight gain (Vitex agnus-castus, 2019). Evening primrose oil can sometimes cause mild side effects including upset stomach, nausea, diarrhea, and headache (Evening primrose oil, 2019). However, this study using combination of 4 types of nutraceuticals showed no side effects because the doses using in this study were lower than the doses of separate nutraceuticals in the previous studies. Using combination of different nutraceuticals which contains more than one type of phytonutrient or phytoestrogen that had synergistic or antagonistic action between each other may decrease the chances of side effects previously reported.

5.2 Suggestion

From this study the results showed interesting clues for in-depth research in the effectiveness of receiving nutraceuticals on the health effects of non-hormone treatment in menopausal as follow.

1) Adjust duration of the treatment program to see earlier efforts as soon as 4 weeks when compared to placebo in reducing menopausal symptoms.

2) Long term effect of using combined nutraceuticals often taking more than 12 weeks for sustainable results and adverse effect.

3) Study the reduction of hs-CRP effect in high level of hs-CRP subjects.

5.3 Conclusion

This experimental, randomized, double-blinded, placebo-controlled clinical trial was aimed to study the effects of oral combined nutraceuticals on menopausal symptoms and hormonal changes in post-menopausal women. The 110 subjects in this study were the post-menopausal women age between 45-60 years old who were amenorrhea at least 12 months and had normal health after taking 1 capsule per day of oral combined nutraceuticals composed of 4 ingredient includes soy isoflavones 100 mg, black cohosh extract 80 mg, chasteberry extract 40 mg and evening primrose oil 500 mg for 6-12 weeks. The results showed that severity of menopausal symptoms including hot flushes , sweating, sleep problems, depressed mood and irritability in treatment group decrease continuously at 6 weeks and 12 weeks interval. Meanwhile, the proportion of the change in severity of each menopausal symptoms in the placebo

groups were too small when compared with the treatment group. Hormone parameters showed no significant difference in FSH, LH, estradiol and SHBG between the treatment group and the placebo group. This study didn't find any adverse effects during procedure. Additionally the treatment group had lower hs-CRP levels in the blood compared with placebo after taking combined nutraceuticals continuously for 12 weeks. Also, in treatment group, LDL-C, triglycerides and hs-CRP were significantly lower after taking combine supplements continuously at 6 weeks and 12 weeks. No difference in total energy and nutrient intake between treatment group and the placebo group .

The effectiveness of using combine nutraceuticals for improving health and reducing the menopausal symptoms occurs after receiving the treatment continuously for at least 6 -12 weeks. This benefit was suitable for those who need to control or reduce menopausal symptoms (hot flushes and sweating, sleep problems, depressed mood and irritability) without side effects.

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Appendix

Appendix A

Study Record Form

Subject ID. _____

Date _____

Study Record Form

The Effects of Oral Combined Nutraceuticals

on Menopausal Symptoms, Hormonal Change

in Post-menopausal Women:

A Randomized, Double-Blind, Placebo-Controlled Trial

1. General Information

- Name Miss/ Mrs._____ Last name _____
- Date of Birth _____ Age _____
- Last menstrual period _____
- Underlying disease _____
- Current medication _____

_____

_____

- Allergic history _____

_____

- History of any herbs/supplements/hormone therapy _____

_____

- History of surgery _____

_____

- Remark _____

_____

2. Compliance ☐ Yes ☐ No

Number of remaining pharmaceutical ……. (Tabs/ Capsules)

3. Health Record

No.	Data	Week 0	Week 6	Week 12	Remark
1	Weight (kg.)				
2	Height (cm.)				
3	BMI (kg/m^2)				
4	Blood pressure (mmHg)				
5	Pulse rate (bpm)				

4. Assessment

4.1 Menopause Rating Scale (MRS)

No.	Data	Week 0	Week 6	Week 12	Remark
A	**Physical Symptoms**				
1	**Hot flushes, sweating** (episodes of sweating)				
2	**Heart discomfort** (unusual awareness of heartbeat, heart skipping, heart racing, tightness)				
3	**Sleep problems**				

No.	Data	Week 0	Week 6	Week 12	Remark
	(difficulty in falling asleep, difficulty in sleeping through, waking up early)				
4	**Joint or Muscular discomfort** (pain in the joints, rheumatoid complaints)				
B	**Emotional symptoms**				
5	**Depressed mood** (feeling down, sad, on the verge of tears, lack of drive, mood swings)				
6	**Irritability** (felling nervous, inner tension, feeling aggressive)				
7	**Anxiety** (inner restlessness, feeling panicky)				
8	**Physical and mental exhaustion** (general decrease in performance, impaired memory, decrease in concentration, forgetfulness)				
C	**Urogenital symptoms**				
9	**Sexual problems** (change in sexual desire, in sexual activity and satisfaction)				
10	**Bladder problems** (difficulty in urinating, increased need to urinate, bladder incontinence)				
11	**Dryness of vagina** (sensation of dryness or burning in the vagina, difficulty with sexual intercourse)				
Total Score					

4.2 Biochemical outcome

No.	Data	Week 0	Week 6	Week 12	Remark
A	**Blood Glucose**				
1	FPG				
B	**Lipid Profile**				
1	Total Cholesterol				
2	TG				
3	HDL				
4	LDL				
C	**Liver Function**				
1	AST				
2	ALT				
D	**Renal Function**				
1	BUN				
2	Creatinine				
E	Inflammatory Marker				
1	hs-CRP				

3.3 Hormone

No.	Data	Week 0	Week 6	Week 12	Remark
1	Estradiol				
2	FSH				
3	LH				
4	SHBG				

3.4 Adverse reaction events observed ☐ No ☐ Yes

No.	Data	Week 6	Week 12	Remark
1	Rash			
2	Pruritus			
3	Dyspnea			
4	Headache			
5	Dizziness			
6	Nausea			
7	Emesis			
8	Abdominal discomfort			
9	Weight increase			
10	Breast distending pain			
11	Vaginal bleeding			
12				
13				

Appendix B

Satisfaction Self-Evaluation Form

The Effects of Oral Combined Nutraceuticals on Menopausal Symptoms and Hormonal Health in Post-menopausal Women	**ID :** ☐☐☐☐ **Abbre :** ☐☐ **Date :** ☐☐ ☐☐ ☐☐ **Day / Month/ Year**
Satisfaction Self-Evaluation Form	

Subject No. _____

Date _____

Name Mrs./Miss……Last name….....

Follow up ☐ **1** ☐ **2** ☐ **3**

(Please fill X in the below table) ☹ ----------> 😐 ----------> ☺

ที่	ประเด็น	Least satisfied 1	Minimally satisfied 2	Moderately satisfied 3	Very satisfied 4	Most satisfied 5
A	Satisfaction of Nutraceuticals					
1	Taste of supplement					
2	Smell of supplement					
3	Size of capsule of supplement					
4	Convenience for keeping					
5	Convenience for taking ex. time/ number to take					
6	Suggestions and opinions _____ _____ _____ _____ _____ _____					

B	Satisfaction of the effect of Nutraceuticals					
ที่	Topic	Least satisfied 1	Minimally satisfied 2	Moderately satisfied 3	Very satisfied 4	Most satisfied 5
1	Sleep problems improve					
2	Hot flushes improve					
3	Joint and muscular discomfort improve					
4	Irritability and anxiety improve					
5	Felling better					
6	Urinating problems improve					
7	Sexual happiness improve					
8	Quality of life improve					
10	Suggestions and opinions _____ _____ _____					

การศึกษาผลของโภชนเภสัชภัณฑ์ต่ออาการวัยทอง และระดับฮอร์โมน ในหญิงวัยหมดประจำเดือน	**รหัส :** ☐☐☐☐
แบบประเมินความพึงพอใจ	**ชื่อย่อ :** ☐☐ **วันที่ :** ☐☐ ☐☐ ☐☐ **วัน / เดือน/ ปี(พ.ศ.)**

Subject No. _____

Date _____

ชื่อ นาง/นางสาว .....นามสกุล.....

การติดตามผลครั้งที่ ☐ **1** ☐ **2** ☐ **3**

(โปรดทำเครื่องหมาย X ในช่องด้านล่าง) ☹ ----------> 😐 ----------> ☺

ที่	ประเด็น	ไม่พึงพอใจเลย 1	พึงพอใจน้อย 2	พึงพอใจปานกลาง 3	พึงพอใจมาก 4	พึงพอใจมากที่สุด 5
A	ระดับความพึงพอใจต่อโภชนเภสัชภัณฑ์					
1	กลิ่นโภชนเภสัชภัณฑ์					
2	รสชาติโภชนเภสัชภัณฑ์					
3	ขนาดเม็ดโภชนเภสัชภัณฑ์					
4	สะดวกต่อการเก็บรักษา					
5	สะดวกต่อผู้ใช้ เช่น เวลา/ จำนวนครั้งที่ต้องกิน					
6	ความคิดเห็นอื่น ๆ เพิ่มเติม _____ _____ _____ _____					

B	ระดับความพึงพอใจต่อผลที่ได้รับ					
ที่	ประเด็น	ไม่พึงพอใจเลย 1	พึงพอใจน้อย 2	พึงพอใจปานกลาง 3	พึงพอใจมาก 4	พึงพอใจมากที่สุด 5
1	ปัญหาการนอนหลับลดลง					
2	อาการร้อนวูบวาบลดลง					
3	ลดอาการปวดกล้ามเนื้อ/ ข้อ					
4	อารมณ์ดีขึ้น หงุดหงิดลดลง					
5	รู้สึกมีความสุขมากขึ้น					
6	ปัญหาเรื่องปัสสาวะลดลง					
7	ความสุขทางเพศดีขึ้น					
8	คุณภาพชีวิตโดยรวมดีขึ้น					
10	ความคิดเห็นอื่น ๆ เพิ่มเติม _____ _____ _____					

Appendix C

Consent Form

หนังสือแสดงเจตนายินยอมเข้าร่วมงานวิจัย

(Consent Form)

โครงการวิจัย การศึกษาประสิทธิผลของโภชนเภสัชต่อสุขภาพผิว ในหญิงวัยหมดประจำเดือน

วันที่ให้คำยินยอม วันที่.....เดือน.....พ.ศ.....
ข้าพเจ้า.....อายุ.....ปี อาศัยบ้านเลขที่.....
ถนน.....หมู่ที่.....แขวง/ตำบล.....
เขต/อำเภอ.....จังหวัด.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตรายหรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่อาจเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว ซึ่งผู้วิจัยได้ตอบคำถามต่าง ๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้น จนข้าพเจ้าพอใจและเข้าร่วมโครงการนี้โดยสมัครใจ

ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมการวิจัยเมื่อใดก็ได้ ถ้าข้าพเจ้าปรารถนาโดยไม่เสียสิทธิในการรักษาพยาบาลที่จะเกิดขึ้นตามมาในโอกาสต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปแบบที่เป็นสรุปผลงานวิจัย

การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็น ด้วยเหตุผลทางวิชาการเท่านั้นและจะต้องได้รับความยินยอมจากข้าพเจ้าเป็นลายลักษณ์อักษร

ผู้วิจัยรับรองว่าหากเกิดภาวะแทรกซ้อนใดๆ ที่มีสาเหตุจากการวิจัยดังกล่าวข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดค่าใช้จ่าย และหรือจะมีการชดเชยค่าตอบแทน ตลอดจนเงินทดแทนความเจ็บป่วยที่ อาจเกิดขึ้นตามเหมาะสม

ข้าพเจ้ายินยอมให้ผู้กำกับดูแลงานวิจัย ผู้ตรวจสอบ คณะกรรมการจริยธรรมการวิจัยในคนและสามารถเข้าไปตรวจสอบบันทึกข้อมูลทางการแพทย์ของข้าพเจ้าเพื่อเป็นการยืนยันถึงขั้นตอน

โครงการวิจัย ทางคลินิก โดยไม่ล่วงละเมิดเอกสิทธิ์ในการปิดบังข้อมูลของการสมัครตามกรอบที่กฎหมายและกฎระเบียบได้อนุญาตไว้

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ ในกรณีที่ข้าพเจ้าไม่สามารถอ่านหนังสือได้ ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้ข้าพเจ้าฟัง จนเข้าใจดีแล้ว ข้าพเจ้าจึงลงนามในใบยินยอมนี้ด้วยความเต็มใจ ข้าพเจ้าสามารถติดต่อผู้วิจัยได้ที่ 137-139 ซอยประดู่ ถนนพระราม 4 แขวงป้อมปราบ เขตป้อมปราบ กทม. 10100 เบอร์โทรศัพท์ 089-792-2206 e-mail: aburame13@hotmail.com โดยบุคคลที่รับผิดชอบเรื่องนี้ คือ นพ.ธีรพงศ์ รัตนตันติกุล ในความดูแลของ ผศ.นพ. พันธ์ศักดิ์ ศุกระฤกษ์, ผศ.นพ. มาศ ไม้ประเสริฐ และ ผศ.ดร. เอกราช บำรุงพืชน์

ลงนาม ..... ผู้ยินยอม

(.....)

วันที่.....เดือน.....พ.ศ.....

ลงนาม ..... ผู้วิจัย

(.....)

วันที่.....เดือน.....พ.ศ.....

ลงนาม ..... พยาน

(.....)

วันที่.....เดือน.....พ.ศ.....

Appendix D

24-hour Diet Recall Record Form

Subject No. _____

Date _____

24-hour Diet Recall Record Form

The Effects of Oral Combined Nutraceuticals on Skin Health in Post-menopausal Women: A Randomized, Double-Blind, Placebo-Controlled Trial

Meal	Food Intake	Food Composition	Amount of Intake

Author Biography

Name-Surname	Teerapong Rattanatantikul
Educational background	2012 Doctor of Medicine (MD) Faculty of Medicine Vajira Hospital, Navamindradhiraj University
Position and Current workplace	General physician Thian Fah Foundation Hospital