2| REVIEW OF LITERATURE

Women considerably live more than half of their lives by adjusting and combating from various biological changes during different transitions about the reproductive cycles.The concern regarding improvement in their quality of life is a point of attention. Women tend to get neglected by the time they age in 40's. The current study was designed with the same vision and efforts have been made to bring betterment of middle aged women's life.

In this chapter, the past and current findings on reproductive life of women, existing knowledge on use of Hormone replacement therapy, phytoestrogen and its importance etc are discussed.

These were concise as following headings and directions.

1. Physiology and endocrinology of menopausal transition
2. Factors affecting early menopause
3. Menopause and related hormones
4. Menopausal symptoms – Physiology, prevalence and comparison
5. Hormone replacement therapy – Pros and cons
6. Alternative approaches to alleviate menopausal symptoms
7. Foods rich in phytoestrogen
8. Mode of action – Estrogen and Phytoestrogen
9. Phytoestrogen – Methods for identification and quantification
10. Effects of food supplement on serum Gonadotropins (FSH, LH), Estradiol and MRS
11. Knowledge and perception of people regarding menopause and its management

1. PHYSIOLOGY AND ENDOCRINOLOGY OF MENOPAUSAL TRANSITION

Variations in length of menses and the inter-menstrual interval and hormonal fluctuations are more common in perimenopause.

Anovulatory cycles have been increased during perimenopause and in some women it results into an inability to produce a preovulatory surge of gonadotrophins after exposure of Estrogen. (Santoro N, 1996)

Along with depletion of ovarian follicles, changes due to aging in non-ovarian tissues such as hypothalamus, pituitary, and uterus may contribute to changes experienced during perimenopause (KS and SN, 2011).

In various reports the changes in hormonal milieu during perimenopause are discussed; where mid cycle estrogen concentrations have been observed to be normal or increased and levels of progesterone and androgens have been observed to be normal or decreased (14-19) (KS and SN,2011)

Longitudinal studies reported that FSH increases as aging and a marked rise occurs as early as the early 40s in normal women.(Lenton EA, 1988)

The increase in FSH that occurs during the menopausal transition has been attributed to a loss of ovarian inhibin B with aging.(KS and SN, 2011; Welt CK *et al*,1999) In perimenstrual period, along with rise in FSH, significant rise in LH is observed though a bit lesser in comparison to FSH (24.25). Thus, in comparison to mid reproductive age women; women in menopausal transition have higher levels of FSH and LH but not lower estrogen levels. (14.25) Therefore, elevated FSH concentrations in the early menopausal transition are most likely not due to low estrogen associated with follicle failure, but are also likely to be caused by other factors, such as inhibin B. (KS and SN, 2011)

An important aspect of the perimenopause is that estradiol levels do not gradually decrease; instead, they fluctuate greatly around the normal range until menopause, when no more responsive follicles are available (Sowers *et al.*, 2008). Santoro N named this response as "roller-coaster" in estrogen production. (Santoro N, 1996)

Estrogen plays an important role in modulating GH (Growth Hormone) secretion in women. There is a positive association between estrogen and GH and in hypoestrogenic environment such as in menopause, there is a decrease in GH secretion. (Ho *et al*, 1987)

Wilshire G *et al.*, (1995) noted that the older reproductive aged women had twice the early follicular phase concentration of estradiol compared with the younger control (368±51 versus 167±20 pmol/L). The elevated estradiol and decreased GH levels in older reproductive age women indicate a trend towards lower levels of insulin-like growth factor 1 (IGF-1). The physiology behind these changes in peri menopausal women is not fully understood.

KS and SN stated that the perimenopause is an important time for a woman to mitigate her risk factors (like weight control, diet and exercise) for cardiovascular disease.

The ovary, adrenal cortex and peripheral conversion of circulating androstenedione and dehydroepiandrosterone (DHEA) to testosterone are three major sources accounting for circulating androgens in the reproductive life of a woman.

Longcope C and Baker S (1993) conducted a study to understand the androgen and estrogen dynamics in relationships with age, weight and menopausal status. They observed that as women traverse the menopause, the inter conversions of androstenedione to testosterone, estrone and estradiol changes. This results into a greater decrease in the concentration of the androstenedione and estradiol (products) than the testosterone and

estrone (precursors). DHEAS is not biologically active unless it is converted to testosterone or estradiol. The decrease in adrenal androgen secretion that occurs during the perimenopause appears to be independent of reproductive aging and instead represents somatic aging.

A change in bleeding pattern is a hallmark of the menopause transition. As women progress through the menopausal transition the menstrual cycle length may shorten before it lengthens and gradually skip menstrual cycles.

Sowers MR (2006) mentioned in his study that sex-steroids are important before menopause to maintain integrity of the skeleton and also are important during the peri- and post menopausal years.

SWAN study revealed that a 4-year period lumbar spine bone mineral density decreased 3.2% among pre- and peri menopausal women who progressed from the early to late menopause transition, 3.9% in surgically menopause women and 5.6% in naturally menopause women. Higher baseline and subsequent high FSH concentration over a 4-year period and low absolute level of estradiol (<35 pg/ml) were associated with lower bone mineral density. Though, measures of baseline estradiol and its 4-year variation were poor predictors of bone mineral density.

McKinlay SM (1996) reported that on an average, women, can expect to experience menstrual irregularity and /or amenorrhea for nearly 4 years before menses cease permanently. The menopausal transition begins when a women experiences either

1) A change in her usual inert cycle interval of >7 days or
2) A skipped menstrual periods

The menopause transition is divided into early and late stages where FSH elevations are greater and more likely to be sustained from cycle to cycle during this time. (Soules MR *et al.*, 2001)

Various longitudinal studies were conducted to understand and brief the endocrinology of menopause in terms of different stages of reproductive aging and hormonal changes across the menopausal transition (MT).A few of them are briefed below.

STRAW System (Stages of Reproductive Aging Workshop)

The Stages of Reproductive Aging Workshop was carried out in Park city Utah in 2001. This was the first attempt to stipulate a nomenclature for the stages of menopausal transition. (Butler l *et al*, 2011; Soules MR *et al.*, 2001) The Figure 2.1 represents the STRAW stages. The STRAW classification separates a women's life into seven segments or stages.

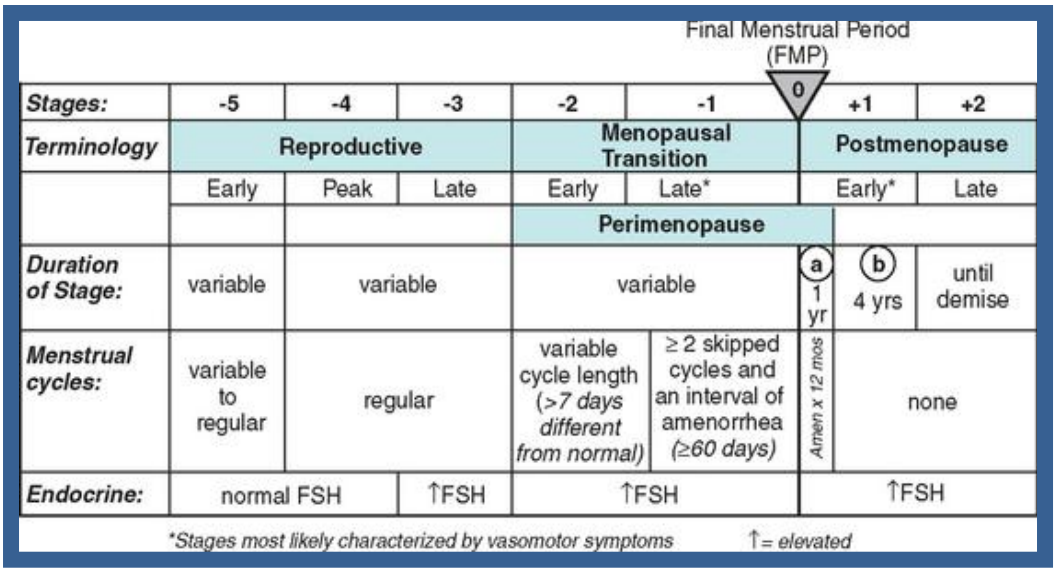


Figure 2.1: Stages of reproductive aging workshop (STRAW)

The stages were defined and characterized as follows:

Stage 0: It is called as final menstruation period (FMP), it anchors the stages -5 to +2

Stage -5 to -3: Reproductive Interval

Stage -5: Early reproductive stage

- menstrual cycles are variable to regular

- FSH is well within range
- Referred as post-menarche period

Stage -4: Peak reproductive stage

- Cycles are regular (25-35 days)
- FSH remains normal
- Duration of this change is variable

Stage -3: Late reproductive stage

- Period of regular cycling when elevated FSH begins to occur.
- Women may experience symptoms associated with perimenopause, including vasomotor symptoms, breast tenderness, insomnia, migraines and premenstrual dysphoria.

Stage -2 and -1: Period of menopausal transition

Stage -2: Early transition period

- Amenorrhea is intermittent and relatively infrequent

Stage -1: late transition period

- Women skip at least two cycles
- Experiences at least 60 days of amenorrhea

Stage +1 and +2: Post menopause period

Stage +1: First five years after the FMP

a) The first year of amenorrhea
b) The next four years
    - Known for accelerated bone loss

Stage +2: Late Postmenopause stage

- Begins five years after the FMP and continues until demise
- FSH remains elevated throughout post menopause, which eventually declining overa long period of time (Butler *et al.*,2011 ; Soules MR *et al.*, 2001)

The STRAW staging primarily applies to women experiencing spontaneous menopause and not those with secondary menopause. It is also less useful for women who are unable to observe a change in their menstrual bleeding patterns, owing to hysterectomy, endometrial ablation, hormonal contraception with suppressed ovarian cycles etc. For such women, the occurrence of menopausal symptoms, due to the fall in ovarian oestrogen production, may provide the first indication of the menopause. (Davis SR, 2015)

Melbourne Women's Midlife Health Project (MWMHP)

A longitudinal study was carried out in Melbourne in women aged 45-55 years to quantify average hormone levels during the menopausal transition to determine their correlates. (Burger *et al.*, 1999) The study was continuing for 9 years and the retention time at the final 9th year of the study was 88%. Results of the study were published in different parts over the years.

The major findings were:

1) On comparison of serum hormone levels between women (n=380) divided into five menstrual cycle-based groups associated with different stages of the menopausal transition.

   Group i – with regular menstrual cycles

   Group ii – Change in menstrual flow, without change in menstrual frequency

   Group iii – Change in frequency, no change in flow

   Group iv – Changes in both frequency and flow

   Group v – three months since their last menstrual period

   This revealed an increase in serum FSH and decrease in estradiol and inhibin were the major endocrine changes associated with menopause transition. (Burger HG *et al.*, 1995)

2) A significant decrease in inhibin B was the first endocrine marker of the early transition (n=380). (Burger HG *et al.*, 1998)
3) FSH was slightly raised but not significantly (n=110) when comparing premenopausal to early peri-menopausal women (who reported changes in cycle frequency within the past year in addition to a bleed within the past 3 months); inhibin B levels were significantly lower in the latter group. (Burger HG *et al.*, 1998)
4) On comparing early perimenopausal to late perimenopause women (who reported amenorrhea for 3 or more of the preceding months), significant falls in E2 and inhibin A as well as elevations in FSH were observed. (Burger HG *et al.*, 1998)
5) The 18 months on either side of the FMP correlated with the greatest change in FSH; levels increased 5 fold during this time from an average of 17.5 – 100.5. (Burger HG *et al.*, 1999)
6) Significantly the association of FSH levels with time to FMP was found to be stronger than the association of age with time to FMP. (Burger HG *et al.*, 1999)
7) The modifying effects of age and BMI were both statistically significant between ages 46-54, log (FSH) increased linearly then flattened by age 56; BMI was inversely related to FSH. (Burger HG *et al.*, 1999)
8) Mean estradiol levels were largely variable during the premenopausal years, than began to decrease after 2 years before the FMP. (Burger HG *et al.*, 1999)
9) Estradiol was affected by age, as log(E2) decreased almost linearly between ages 48 -56 years, but the magnitude of change was less than for FSH; BMI was not observed to be related to E2. (Burger HG *et al.*, 1999)

10) Total serum testosterone was not found to change during the menopausal transition; DHEAS did not change relative to FMP but did decrease with age. (Burger HG *et al.*, 2000)

Study of Women's health Across the Nation (SWAN)

SWAN is a cohort-study started in 1995, which includes the participants from community-based sample of African-American, non-Hispanic Caucasian, Chinese, Hispanic, and Japanese women from multiple cities across the country. SWAN has a number of sub studies that assess bone density, psychiatric morbidity, sleep and cardiovascular markers such as carotid intimal medial thickness and coronary calcium accrual in relation to menopause. The major contributions of SWAN have served to clarify the relationships between menopausal stages, hormones, and intermediate disease outcome. The key findings of the study are:

1. A strong association with progression from the early to late menopausal transition and increased hot flushes, depressive symptoms and major depression. (Butler L and Santoro N, 2011; Gold *et al.*., 2006; Bromberger *et al.*., 2010)
2. Serial study of bone mineral density reflects a similar trajectory to symptoms in that the late menopausal transition is the stage at which bone demineralization becomes detectable. (Finkelstein *et al.*., 2008)
3. Transient short term memory deficits associated with the late transition and amenable to hormone treatment, but only if it is given prior to the FMP. (Greendale *et al.*., 2009)
4. A general trend towards increased total cholesterol, LDL, and apolipoprotein B associated with progress through the transition and, a loss of the protective effect of HDL as women become

postmenopausal. (Matthews *et al*.., 2009; Woodard *et al*., 2010; Butler L and Santoro N, 2011)

Penn Ovarian Aging System (POAS)

The longitudinal Penn Ovarian Aging Study (POAS) that took place from 1995 to 2007 included a cohort of 436 women identified by random digit dialing in Philadelphia County, Pennsylvania (Freeman *et al*., 2010). The group included equal numbers of African American and white women (n=218) aged 35 to 47, all of whom had regular menstrual cycles for the previous three cycles, intact uterus, and at least one ovary (Freeman *et al*., 2010).

The data were collected during twelve assessment periods, the first 6 at approximately 8to9month intervals, and the remaining conducted annually with a 2years gap between periods 10 and 11 (Freeman *et al*., 2010). Blood was drawn for hormone level assessment during days 1–6 of the cycle in two consecutive menstrual cycles or 1 month apart in noncycling women, therefore a maximum of 24 hormone samples per participant (Freeman *et al*., 2010).

POAS focused on predicting entry into the earliest stages of the menopausal transition. The POAS was the first study to determine associations between depression and hormonal changes during the transition to menopause.

They found an increased likelihood of depressive symptoms during the transition to menopause that decreased after menopause (Freeman *et al*., 2004). These symptoms were almost twice as likely in the early transition phase and decreased with age (Freeman *et al*., 2004). This was consistent with earlier SWAN findings that early perimenopausal women had higher rates of psychologic distress (Bromberger *et al*., 2005). This likelihood was lower for those with a rapidly increasing FSH but higher for those

with increasing estradiol, which occurs in the early transition. (Freeman *et al*., 2004; Santoro N., 1996)

They later had similar findings with a 9 year longitudinal assessment of the same cohort: depressed mood was again found to be increased in the early menopausal transition and then significantly associated with progression through stages of the transition after adjusting for other risk factors. Again, incidence of depressed mood significantly decreased post menopause (Freeman *et al*., 2007).

A comparison was made for the staging definitions given by STRAW, SWAN and PENN-5 definition by Gracia *et al* (2005). The study focused on determination of how well each staging system could be validated by corresponding hormonal changes (Table 1). The women who had regular 22-35 day cycles for 3 months prior to enrollment and ages 35-47 (n=427) were followed for 5 years and total 2263 observations were made. All women kept a menstrual calendar and had blood sampled for hormone assays (inhibin B, FSH, LH, estradiol, DHEAS and testosterone) between day 1 and 6 of two consecutive menstrual cycles.

They observed the following:-

- Significant differences in mean inhibin B and FSH levels between the premenopausal and early transition stages of each definition and extra stages added to the PENN-5 system
- Significant differences in LH in the earliest stages of SWAN and STRAW that were not detected in PENN-5 definition
- No significant differences in estradiol levels among the premenopausal and early transition stages using any of the staging system

- No significant differences in testosterone or DHEAS between adjacent stages of any of the system

They noted an additional stage in the early transition period have shown correlation with significant changes in inhibin B and FSH levels, this suggests that ovarian reserve can be observed to decline well before overt cycle abnormalities occur. These subtle changes imply that even a single change in cycle length for women in the age group of 35-44 should be taken seriously by the physician and considered a reason for counseling patients about the possibility of an early menopausal transition or a reduction in fertility.

Finch CE (2014) stated that estrogens gradually decline very late in the human perimenopause as the follicular pool becomes exhausted. He observed that average follicular phase blood E2 was 30% higher in peri menopausal versus the premenopausal women.

Prior (1998) describes “the paradox of endogenous ovarian hyper stimulation” where declines are the larger trend in estrogen production as menopause approaches. Hyper-estrogenic cycles may be intersperse with cycles with lower estrogens, suggested by the 7-fold higher frequency of episodes of high unopposed estrogens in peri menopausal than regularly cycling younger women.

Perimenopausal women show sporadic impaired hypothalamic functions. Keenan *et al*., (2003) and Gill *et al*., (2002) concluded the negative feedback of E2 on LH and FSH has not shown impairment in postmenopausal women while perimenopause includes sporadic, transient impairment of the LH surge. (Finch CE, 2014)

Table 2.1: Menopausal Status Definitions

STRAW definition		PENN-5 definition		ReSTAGE definition	
Premenopause (stages -5 to -3)	Regular cycles, with no change in cycle length	**Premenopause**	Regular cycles, with no change in cycle length	**Premenopause**	Regular cycles, with no change in cycle length
Early transition (Stage -2)	1 cycle length change (≥ 7 days)	**Late premanopause**	1 cycle length change (≥ 7 days)	**Early transition**	1 cycle length change (≥ 7 days)
		Early transition	≥2 cycle length changes (≥7 days)		
Late transition (Stage -1)	2-11 months of amenorrhea	**Late transition**	3-11 months of amenorrhea	**Late transition (0.85 probability of FMP within 5 years)**	Ages 45-49 years: 60 days to 11 months of amenorrhea: ages 40-44 years: at least 60 days of amenorrhea + 60 days of amenorrhea within the next 10 bleeding segments
Postmenopause (stages +1, +2)	≥12 months of amenorrhea	Postmenopause	≥12 months of amenorrhea	Postmenopause	≥12 months of amenorrhea

To understand the hypothalamic-pituitary response to estrogen SWAN investigators carried out the Daily Hormone Study (DHS).

Investigators focused on 160 women who did not show luteal activity for ovarian and pituitary hormones daily, with hormones assayed in urine (estrogen conjugates, E1c) and LH. These anovulatory women were classified into three groups based on their hormonal pattern.

Group 1: had both an estrogen increase and LH surge within 2 days. Indicating normal ovarian function and a normal hypothalamic-pituitary response to estrogen

Group 2: had an estrogen increase only (this surge of estrogen was adequate to elicit a surge in younger ovulating women. Indicating appropriate hypothalamic-pituitary response to an estrogen

Group 3: had neither of the above characteristics. These women had similar estrogen levels as the other groups throughout the cycle but lacked the estrogen peaks.

They stated the percentage of days with hot flushes or night sweats were significantly higher for group 3 women than for either group 1 or group 2 women. (Butter *et al*., 2011; Finch CE, 2014)

The failure of estrogen to elicit LH surge in group 2 suggests that a decrease in estrogen sensitivity accompanies the menopausal transition.

This decreased sensitivity to estrogen is consistent with the fact that exogenous estrogen is therapeutic for perimenopausal women. (NAMS, 2004)

Finch CE also mentioned that the levels of estrogens and androgen may vary independently because of the distinct steroidogenic pathways of adrenal and ovary and their different hormonal inputs. Plasma levels of sex hormone binding globulin (SHBG) a hepatically secreted

glycoprotein, may also be important in menopausal transition. The level of free androgens in plasma varies with SHBG levels, which binds both androgens and estrogens with high affinity. SHBG levels are also influenced by individual variables like obesity lowers it levels whereas oral contraceptives increase its levels. (Hammond GL, 2011)

To summarize, the early menopausal transition, is characterized by highly variable patterns of gonadotropin and sex steroid output. As women progress through the transition, follicle failure appears to occur, and sex steroid production diminishes dramatically but intermittently. Eventually, menstrual cycle cease, but evidence of estrogen production occurs for a period of 6 months to 2 years, after which time women achieve a steady state of hypergonadotropic hypogonadism.These indicate that the initial loss of restraint on FSH secretion leads to the early transition patterns. Once follicle numbers become insufficient to sustain folliculogenesis, uncompensated ovarian function can be highly erratic and may well account for the symptomatology that so often accompanies the menopausal transition. (Butler *et al*., 2011)

2. FACTORS AFFECTING THE EARLY PERIMENOPAUSE

It is a major challenge to distinguish the effects of menopausal transition on a woman's health and quality of life (QoL) from the effects of ageing for cross-sectional, observational studies. Moreover, other major life events can occur during midlife, such as needing to care for elderly parents and children leaving home permanently for studies/job or any other reason.

Findings from a study in the United States indicate that the perimenopausal stage lasts on average for almost 4 years, although for some women it can last much longer, with a longer perimenopause being associated with a higher rate of medical consultations. (McKinlay SM *et al*., 2008; Davis SR *et al*., 2015)

Longitudinal analysis of data from women in the Medical Research Council 1946 British birth cohort has unraveled other effects on Quality of Life (QoL). After adjusting for age, life events and a range of other socioeconomic and lifestyle factors, two aspects of QoL declined with the menopausal transition: namely, perceived physical health (including energy levels) and psychosomatic status (such as nervous emotional state and ability to concentrate). These changes were associated with a longer duration of perimenopause. (Mishra G and Kuh D, 2006; Davis SR *et al*., 2015)

Few common casuses which are thought to be associated with onset of early menopause are:

- Hereditary factors - menopause has a strong genetic link. The age at which a woman's mother reached menopause is a good predictor of her approximate age of menopause.
- Ethnicity – women from certain ethnic groups have menopause earlier than others; while average age of menopause for Caucasian women is 51 years, the same is 46 years for Indian women.

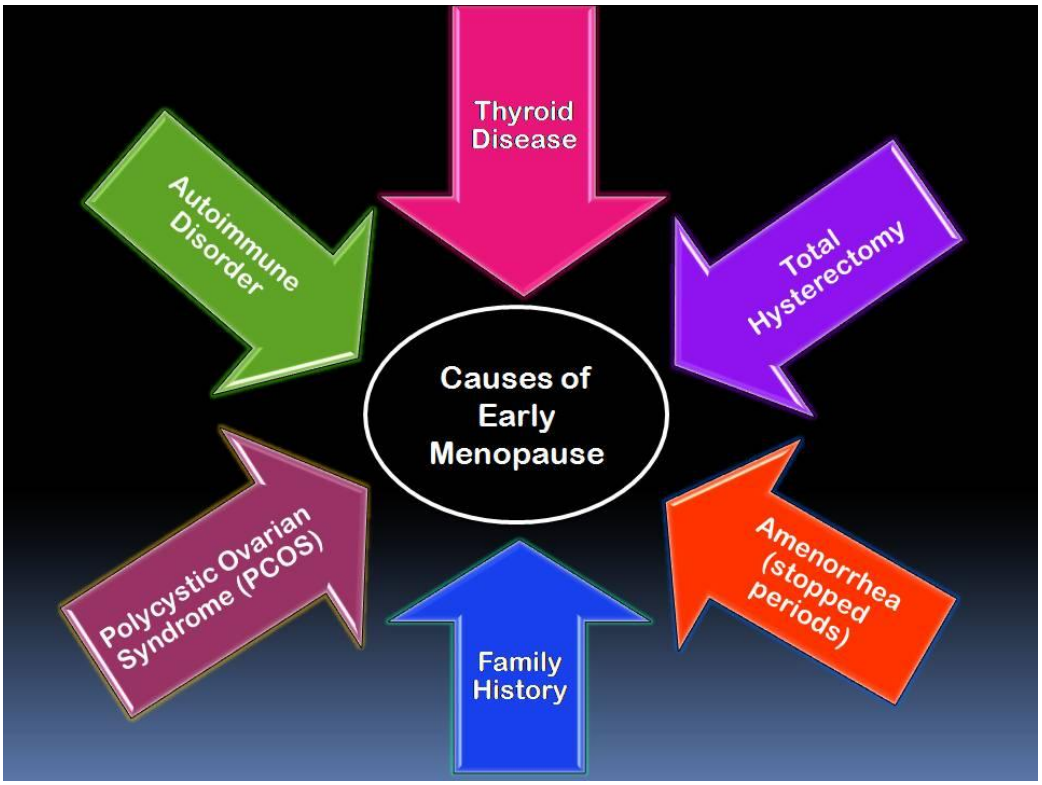


Figure 2.2: Common causes of early menopause

- Smoking – women who smoke are likely to experience menopause earlier than non-smokers.
- Chemotherapy – Chemotherapy accelerate the onset of menopause by damaging the normal ovarian tissue.

- Ovarian Surgery or Hysterectomy – Women who have removed one ovary (single oophorectomy) or both ovaries (bilateral oophorectomy) and a removal of uterus (hysterectomy) have a reduced amount of estrogen and progesterone, and finally leads to early and immediate menopause.
- Autoimmune Disease – these occur when the immune system attacks a part of the body, mistaking it for an invader. Inflammation caused by some autoimmune diseases, such as thyroid disease and rheumatoid arthritis can affect the ovaries. This leads to menopause.

Gold *et al* (2006) analyzed data from a large, multicenter study – Study of Women's Health across the Nation (SWAN) of a multiracial/multiethnic sample of a number of factors with age at natural menopause by using multivariate survival statistical techniques to adjust simultaneously for the effects of multiple factors. They compared menstrual status with respect to key demographic, lifestyle, and health characteristics by chi-square statistics. They performed bivariate Kalpan-Meier survival analyses and Cox proportional hazards regression analysis to evaluate the relation of categorical and continuous variables, respectively, to age at menopause. Data showed current smoking, lower educational attainment, not being married, not being employed, and having a history of heart disease were all significantly, independently associated with earlier menopause, while ever using oral contraceptives, being parous, and Japanese race/ethnicity were significantly, independently associated with later age at natural menopause after adjustment for site and each variable

for all other important variables. Hispanics had a significantly earlier menopause than Caucasians women (Table 2.2).

They mentioned that women who smoke stop menstruating 1-2 years earlier than comparable non-smokers and may have a shorter menopause and heavy smokers have an earlier menopause than light smokers. Polycyclic aromatic hydrocarbons in cigarette smoke are toxic to ovarian follicles and could result in their loss and thus in earlier menopause in smokers.

Social and physical stresses are also associated with amenorrhea and reproductive dysfunction. Low socio-economic status or low educational level may be causative factors and markers for elevated stress.

Table 2.2: Findings from the SWAN study - Adjusted hazard ratios (and 95% CI) and demographic, lifestyle, and health factors affecting median ages at natural menopause (1995-1997)

Characteristics	Early and Late perimenopausal (n=4173)	Adjusted hazard ratio	95% CI	Median age at menopause (years)
Race/ethnicity				
African American	1187			
Caucasian	2122			
Japanese	213			
Chinese	177			
Hispanic	474			
Educational attainment				
<12 years	421	1.48	1.26, 1.773	51.0
High school/GED certificate	1346			
Some college	1094	1.29	1.10, 1.51	51.5
College graduate	692	1.15	0.96, 1.38	51.5
Graduate/professional school	620	1.00		51.7
Difficulty paying for basics				
Very difficult	537			
Somewhat difficult	1388			
Not at all difficult	2235			
Employment				
No		1.20	1.08, 1.33	51.1
Yes		1.00		51.4
Marital status				
Never married	554	1.16	0.99, 1.36	51.0
Married/living as married	2644	1.00		51.7
Separated	220	1.27	1.14. 1.41	51.0
Widowed	127			
Divorced	618			
Live births				
None	648	1.00		51.2
Any	3519	0.80	0.70, 0.93	51.3
Physical activity				
Much less	260			
Somewhat less	676			
Some	1639			
Somewhat more	939			
Much more	566			

(Contd.)

Characteristics	Early and Late perimenopausal (n=4173)	Adjusted hazard ratio	95% CI	Median age at menopause (years)
Smoking				
Never	2120	1.00		51.4
Former	1009	1.04	0.92, 1.17	51.7
Current (no. of cigarettes/day)				
<10	269	1.17	0.97, 1.42	51.1
10-19	301	1.70	1.44, 2.00	50.2
≥20	430	1.63	1.40, 1.89	50.8
Body mass index (kg/m2)				
<19	291			
19 to 26.9	2152			
27 t0 31.9	903			
≥32	827			
Use of oral contraceptives				
No	1217	1.00		51.0
Yes (no. of years)		0.84	0.76, 0.93	51.6
<1	661			
1 to <3	701			
3 to <5	592			
≥5	975			
History of BP				
No	3219			
Yes	951			
History of diabetes				
No	3923			
Yes	246			
History of Heart Disease				
No	4084			
Yes	88			

They observed a trend of increasing age at menopause with increasing number of live births, but the trend was not strongly monotonic. Since onset of menopause is theorized to be related to the rate of loss of oocytes and thus to the occurrence of ovulatory cycles, the proposed mechanism by which parity and use of oral contraceptives may result in later age at natural menopause is by reducing ovulatory cycles earlier in life and thus preserving oocytes longer, resulting in later menopause.

Dr. Miache Seibel, Professor at University of Massachusetts Medical School (Founder of My Menopause Magazine) has listed certain factors affecting age at menopause, which are summarized as follows. (July 10, 2013)

Table 2.3: Factors affecting to early or late menopause

Later Onset	Earlier Onset
Larger body mass	Smoking
Delivering many babies	Not delivering a baby
Chinese or Japanese Ethnicity	Medically treated depression
	Toxic chemical exposure
	Childhood cancer treated pelvic radiation or certain chemotherapy
	Ovarian surgery
	African or Hispanic Ethnicity
	Hysterectomy with ovaries left

The findings of research carried out by Kok HS *et al*., 2006 suggests that pre-existing risk factors, such as raised total serum cholesterol and blood pressure, are also associated with earlier menopause. (Davis SR, 2015)

Overall, however, each additional year of later menopause is linked with a 2% reduction in all-cause mortality. (Ossewaarde ME *et al*., 2005; Mondul AM *et al*., 2005, Davis SR, 2015)

A population based study conducted in Sweden; the Women's Health in Lund Area study, found that after controlling for other variables, the frequency and severity of hot flushes was more almost 3-fold higher in women who had oophoerectomy, was over 50% higher in women drinking large amounts of alcohol, and was 30% higher in women who gained weight. This study also assessed vaginal dryness, which is the symptom commonly, associated with atrophy of estrogen-sensitive tissue, although urinary difficulties such as stress incontinence can also occur (Li *et al*., 2003).For the postmenopausal women in this Swedish cohort,

vaginal dryness was almost 2 fold higher in the women age 58–64 y than in women 50–53 y of age.

However, having menopause later (after age 53 y) was associated with a lower likelihood of vaginal dryness than having menopause at earlier age (Li *et al*., 2003). It appears that women who have early menopause may suffer more symptoms over a longer period of time, which may be linked to lower endogenous estrogen production or an earlier shutoff of estrogen production. Obesity and larger waist circumferences were associated with less vaginal dryness presumably due to higher estrogen and androgen hormones. (Li *et al*, 2003; Wylie-Rosett J, 2005)

They also studied the relationship between obesity and reproductive hormone levels in the menopausal transition period using linear regression models with data from a larger group of women (Freeman *et al*., 2010). They compared hormone levels between obese and non obese women at different stages of the transition. The study included all 436 participants for 12 years; 137 women discontinued the study before the study ended (Freeman *et al*., 2010). Premenopausal obese and overweight women had significantly lower estradiol levels compared with non obese women; this was independent of age, race, and smoking (Freeman *et al*., 2010). In the postmenopausal period, however, obese women had relatively higher estradiol levels (Freeman *et al*., 2010). Their inhibin B findings suggested an earlier decline in ovarian reserve in obese women: premenopausal obese women had significantly lower levels than premenopausal non obese women but this actually reversed in the late transition stage. (Freeman *et al*., 2010)

Somewhat counter intuitively, FSH was found to be lower in postmenopausal obese compared with non obese women and not

significantly different early in the menopause transition. (Freeman *et al*., 2010) In addition to BMI, waist circumference and waist to hip ratio were examined; These correlations were similar to the BMI findings. (Freeman *et al*., 2010) Taken together, the data suggest that obesity adversely affects markers of ovarian aging that come from the ovary (i.e., estradiol, AMH, inhibin), such that it appears obese women have less ovarian reserve, but pituitary hormones (FSH, and in some studies, LH) are lower in obese women relative to non obese women, suggesting that both hypothalamic pituitary suppression and reduced ovarian function characterize obesity. Despite these differences in pituitary and ovarian hormones between obese and non obese women, there is no observed difference in age at menopause between the two groups.

The diuretic effects of caffeine and alcohol can exacerbate urinary symptoms such as nocturia.Smoking and obesity increase the risk of developing incontinence. (Brockie J, 2006)

3. MENOPAUSE AND RELATED HORMONES

KS and SN mentioned a study carried out by Van Voorhis and colleagues who studied reproductive hormone production and menstrual bleeding pattern in a large sub cohort (the Daily Hormone Study) of the SWAN participants aged 42-52 (Van Voorhis BJ, 2008). They found that 20% of all cycles are Anovulatory. They observed that early in the menopause transition the cycle lengths going to be short (<21 days) whereas during late menopause transition the cycle length interval (36+ days) were long. Both short and long cycles as well as short and long duration of menstrual bleeding were frequently associated with anovulation (Van Voorhis BJ, 2008).

Sower MR *et al* (2008) conducted a study to identify menopause transition stages using acceleration or deceleration patterns of FSH rates of change from the late reproductive years to post menopause. It was a cohort of Caucasian women (n=664) during their young and mid adulthood, where data collected for the 14-year period from 1992-1993 through 2006-2007.On average, participants contributed more than nine annual FSH data points of a total possible 11 annual points.

Menopause status was based on the regularity of menstrual bleeding in the year before the study visit. A woman was classified as premenopausal if she had no increase in menstrual irregularity in the previous year. Perimenopause was defined as having menstrual irregularity and having nine or fewer menstrual cycles in a 12-month time period. Post menopause was characterized as having at least 12 consecutive months of amenorrhea with no alternative physiologically normal explanation such as pregnancy or lactation. The results revealed the cohort median age was

38 year at baseline (1992-1993) and it was 51.9 year at the 2006-2007. The baseline median cohort body mass index was 23.8 kg and 27.1 kg 14 years later. The median age at menarche was 13 year and the median age at FMP was 50.5 year.

Over the time period, the number of women who were premenopausal declined from 70.6% at baseline 23.8% at 2006-2007 visits; surgical menopause frequency changed from 4.4% of the cohort at baseline to 20.2% at the 2006-2007 visits.

Four different stages were identifiable in relation to time to the FMP using measures of acceleration and deceleration and piece-wise modeling of FSH rates of change (with 95% confidence interval).

Results revealed FSH stage 1 was a period of gradually increasing FSH rates of change that ended 7 year after the FMP.

At the node to FSH stage 2, there is significant acceleration to a greater FSH rate of change. This rate of change remained relatively constant during the time interval from -7 to -2 year before the FMP. During this period, the FSH levels increased, on average, from 15 to 33 mIU/ ml.

The node at FSH stage 3 marked an acute increase in the FSH rate of change; this increased rate was observed between -2 to +1 year around the FMP during which time the average FSH level rose from 34 to 54 mIU/ ml.

At the FSH stage 4 and commencing 1 year after FMP, there was downward shift in the FSH rate of change that resulted in a plateau of FSH levels.

The results indicated that there is a shift from premenopause FSH rate of change stability to early perimenopause stages at age 40and 42 year and that at age 45 year, there is a major acceleration in the rate of FSH change

thought to be the signal that folliculogenesis is increasingly compromised.

Hale *et al* (2007) and Robertson *et al* (2009) reported that changes in serum FSH,LH,estradiol, progesterone, inhibin A, inhibin B, and anti-Mullerian hormone (AMH) levels with age are due to the age related decline in ovarian follicle reserve, causing a decrease in ovarian factors (e.g. inhibin B) that are critical in the regulation of ovary:pituitary feedback and a secondary decline in luteal function. A rise instead of fall in estradiol during the mid and late luteal phases was also observed.

Further, Robertson *et al* (2009) used a multiple linear regression analysis to understand the 1) independent relationships between serum levels of ovarian hormones (estradiol, progesterone, inhibins A and B, AMH)and both pituitary hormones (FSH and LH) as assessed in simultaneously measured samples; 2) independent relationships between serum levels of ovarian hormones (estradiol, progesterone, inhibins A and B,AMH)and both pituitary hormones (FSH and LH) after the application of a 3-d time-lag; and 3) which of the ovarian hormones best predicts changes in FSH and LH as a basis for assessing their role in the feedback regulation of the two gonadotropins.

In this study, 21 mid reproductive age control women (aged 21–35 yr) with regular menstrual cycles and 56 women (aged 45–55 yr) with variable cycle characteristics **(**late reproductive age with regular cycles and early and late menopausal transition, as defined using the STRAW classification (5), were recruited from Australia and Blood was collected three times weekly throughout one entire cycle and the initial stages of

the succeeding cycle. Serum LH, FSH, estradiol, progesterone, inhibin A, inhibin B, and AMH were measured, and the results were presented as means within 3-d windows that were centered on the mid cycle LH surge.

The correlation coefficients between ovarian and pituitary hormones as assessed in simultaneously obtained samples from women of mid and late reproductive ages are presented in Table 2.4.

Analyses of data from simultaneously obtained samples within each phase of the menstrual cycle revealed a strong inverse relationship ($P<0.001$) between FSH as the independent variable and both estradiol and AMH in the follicular phase of ovulatory cycles. This relationship was not altered with age. Inhibin B showed a significant negative relationship with FSH ($P=0.008$) in the mid follicular stage of the cycle, although at lower significance ($P=0.068$) when tested over the combined stages of the follicular phase.

The associations for the lagged prediction of ovarian hormones by FSH and LH were also investigated. Significant positive lagged associations were observed in the follicular phase between inhibin A and LH [0.38 (0.09 to 0.67)], and estradiol and LH [0.35 (0.09 to 0.61)], whereas a significant negative lagged association was noted between AMH and FSH [-0.32 (-0.62 to -0.02)] in the follicular phase, but not LH. Inhibin A and B showed no significant relationships.

Table 2.4: Correlation coefficients between ovarian and pituitary hormones as assessed in simultaneously obtained samples in different stages of the menstrual cycle (n=42-54)

				R	P
Follicular Phase					
FSH	Vs	Estradiol	EF	-0.24	Ns
			MF	-0.76	P<0.001
FSH	Vs	progesterone	EF	0.08	Ns
			MF	0.13	Ns
FSH	Vs	LH	EF	0.75	P<0.001
			MF	0.68	P<0.001
FSH	Vs	Inhibin A	EF	-0.07	Ns
			MF	-0.04	Ns
FSH	Vs	Inhibin B	EF	-0.56	P<0.001
			MF	-0.49	P<0.001
FSH	Vs	AMH	EF	-0.52	P<0.001
			MF	-0.40	P<0.01
Inhibin B	Vs	AMH	EF	0.60	P<0.001
			MF	0.39	P<0.001
estradiol	Vs	Inhibin A	EF	0.86	P<0.001
			MF	0.49	P<0.001
Luteal Phase					
FSH	Vs	LH	ML	0.78	P<0.001
FSH	Vs	AMH	ML	-0.34	P<0.05
FSH	Vs	progesterone	ML	-0.37	P<0.01
LH	Vs	Estradiol	LL	0.36	P<0.01
LH	Vs	AMH	ML	-0.15	Ns
LH	Vs	progesterone	ML	-0.55	P<0.001
AMH	Vs	progesterone	ML	0.42	P<0.01
estradiol	Vs	Inhibin B	ML	0.45	P<0.001
EF – Early follicular phase, MF – Mid follicular phase, LF- late follicular phase, EL – Early luteal phase, ML-Mid Luteal phase, LL – Late luteal phase, ns – not significant					

FSH (but not AMH or inhibin B) in the mid follicular phase predicts estradiol positively [0.41 (0.12:0.70)] and progesterone negatively [-0.35 (-0.60 to -0.092)] in the mid luteal phase.

Both these associations were significant in the more than 40 yr age group [estradiol, 0.41 (0.12 to 0.70); progesterone, -0.40 (-0.053 to -0.75)], but not in the less than 40 yr age group.

When comparisons were made within cycle in the follicular phase between ovarian hormone levels and pituitary hormone levels obtained 3 d later (lagged samples), inhibin B ($P<0.001$) and AMH ($P<0.001$), but not estradiol, progesterone, or inhibin A, showed a strong negative association with FSH.

Overall, they concluded estradiol (a regulator of overall FSH "tone or setting") and inhibin B (the major negative feedback factor) appear to be the main factors regulating FSH across age and within cycle.

AMH is not an ovarian feedback regulator of pituitary FSH/LH, its marked inverse association with FSH is surprising and may yet indicate an unrecognized role.

Many of the menopausal symptoms are similar to the symptoms of hypothyroidism. Due to this reason, likelihood of hypothyroidism symptoms in mid age group misinterpreted as menopausal symptoms and hypothyroidism may remain undetected. This may lead to health hazards like hyperlipidemia, atherosclerosis and heart disease.

Joshi *et al*., (2011) carried out a study in Nagpur to find out the prevalence of hypothyroidism in perimenopausal and postmenopausal women and the correlation of menopausal symptoms with hypothyroidism. Out of 220 women screened, 22 were suffered from subclinical hypothyroidism (TSH high, free T4 normal). He observed that out of 94 (postmenopausal) women who had score more than 8, sixteen (16.6%) women had subclinical or clinical hypothyroidism and out of 106 (perimenopausal) women with lower score between 1 and 8, nine (8.49%) women had subclinical or clinical hypothyroidism, indicative of though

women with high score are more likely to suffer from hypothyroidism, low score does not preclude the possibility of hypothyroidism. One woman with overt hypothyroidism (TSH high, free T4 low) had very high value of TSH (56 mIU/l) and high MRS score who correlated these symptoms with menopause.

He concluded that women in postmenopausal and perimenopausal age group should be investigated for hypothyroidism irrespective of presence high or low score on menopause rating scale to avoid serious consequences of hypothyroidism in postmenopausal women.

4. MENOPAUSAL SYMPTOMS – PHYSIOLOGY, PREVALENCE AND COMPARISON

Not only in India, over the world menopausal health needs an attention and a matter of priority due to rising population of menopausal women and increase in life expectancy. This demands large and massive efforts to educate and make the women aware of menopausal symptoms and its management. This facilitates in early recognition of symptoms, reduction of discomfort and fear, enables to seek appropriate and required guidance and medical care. During menopausal transition women experience different combination of symptoms like irregular menses, hot flushes, night sweats, insomnia (sleeping problems), forgetfulness, headaches, pain in joints, mood swings, irritability, depression, anxiety, urinary/bladder problems, vaginal dryness, sexual problems, etc.

The literature survey brings out various facts scattered on this globe bringing all the women under one umbrella. It was very interesting to observe, irrespective of the races, issues and concerns women faced biologically around the world remained more or less similar. More interesting fact observed here were some of the non-biological factors also contributed towards risk analysis in a positive way.

Hot flushes

A complex interaction between estrogens, progesterone, and neurotransmitters may underlie the thermoregulatory changes involved in hot flushes.

Sleep Disturbances

In perimenopause, the association was reported to sleep disturbance and mood swings, anxiety, confusion. This is in accordance with age related decrease in total brain serotonin, its decreased level is associated with many psychological and sleep disorders.

Krajewska K *et al* (2007) conducted a comparative analysis of quality of life of women in menopause period in Poland, Greece, and Belorussia using MRS scale. The women after 45 years were enrolled from Poland (n=55), Belorussia (n=50) and Greece (n=85). He found significant ($p<0.001$) differences for marked complaints in MRS scale reported by almost 14.4% women from Greece compared to complaints by 9%women from Belorussia and 9.5% from Poland. Moderate complaints were reported by 32.56%women from Poland, 34% from Belorussia and by 28.55% women from Greece. Severe complaints were reported more rarely in 1.6% Greek women compared with 2.6% Belorussia and 3% Poland respondents; these were not significant findings. The author did not observe any significant difference in reported complaints with respect to no complaints, and different severity levels i.e., mild, moderate, marked and severe between women from Poland, Greece, and Belorussia.

A cross-sectional study in Kuching, Malyasia (Rahman *et al*, 2010) also used MRS as a tool to assess menopausal symptoms in middle age women. He reported the most prevalent symptoms were joint and muscular discomfort (80.1%), physical and mental exhaustion (67.1%) and sleeping problems (52.2%). The prevalence of menopausal symptoms in this study correspond to other studies on Asian women, however the presence of classical menopausal symptoms of hot flushes and sweating (41.6%) were noted to be lower to studies on Caucasian women. Other symptoms noted were irritability and dryness of vagina (37.9%), anxiety (36.5%), and depressive mood (32.6%); followed by sexual problem (30.9%), bladder problem (13.8%) and heart discomfort (18.3%).

Viewing to differences reported at different menopausal transitions, he reported that the perimenopausal women had the most significant somatic complaints compared to post menopausal and pre menopausal women,

while postmenopausal women had the most significant urogenital symptoms compared to pre and perimenopausal women.

Similar kind of study was carried out by Chedraui P (2007) among healthy middle age Ecuadorian women. Mean age of women was 45.1±3.1 years, where 40.6% were premenopausal, 48% were perimenopausal and 11.4% were postmenopausal women. He reported the most prevalent symptoms were muscle and joint problems (77%), depressive mood (74.6%), sexual problems (69.6%), hot flushes (65.6%) and sleeping disorders (45.6%). Peri- and postmenopausal women significantly have high MRS when compared to premenopausal. Efforts also have been made to find out the associated risk factors for high MRS like age, socio-demographic profile and other chronic conditions etc. He reported the women with lower education level presented higher somatic and psychological scoring in comparison to their counterparts. Sexually inactive women presented higher total MRS as well as somatic, psychological, and urogenital scoring. This may be due to estrogen exerts a positive effect over the urogenital system. The vagina, vulva, urethra and trigove of the bladder all contain estrogen receptors and undergo atrophy when estrogen levels decrease. Sexual dysfunctions among climacteric women are not only correlated to hypoetsrogenic anatomical and physiological changes upon the urogenital system y*et al*so to masculine sexual dysfunctions and antidepressant use factors more prevalent as women ages. (Yanez D, 2006) He opined in the study population age, the menopause, sexual inactivity and education level were independent risk factors predicting more severe menopausal symptoms.

The study conducted on Pakistani post menopausal women (n=50) (Mazhar SB, 2009) reported the most prevalent symptoms were hot flushes (90%), sleep disturbances (89%) followed by palpitations (42%). Urogenital symptoms like sexual problems (18%) and bladder problems (12%) were reported least frequently. The menopause rating scale ranged from 9 to 21 with a mean of 12.

A systematic review was conducted by Palacios S *et al*., (2010) on onset of menopause and symptoms in Europe, North America, Latin America and Asia to see the differences in the age of onset of menopause and in the prevalence of climacteric symptoms across different geographical areas. The median age at menopause in Europe ranges from 50.1 to 52.8 years, in North America from 50.5 to 51.4 years, in Latin America from 43.8 to 53 years, and in Asia from 42.1 to 49.5 years. The frequency of vasomotor symptoms ranges from

74% of women in Europe, 36–50% in North America, 45–69% in Latin America and 22–63% in Asia, as reported in different, large, epidemiological studies. These vary depending on the geographical region, selection of criteria, and method of symptom identification. The review concluded that both in Asia and Latin America, women of poorer socioeconomic status have significantly earlier onset of menopause. Within a geographical region, there are ethnic differences in menopause symptoms. Due to differences in study methodologies, firm conclusions were not possible. However, regional differences in age at menopause and in climacteric symptoms are important to consider and provide the foundation for an informed approach to the management of menopause and an understanding of its impact on women's health in the different regions of the world.

A community based cross-sectional study was conducted by Dutta R *et al*., (2012) on Postmenopausal women of rural area in the Poonamallee block of the Tiruvallur district in Tamilnadu. The mean age of the study population was 50.20 years and the mean age at menopause was 44.49 years. The overall prevalence of any one post menopause symptom was 88.1%, of the vasomotor symptom was 60.9%. While 24.7% women reported symptom of depressive mood, 35.4% anxiety, 9.1% irritability, 40.1% sleep related problems, 11.75 urinary symptoms, 20% joint pain. In the study population the awareness of hormone replacement therapy was very low, only 3.2% of women had heard of this before and these were women with high education. The alternative therapies which were used by the women to alleviate their symptoms were biologically based therapies, such as botanical medicines, dietary supplements, vitamins, minerals etc. The author opined the use of appropriate therapy should be encouraged whenever required. Intensive health education is required for women who are in the post-menopausal phase of their lives, for their family and for the community at large.

To determine the effectiveness of menopause on depression and insecurity, a study was conducted by Pradhan and Srivastava (2003). A total of 100 women (fifty working and fifty non working) women aged between 48-52 years were enrolled from Haridwar district. Results show that the mean insecurity scores of working women were 31.29 and that of non-working women were 31.38. The mean scores of inferiority for working women were 37.68±12.73 and that for the non working women was 37.70±12.18. The depression scores were 106.77±45.69 and 114.5±39.07 for working and non working women respectively. They reported non significant differences between the means on all the variables (inferiority, insecurity and depression). They compared their

results with the study carried out by Lenon (1987) and suggested that menopausal status may not be associated with depressive symptoms either directly or indirectly through traditional gender roles. The study concluded that working and non working women in relation to menopause do not show any significant difference on three variables i.e., depression, inferiority and insecurity.

An observational, cross-sectional study on menopausal women from Jammu city was carried out by Sharma S *et al* (2007) to evaluate menopausal symptoms in middle age women and to evaluate the correlation of age on these symptoms. Total 117 menopausal women were enrolled and were distributed in the following age, 40-44 years (n=27), 45-50 years (n=30) and above 50 years (n=60). Mean age of study population was 47.35 years. The most prevalent symptoms were fatigue and lack of energy (72.935), headache (55.9%), hot flushes, cold sweats, cold hand and feet (53.86% each) and weight gain (43.13%). Mean number of menopausal symptoms in three age groups were as (mean±SD) 10.53±7.33, 7.70±6.76 and 14.50±10.77 in 40-44 years, 45-50 years and >50 years age groups respectively. These revealed that at transition of menopause and in post menopausal period the number of symptoms were more and in-between numbers of complaints were less. The correlation of age and menopausal symptoms revealed that the vasomotor symptoms were high with the lesser age at menopause and MDSM (mean duration since menopause). While psychological and rheumatic complaints were more prevalent with increasing age (later menopause) and MDSM. The researcher reported the similar trend was observed in the study by Bagga A (2004) with less number of complaints but the different scenario was observed in women from USA. They

emphasized on such regional studies to help and educate the women regarding an early identification of common menopausal symptoms.

Kaur S *et al* (2004) conducted a study on women aged 40-60 years from Chandigarh city, India to see the effect of menopause on their lives. Of the 725 women enrolled, 298 (41%) had attained menopause, 47 (6.5%) were in transition and 43 (5.9%) had undergone hysterectomy. Mean age of the study population was 51.86±4.3 years and average per capita monthly income was rupees 1126.5±799.49. Among these women, transitional period lasted for 1-12 months in 48.7% of respondents and for 1 year or more in 20.8%, while 30.5% were never experienced any transitional changes. The symptoms reported in this phase were delayed periods (37.6%), heavy bleeding (13%) or scanty periods (7%) or any combination of these. Majority of women (76%) did not experience any tension or apprehension on attainment of menopause or during the transition phase. Of those who reported tension, it was significantly more in women who attained menopause at a younger age (40-50 years) compared to between 50 and 60 years. On attaining menopause, they experienced the symptoms like diminished acuity of vision (n=66, 22.1%), joint pain/body ache and swelling on body/feet (n=59, 19.8%), high blood pressure(n=22, 7.4%), headache (n=16, 5.4%), hot flushes (n=15, 5%) and sweating (n=9, 3%). The rest of the respondents (n=66, 22.1%) reported other symptoms such as leucorrhea, weight gain, irritability, breathlessness, vulval itching and sexual problems. Most of the women (n=281, 94.3%) welcomed the cessation of menses and overall menopause was viewed positively by study population.

Ahsan M *et al* (2015) conducted a study in Patna to compare the frequency and severity of menopausal symptoms during perimenopause

(n=92) and post menopause (n=95) using the MRS. The mean age of perimenopause group was 43.45±2.02 years and that of post menopausal group was 48.52±2.27 years, while the mean age of menopause was 45.29±2.17 years. The mean total MRS score of the peri menopausal and post menopausal groups were 21.4±5.11 and 20.01±3.99 respectively. The mean score for somatic psychological scale ($p<0.01$) was higher in peri menopausal women in comparison with postmenopausal women. On contrast, the mean score for urogenital subscale was significantly higher by 28% ($p<0.001$) in post menopausal women.

For the somatic symptoms, among perimenopausal women, 66.3% reported hot flushes and/or night sweats, 35.875 had severe to very severe symptoms, 49.73% sleep problems. Though few post menopausal women (52.63%) reported such symptoms, they were mild to moderate.

All of peri menopausal women had muscle and joint pains and 46.74% had severe to very severe symptoms, this rose markedly to 90.53% post menopausal women reported such severity ($p<0.001$).The significant high psychological subscale in perimenopause women compare to postmenopausal women may be due to the decrease in the fluctuation of estrogen levels.

Kakkar V *et al* (2007) carried out a research to study the variation of MRS with age, working / non working and educated/ uneducated status in a cohort of north –Indian subpopulation and look for the possible reason for the incurred variations. The average age at menopause of study population was 48.7±2.3 years, based on which the women (n=208) were divided into peri (35-45 years), early menopause (46-51 years) and the post menopausal (52-65 years) groups. In case of peri menopausal women the psychological symptoms were predominant with respect to the somatic and urogenital scores ($p\leq0.001$). To justify this, the author

opined that this may be due to unstable and highly variable E2 levels reported in the peri menopausal phase, which modulates neurotransmitters that control mood, especially serotonin and non-epinephrine, a heightened sensitivity towards perceiving the psychological symptoms by the perimenopause women. The somatic symptoms were found to step up to each phase during the transition from peri to post menopause. The urogenital symptoms like vaginal atrophy, vaginal discomfort, dysuria, dysponeuria, UTI (urinary tract infections) etc. were highly prevalent amongst the women in post menopause. The symptoms score $\geq$7 indicate the severity for psychological and somatic scale while $\geq$4 indicates the severity for urogenital scale. A significantly higher percentage of peri menopausal women (36%) showed a P score of $\geq$7, while a higher percentage of post menopausal women showed S score and U score $\geq$7 (>40%, $p \leq 0.001$).

There was a significant difference between the P (psychological) score of $\geq$7 between working (44%) and non-working women ($p \leq 0.001$). While somatic symptoms were significantly higher (at $p \leq 0.017$) in the non-working women, which may be attributed to the secondary life style and lesser emphasis on the dietary and life style changes (walking, exercise), generally observed in the case of non-working women.

The urogenital symptoms were more prevalent among the non-working group indicating either a compromised hygiene or an inability to contact a physician and take required treatment. Education plays an important role to maintain a better health status during the menopausal transition phase. Educated women showed a lower incidence of psychological and somatic symptoms ($p<0.001$). Finally, researcher concluded that an interaction with a counselor and suitable advice about the non-therapeutic options can help alleviate the problems in younger (perimenopausal) women. And

emphasis was given to the importance of counseling in improving the overall state of menopausal women.

Vasomotor symptoms are among the most frequently reported physiological symptoms during and after menopause (Utian WH, 2005; Thurston RC and Joffe H, 2011; Davis SR 2015)but their prevalence among women in developed nations ranges from 30% to 75%. (Gartoulla P *et al*., 2014; Gold EB *et al*., 2006; Freeman AW *et al*., 2007; Davis SR, 2015)

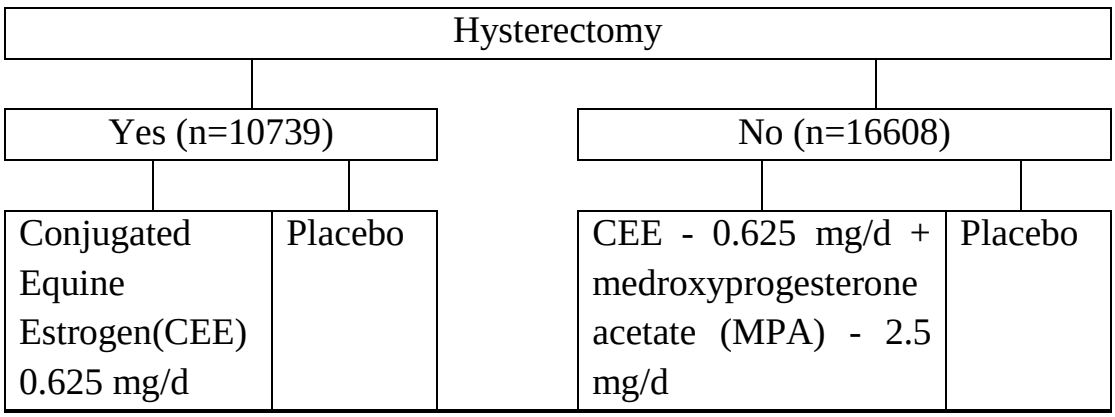
A systematic review of the prevalence of menopausal symptoms in Asian countries found a predominance of other physical symptoms over vasomotor and psychological symptoms. Furthermore, vasomotor symptoms occurred in premenopausal, perimenopausal and post-menopausal women. However, most studies that were considered for this systematic review showed low external and internal validity when evaluated by a risk of bias tool. Thus, further studies of representative samples and using validated questionnaires are needed to clarify the prevalence of menopausal symptoms in Asian women.

5. HORMONE REPLACEMENT THERAPY – PROS AND CONS

The onset of menopause or the surgical removal of the uterus in women causes hormonal imbalance and calls for Hormone Replacement Therapy (HRT). HRT is sometimes recommended when there is a fluctuation in the estrogen and progestin levels. HRT is prescribed for a woman to alleviate acute symptoms of menopause and to avoid the risk of osteoporosis. Women who have had their uterus removed surgically are also put on estrogen therapy. HRT is prescribed in the form of pills, tablets, and skin and vaginal creams.

The Women's Health Initiative (WHI) was a major 15-year research program to address the most common causes of death, disability, and poor quality of life in postmenopausal women – CVD, cancer and osteoporosis. It consisted of three clinical trials and one observational study, which were designed to study the test effects of postmenopausal hormone therapy, diet modification, Calcium and Vitamin D supplementation on heart diseases, fractures, and breast and colorectal cancer. (WHI, Wikipedia, 2015)

The WHI's study design was as follows:



Results of the Women's Health Initiative Hormonal trials were listed as follows (Table 2.5).

Table 2.5: Results of WHI's Hormonal trails

OUTCOME CONDITION	ESTROGEN ALONE	ESTROGEN PLUS PROGESTIN
Myocardial infarction	No significant difference found between treatment arms	Risks was 29% higher than in the placebo group
Stroke	Risk was significantly higher than in placebo group with an increase of 12 strokes per 10,000 person years	Risk was 41% higher than in the placebo group
Blood clots	Did not appear to increase or decrease risk	The overall rate of increase was two-fold higher compared to the placebo group. Compared with those on placebo, risk was fourfold during first 2 years.
Breast cancer	Trend for possible reduction	Risk increased by 24% after 4 years, regardless of age, family history or race
Colorectal Cancer	No difference found between treatment arms	Risk was 37% lower than in the placebo group
Ovarian cancer	Not applicable	Trend for higher rate than in the placebo group, which was not statistically significant.
Osteoporosis	There were 6 fewer hip fractures per 10,000 person years than in the placebo group	Hip fractures were 34% lower and total fractures were 24% lower than in the placebo group.

...**(Contd.)**

OUTCOME CONDITION	ESTROGEN ALONE	ESTROGEN PLUS PROGESTIN
Cognitive impairment	Trend for higher rates of dementia than in the placebo group, which was statistically significant.	Women on all forms of hormone replacement therapy had increased risk of mild cognitive impairment or probable dementia.
Quality of life	No differences found between treatment arms.	Some trends noted but no clinically significant effects on physical or emotional health, pain, energy, sleeping or sexual satisfaction.

(Source: Wylie-Rosett J, 2011)

The data revealed contrasts between several of the hypothesized risks and the observed attributable risks, which are instructive in demonstrating the distinct differences between the HT trial findings and those of previous observational studies.

The dramatic shift in use and prescription of HRT had shown a dramatic shift from 1995-2003 in response to publication of data (2002) from the Women's Health Study and Million Women Study.

Table 2.6: Summary of the hypothesized and observed risks of specific clinical outcomes

Clinical Outcome	Hypothesized Effect on risk	E+P treatment group			E-alone treatment group		
		HR	95% CI	AR	HR	95% CI	AR
CHD	Decreased	1.24	1.00-1.54	+6	0.95	0.79-1.15	-3
Stroke	Decreased	1.31	1.02-1.68	+8	1.37	1.09-1.73	+12
Pulmonary embolism	Increased	2.13	1.45-3.11	+10	1.37	0.90-2.07	+4
Venous thromboembolism	Increased	2.06	1.57-2.70	+18	1.32	0.99-1.75	+8
Breast cancer	Increased	1.24	1.02-1.50	+8	0.80	0.62-1.04	-6
Colorectal cancer	Decreased	0.56	0.38-0.81	-7	1.08	0.75-1.55	+1
Endometrial cancer	-	0.81	0.48-1.36	-1		N/A	N/A
Hip fractures	Decreased	0.67	0.47-0.96	-5	0.65	0.45-0.94	-7
Total fractures	Decreased	0.76	0.69-0.83	-47	0.71	0.64-0.80	-53
Total mortality	Decreased	0.98	0.82-1.18	-1	1.04	0.91-1.12	+3
Global index	-	1.15	1.03-1.28	+19	1.01	1.09-1.12	+2
Diabetes	-	0.79	0.67-0.93		0.88	0.77-1.01	
Gallbladder disease	Increased	1.59	1.28-1.97		1.67	1.35-2.06	
Stress incontinence	-	1.87	1.61-2.18		2.15	1.77-2.82	
Urge incontinence	-	1.15	0.99-1.34		1.32	1.10-1.58	
Peripheral artery disease	-	0.89	0.63-1.25		1.32	0.99-1.77	
Probable dementia	Decreased	2.05	1.21-3.48		1.49	0.83-2.66	

(Source: https://en.wikipedia.org/wiki/Women%27s_Health_Initiative)

The total annual hormone prescriptions in the United States increased from 58 million in 1995 to 90 million with an annual increase of 15 million per year. The prescriptions remained stable until 2002 when the results of the WHI were released. The number of prescriptions dropped by 66% for Prempro (the estrogen plus progestin combination used in the

WHI) and by 33% for Premarin (the conjugated equine estrogen [CEE] used in the WHI).

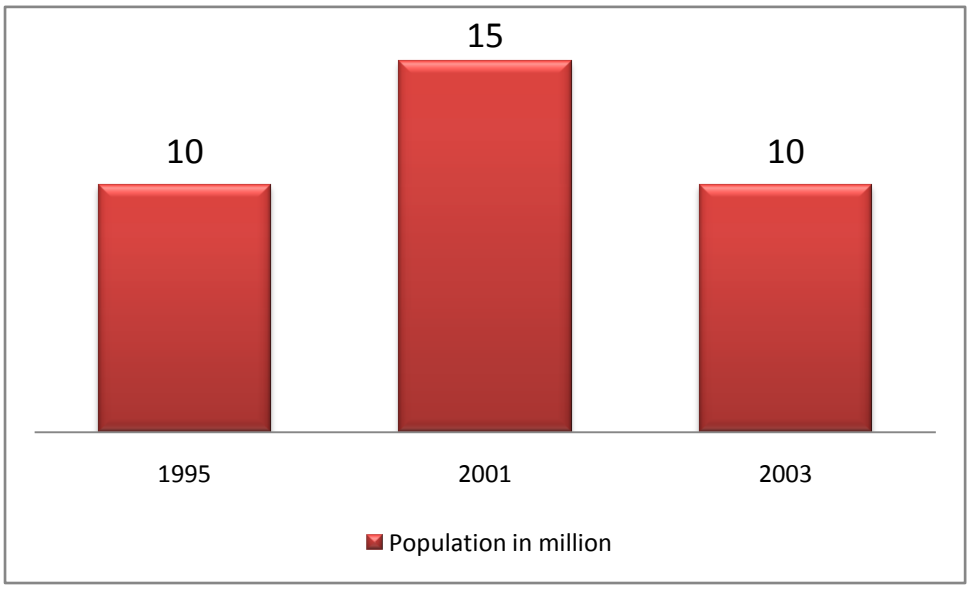


Figure 2.3: Post menopausal Estrogen Prescription in US (1995-2003)

The total number of women for whom postmenopausal estrogen was prescribed rose from 10 million in 1995 to a peak of 15 million in 2001, and fell back to 10 million in 2003 (Figure 2.3). (Hersh *et al*, 2004)

International Menopause Society (IMS)(2003) reviewed available information from observational studies, randomized clinical trials (RCTs) and pre-clinical research, which include WHI study along with Heart and Estrogen/progestin Replacement Study (HERS) and the Estrogen Replacement and Atherosclerosis Study (ERAS), which utilized the same hormonal regimen to addresses the validity of these RCTs. The MWS, a recent prospective cohort analysis was less considered for review because it focused on the potential for breast cancer induction by HT.

The WHI's publication indicated that, by design, symptomatic women were limited to ~10% ofthe study population7. The HERS and

ERAStrials, by design, excluded younger women. Theaverage ages of women in the WHI, HERS andERAS trials were 63.3, 67 and 65 years, respectively.Results in such populations cannot,and should not, be generalized to women whoare unlike those tested (i.e. younger womenearly in menopause).

The MWS is an observational study of UK women volunteering for a national breast screening program. It reported that all types of HT regimens induce an increase in breast cancer risk, starting from the 1st year of use. In addition, the risk disappears from 1 to 5 years after the withdrawal of HT. The appearance of significant risk in the 1st year strongly suggests that the surplus of breast cancers arose from observational bias and was not induced by the hormones.

The Executive Committee has identified crucial differences between the experimental populations in the two different types of studies, which tend to be neglected during minute consideration of the outcomes. In the observational studies, the hormones were prescribed for women in the menopausal transition, most of whom were symptomatic, and who were generally 55 years of age or less at the time of starting treatment.

On the contrary, in the three RCTs, the HT was started at 55 years or older in 89% of the subjects. Overall, the women in the observational trials were mainly patients in the menopausal transition who sought help for symptomatic hormone deficiency, while the women in the RCTs were, by design, recruited subjects who were largely past the point of being symptomatic, indicating an altered physiological status that could be related to differences in outcomes.

The Executive Committee recommends the continuation of presently accepted global practice, including the use of estrogen + progestin, or estrogen alone in the case of women who have undergone hysterectomy, for the relief of menopausal and urogenital symptoms, avoidance of bone-wasting and fractures, and atrophy of connective tissue and epithelia.
Each patient must be counseled on the current data on the risks and perceived benefits of HT so that she can make appropriate, informed, individual decisions about continuing or stopping treatment.

The 2003 position statement from North American Menopause Society addressed alternatives to estrogen therapy. The specific recommendations included, "First consider lifestyle changes, either alone or combined with a nonprescription remedy, such as dietary isoflavones, black cohosh, or vitamin E. The declining usage of estrogen therapy is likely to increaseresearch that addresses how micronutrients, phytoestrogens, andother food/herb derived compounds affect menopausal symptoms. (Wylie-Rosett J, 2011)

6. ALTERNATIVE APPROACHES TO ALLEVIATE MENOPAUSAL SYMPTOMS

The menarche, menstruation and menopause are seen as normal phases that affect women in their passage through life, and may need supportive treatment during each transition.

Baseline data from participants in the SWAN study indicate that 48.5% had used at least one complementary or alternative therapy during the preceding year. (Bair *et al*, 2002) Complementary and alternative therapy users were more likely to be younger, have more education, be white, and reside in California than the nonusers. (Wylie-Rosett J, 2005)

Herbal therapies were more likely to be selected by the Chinese American women than other groups, but emotional stress and anxiety were associated with herbal therapy among the Japanese American women as well. (Bair *et al*, 2002)

Micronutrient and herbal supplements that are commonly used to treat menopausal symptoms include vitamin E, black cohosh, soy, and other phytoestrogens, which are used to treat the vasomotor symptoms. Other herbal treatments such as ginkobiloba, ginseng, and St. Johns wort have been used for mood related symptoms, and valerin has been used for sleep disturbances associated with menopause. (Wylie Rosett J, 2005)

A randomized crossovertrial (4 wk per treatment condition) conducted in 120women treated for breast cancer found that Vitamin E (800 IU)resulted in fewer hot flush per day than the placebo (Barton *et al*., 1998; Wylie Rosett J, 2005).

Ueda M (2004) conducted a study on symptomatic women aged 40-60 years, and noted a 12-week exercise and education program helped

significantly to alleviate menopausal symptoms. Higher education, vigorous physical exercise and full-time employment were associated with a lower risk of vasomotor symptoms. (Brockie J, 2006) This in turn helps to prevent a domino effect leading to insomnia, tiredness and low mood.

These findings were on same trend noted in earlier days by Menditto A (1999). Yaffe *et al*., 2001 also mentioned that physical activity helped to protect elderly women from cognitive decline.

Huntley (2003) conducted a systematic review to evaluate the benefit of herbal medicinal products for the treatment of menopausal symptoms from 18 randomized controlled trials. These review examined use of black cohosh (n=4), red clover (n=4), kava (n=3), dong quai (n=1), evening primarose oil (n=1), ginseng (n=1), and combination products (n=4) for the reported outcome measures related to the physical or physiological impact of menopause. The review revealed poor methodology for trails involving black cohosh. The studies involving red clover found beneficial for more severe menopausal symptoms. The safety concern associated with the use of kava, limits its application as a therapeutic alternative. The evidence was inconclusive for the other herbal medicinal products reviewed.

Another review on alternative approach to the menopause was carried out by Brockie J in 2006. He included lifestyle and dietary changes, over-the-counter nutritional supplements and complementary therapies.

Many lifestyle and dietary changes need to be adopted early on to create good habits and maximize the benefits later in life.

Table 2.7: The possible benefits and risks of diet and lifestyle on menopause symptoms and long-term postmenopausal health

	Adverse effects	Probable beneficial effects
Smoking	Increases vasomotor symptoms Associated with lower bone density Cardiovascular risks Increased risk of urinary incontinence	
Alcohol	Cardiovascular risks (if intake excessive) Associated with lower bone density and increased fracture risk Increases vasomotor symptoms Increases urinary symptoms Weight gain	Cardiovascular benefits (in moderate amounts)
Caffeine	Lower bone density Insomnia Increases urinary symptoms	
Exercise	None known with moderate exercise	Reduces vasomotor symptoms Improves mood Improves insomnia Osteoporosis prevention Cardiovascular benefits Helps weight control Protects against cognitive decline in the elderly

(Contd.)

Kegel's exercises		Improvement in urinary symptoms
Diet (e.g., phytoestrogens, vitamin and mineral supplements)	Uncertain/dependent on diet	Improves vasomotor symptoms Osteoporosis prevention Cardiovascular benefits
Stress/hot environment	Increases vasomotor symptoms	

(Source: Brockie J, 2006)

Alcohol, caffeine, smoking, obesity and current weight gain, hot spicy food and drinks, a hot environment and stress are known trigger factors for vasomotor symptoms.

Due to fear and dislike of adverse effects as well as these possiblelongterm risks of HRT, many women have turned to complementary and alternative medicines, hoping that these might relieve menopausal symptoms.

The different approaches which are in application for relief from menopausal symptoms are summarized below.

1. Herbalism

Variety of herbal products was available in US and about 80% of them remained unlicensed. There is widespread belief by the publicthat 'natural' means harmless, but herbs can contain potent chemicals and should be used with caution. Women with a contraindication to HRT, such as a history of breast cancer or venous thromboembolism, should be discouraged from using them. There are a number of known drug–herb interactions and practitioners should caution patients against mixing herbs and pharmaceutical drugs.

2. Black Cohosh

Black cohosh is an herb sold as a dietary supplement in the United States. It is used for hot flushes and other menopausal symptoms.

Black cohosh contains a number of compounds with potential bioactivity including tritepene, glycosides, resin, salycilates, isoferulic acid, sterols, and alkaloids. Black cohosh does not appear to alter the hormonal pattern associated with menopause, low estrogen accompanied by elevated luteinizing hormone (LH), and follicle stimulating hormone (FSH). (Wylie-Rosett J, 2005)

Warnecke G (1985) conducted a randomized, double-blind, placebo-controlled trial of 80 menopausal women. And he compared 8 mg/day of a black cohosh extract (as two 2mg tablets of remifemin twice daily) with placebo or conjugated estrogens (0.625 mg/day). The scores of Kupperman index and the Hamilton anxiety scale were recorded at baseline and after 12 weeks of supplementation. After 12 weeks, he reported that both scores were significantly lower in the treated group than in the placebo group. To some extent, the participants who used black cohosh showed a better score than those received the estrogen treatment. In this, hot flushes were separately scored from other symptoms. The findings revealed that daily hot flushes decreased from 4.9 to 0.7 in the black cohosh group, 5.2 to 3.2 in the estrogen group, and 5.1 to 3.1 in the placebo group.

Although preliminary evidence encouraging, the currently available data are not sufficient to support a recommendation on the use of black cohosh for menopausal symptoms.

3. Acupuncture

Acupuncture therapy had shown a significant reduction in vasomotor symptoms in women with previous breast cancer taking tamoxifen:the benefit continued for three months after treatment. (Wyon *et al*., 2004)

4. Homeopathy

Thompson and Reilly (2003) showed that homeopathy significantly reduced menopausal symptoms, including vasomotor symptoms, fatigue and mood disturbances in women with a history of breast cancer. It is one of the most accepted therapies (it is sometimes available on the National Health Service) and remedies are widely available for self treatment.

5. Natural progesterone creams

Skin creams containing natural progesterone, extracted mainly from soy and yams, have been in use since 20 years. Reported an improvement in vasomotor symptoms but there was no effect on bone. The plasma levels achieved using the creams appear inadequate to offer endometrial protection when used in conjugation with estrogen.

6. Phytoestrogen

Active nutritional ingredient can elicit beneficial physiological responses when proper doses are taken. Phytoestrogen is one of them, which has stimulated great interest in recent years.

Phytoestrogens are compounds found in plants to differing degrees. There are many different types, with a variety of activities: estrogenic, anti estrogenic, antiviral, anti carcinogenic, bactericidal, antifungal,

antioxidant, anti mutagenic, antihypertensive, anti-inflammatory and anti proliferative. They are structurally similar to estradiol, less potent but may mimic its action in the body.

The benefits of isoflavones are broad and varied. Studies have shown that they decrease serum cholesterol, and therefore enhance heart health (Anderson *et al*., 1995). It has also been shown that isoflavones are molecularly structurally similar to estrogen and, therefore, have demonstrated mild estrogenic activity. One of the benefits of this is that they alleviate menstrual cycle and menopausal symptoms. (Washburn *et al*., 1999; Xu *et al*., 1998) This may due to a result of their estrogenic activity, isoflavones help maintain bone mass in post-menopausal Women. (Potter S M, Baum J A, and Teng H, *et al*.) Isoflavones alone may also reduce or prevent various symptoms related to the onset and duration of menopause, including hot flushes and osteoporosis.

Complementary and alternative medicines have become popular choices in the treatment of menopausal symptoms. There is no scientific evidence to show any benefits from aromatherapy, hypnosis, yoga and massage on menopausal symptoms. However, they are helpful in stress reduction and this can have a domino effect on other symptoms.

With limited access to complementary and alternative medicine under the National Health Service, using alternative therapies can be costly and women on lower incomes can be disadvantaged.

Table 2.8: List of evidences for complementary and alternative medicine approaches for management of menopausal symptom

Botanical Remedies	Have been tested in short term with little to mild efficacy over placebo Trials are limited by variability of products, menopause populations and inclusion criteria
Black Cohosh	A plant or part of a plant used for its flavor, scent, or potential therapeutic properties. Includes flowers, leaves, bark, fruit, seeds, stems, and root. A study funded by the National Center for Complementary and Alternative Medicine and the National Institute on Aging found that black cohosh, whether used alone or with other botanicals, failed to relieve hot flushes and night sweats in postmenopausal women or those approaching menopause. Other research suggests that black cohosh does not act like estrogen, as once was thought. (National Center for Complementary and Alternative Medicine. National Institutes of Health State of the Science Conference, 2005.) United States Pharmacopeia experts suggest women should discontinue use of black cohosh and consult a healthcare practitioner if they have a liver disorder or develop symptoms such as abdominal pain, dark urine, or jaundice. There have been several case reports of hepatitis, as well as liver failure, in women taking black cohosh. It is not known if black cohosh was causative. Although these cases are very rare and the evidence is not definitive, scientists are concerned about the possible effects of black cohosh on the liver.

Dong quai (Angelicasinensis)	Efficacy has not been demonstrated to be significantly different from placebo. (National Center for Complementary and Alternative Medicine. National Institutes of Health State of the Science Conference, 2005.) Dong quai is known to interact with and increase the activity of warfarin, potentially increasing the risk of bleeding complications.
Ginseng (Panaxginseng orPanax quinquefolius)	Ginseng may help with some menopause related symptoms, such as mood symptoms and sleep disturbances, and enhance overall sense ofwellbeing; however, it has not been found helpful for hot flushes. (National Center for Complementary and Alternative Medicine. National Institutes of Health State of the Science Conference, 2005.)
Kava (Pipermethysticu m)	Kava may decrease anxiety, but there is no evidence that it decreases hot flushes. It is important to note that kava has been associated with liver disease. The U.S. FDA has issued a warning to patients andproviders about kava because of its potential hepatotoxicity. (National Center for Complementary and Alternative Medicine. National Institutes of Health State of the Science Conference, 2005.)
Oil of EveningPrimrose	No significant decrease in hot flushes in one trial. (Chinoy R *et al*., 1995) May potentiate seizure side effects in some medications (e.g., phenothiazines). (Alexander IM., 2009)
Red clover (Trifoliumpratens e)	Controlled studies have found no consistent or conclusive evidence that red clover leaf extract reduces hot flushes. Clinical studies in women report few side effects, and no serious health problems have been discussed in the literature. However, there are some cautions. Some studies have raised concerns that red clover, which

	contains phytoestrogens, might have harmful effects on hormone sensitive Tissue (for example, in the breast and uterus). (National Center for Complementary and Alternative Medicine. National Institutes of Health State of the Science Conference, 2005.)
Soy	The scientific literature includes both positive and negative results on soy extracts for hot flushes. When taken for short periods of time, soy extracts appear to have few if any serious side effects. However, long term use of soy extracts has been associated with thickening of the lining of the uterus. (National Center for Complementary and Alternative Medicine. National Institutes of Health State of the Science Conference, 2005.)
Other CAN Approaches	
Acupuncture	Results have been mixed. Of eight studies published between 1995 and2008, three documented a significant decrease in hot flush severity;none of the others did. A recent metaanalysis concluded that convincing evidence for the use of acupuncture for hot flushes waslacking. However, placebo effects and relaxation achieved with this wellacceptedcomplementary therapy may reduce hot flushes enough to bebeneficial for some women. (Alexander IM., 2009)
Yoga	Pilot trials show benefit. (Pinkerton *et al*., 2009)
Homeopathy Magnetic therapy Reflexology	Efficacy has not been demonstrated to be clinically significant comparedwith placebo. (Pinkerton *et al*., 2009)

(Source: Menopause Care Collaborative, Pfizer Inc, 2013)

7. FOODS RICH IN PHYTOESTROGEN

The major phytoestrogen groups are isoflavones, coumestans, and lignans. They are weak hormones found in many palnts and are strongly believed to play several important roles in the body. (Perspective: Phytochemicals and herbal supplements in health and disease)

Various foods have been reported having estrogenic activities, where few conflicts in the findings are noted. Knuckles and colleagues (1976) found trace amounts of coumestrol in snow peas (frozen), Brussels sprouts (frozen), and spinach leaf (frozen) and also in soybeans (dry), soybean meal (defatted dry), soybean concentrate (dry), and soybean isolate(dry). In contrast, Franke and colleagues (1994) did not detect coumestrol in these last four soybean products.

Soy products, rich in isoflavones, have been use by women during perimenopause to help alleviate some of the side effects of diminished natural estrogen in body. Isoflavone-genistein along with lignans and some other flavonoids, inhibit tumor formation and proliferation. (Perspective: Phytochemicals and herbal supplements in health and disease)

Thompson *et al*., 2006 made an effort to develop a database of nine major phytoestrogens simultaneously analyzed in the same food relevant to Western diets that can be used to estimate their intakes in epidemiological and clinical studies. One hundred twenty one food samples were selected and analyzed for isoflavones (genistein, daidzein, glycitein, formononetin), lignans (secoisolariciresinol, metairesinol, pinoresinol, lariciresinol), and coumestans (coumestrol) using gas chromatography-

mass spectrometry methods. Data were presented on an as is (wet) basis per 100 g and per serving.

Based on group averages on an as is basis per 100 g, the food groups in decreasing order of total phytoestrogens are nuts and oilseeds, soy products, cereals and breads, legumes, meat products and other processed foods, vegetables, fruits, alcoholic and nonalcoholic beverages (Table 2.9).

Soy products (particularly soy beans and soy nuts) contained the highest concentration of total isoflavones, primarily as daidzein and genistein, followed by legumes, meat products and other processed foods (because of their soy contents), cereals and breads, nuts and oilseeds, vegetables, alcoholic beverages, fruits, and nonalcoholic beverages.

All analyzed foods contain lignans and, except for soy or soy containing products, most have higher concentrations of lignans than isoflavones. On an as is (wet) basis per 100 g, decreasing amounts of total lignans are found in nuts and oilseeds, cereals and breads, legumes, fruits, vegetables, soy products, meat products and other processed foods, alcoholic, and nonalcoholic beverages.

In decreasing order within each group, the richest sources of total lignans among nuts and oilseeds are flaxseed, sesame seed, sunflower seed, pistachios, and chestnuts; among cereal products, other than those containing flaxseed, are rye bread and sesame bread;

Table2.9: Phytoestrogen content of foods as consumed (wet weight) per 100g and per serving (µg)

Food Item 100g Serving (g)	FOR	DAI	GEN	GLY	MAT	LAR	PINO	SECO	COU	Total ISO	Total LIG	Total PE
Soy products												
Mean (Per 100g)	8.1	8580.9	10453.1	737.3	0.8	19.7	10.6	23.2	3.3	19779.3	54.3	19837.0
SD (per 100g)	15.4	15080.3	13073.4	1116.6	1.0	27.5	22.6	22.4	9.4	28851.9	68.8	28920.6
Mean (per serving)	2.6	3484.4	4194.7	291.3	0.3	8.7	4.4	9.7	1.2	7973.0	23.0	7997.2
SD (per serving)	4.7	6342.3	5191.5	399.0	0.3	11.7	9.8	11.1	2.9	11789.7	30.9	11819.5
Legumes												
Mean (Per 100g)	1.4	172.6	190.5	37.8	1.3	13.8	62.1	14.6	9.9	402.2	91.9	504.0
SD (per 100g)	1.9	591.6	637.8	135.7	3.9	31.0	215.1	24.8	35.2	1364.9	248.1	1365.3
Mean (per serving)	0.6	36.6	41.0	8.0	0.8	7.3	37.3	5.2	1.9	86.1	50.5	138.5
SD (per serving)	0.8	124.1	133.7	28.5	2.4	19.0	131.4	6.5	6.7	286.2	151.9	312.0
Nuts and oil seeds												
Mean (Per 100g)	4.6	15.7	33.9	5.2	23.4	356.1	635.7	31324.0	5.0	59.4	32339.1	32403.4
SD (per 100g)	9.3	24.3	49.9	15.3	51.7	791.1	1873.6	103719.3	12.7	91.0	104548.5	104639.9
Mean (per serving)	1.8	5.4	12.2	2.2	9.1	141.8	221.6	13463.9	2.0	21.6	13836.3	13859.9
SD (per serving)	4.0	8.9	20.7	6.6	20.6	335.3	637.6	44600.9	5.5	38.2	44973.2	45013.7
Vegetables												
Mean (Per 100g)	16.2	1.1	3.9	0.2	1.4	18.2	31.0	7.6	0.2	21.4	58.2	79.8
SD (per 100g)	76.8	2.1	8.7	0.4	3.7	27.1	98.3	11.3	0.6	78.5	116.7	143.4
Mean (per serving)	1.7	0.2	0.8	0.1	0.4	4.3	5.2	2.1	0.0	2.7	12.1	14.8
SD (per serving)	7.7	0.4	1.9	0.1	1.4	7.2	16.5	3.1	0.1	8.0	20.4	22.1
Fruits												
Mean (Per 100g)	1.1	1.2	3.2	0.3	0.5	14.9	25.1	26.2	0.5	5.7	66.6	72.8
SD (per 100g)	2.8	1.6	4.8	0.3	0.5	28.4	48.4	42.9	1.0	9.0	112.3	119.7

Mean (per serving)	0.7	0.7	1.4	0.1	0.6	7.7	12.1	11.5	0.2	2.9	31.9	35.1
SD (per serving)	1.3	0.8	1.8	0.1	1.4	10.8	21.0	15.2	0.4	3.5	42.6	45.4
Cereals and breads												
Mean (Per 100g)	0.9	82.3	143.8	3.2	0.4	8.0	5.9	868.8	0.2	230.1	883.1	1113.3
SD (per 100g)	1.3	270.6	457.2	9.2	0.5	7.3	10.8	2140.9	0.2	736.8	2145.1	2251.9
Mean (per serving)	0.4	44.1	76.9	1.7	0.1	3.6	3.0	421.0	0.1	122.9	427.7	550.6
SD (per serving)	0.5	146.2	246.9	5.0	0.1	3.6	6.1	1046.4	0.1	397.9	1048.7	1112.0
Meat products and other processed foods												
Mean (Per 100g)	31.0	63.0	149.7	0.2	0.2	4.9	2.8	27.6	0.4	244.0	35.5	279.9
SD (per 100g)	106.2	209.6	509.2	0.3	0.3	10.6	8.6	90.6	1.0	719.7	109.7	741.3
Mean (per serving)	9.4	35.2	83.8	0.1	0.1	2.3	1.0	8.9	0.1	128.5	12.3	141.0
SD (per serving)	31.8	117.4	285.1	0.1	0.1	3.8	2.6	27.1	0.3	401.5	32.7	405.3
Beverages, nonalcoholic												
Mean (Per 100g)	0.3	0.4	0.8	0.1	0.1	0.9	0.8	4.5	0.1	1.5	6.3	7.9
SD (per 100g)	0.3	0.6	1.7	0.1	0.1	0.7	1.2	3.0	0.1	2.3	3.3	4.5
Mean (per serving)	0.7	1.0	1.9	0.2	0.2	2.2	2.0	11.5	0.2	3.8	15.9	19.9
SD (per serving)	0.7	1.5	4.2	0.2	0.2	1.9	2.9	7.5	0.2	5.8	8.2	11.2
Beverages, alcoholic												
Mean (Per 100g)	4.9	1.2	1.4	0.2	0.2	3.2	0.3	11.9	0.0	7.6	15.5	23.1
SD (per 100g)	5.2	0.3	1.0	0.1	0.2	3.0	0.2	12.6	0.0	6.4	15.7	22.1
Mean (per serving)	8.8	2.9	2.5	0.3	0.3	6.0	0.6	21.0	0.1	14.4	27.9	42.3
SD (per serving)	9.0	1.4	1.8	0.2	0.3	5.0	0.3	22.1	0.0	10.4	27.0	37.5

Abbreviations are as follows: FOR-formononetin; DAI- daidzein; GEN- genistein; GLY- glycitein; MAT- matairesinol; LAR- lariciresinol; PINO- pinoresinol; SECO- secoisolariciresinol; COU- coumestrol; ISO- isoflavones; LIG- lignans; PE- phytoestrogens; SD- standard deviation

among legumes are hummus, mung bean sprouts, mung beans, and white (navy) beans; among fruits are dried apricots, dried dates, dried prunes, peaches, and strawberries; among vegetables are garlic, olive oil, winter squash, collards, and broccoli; among soy products are soy beans, soy nuts, and textured vegetable protein; among meat products and other processed foods are black licorice, protein bar, and pizza. Among the alcoholic beverages, red wine is the richest source, having higher total lignan (and isoflavone) values than white wine or beer.

With few exceptions, matairesinol is the least concentrated lignan in foods. Compared with the other lignans, secoisolariciresinol was found in the highest concentration in 63 foods, lariciresinol in 44 foods, and pinoresinol in 14 foods. Coumestan, measured as coumestrol, is generally present in very low concentrations with the food groups containing decreasing amounts as follows: legumes, nuts and oilseeds, soy products, fruits, meat products and other processed foods, vegetables, cereals and breads, nonalcoholic beverages, and alcoholic beverages. (Thompson *et al*., 2006)

This study demonstrated that phytoestrogen sources, particularly of the lignans, are diverse. The lignan levels are often substantial and, therefore, a variety of plant foods can contribute significantly to phytoestrogen intake in the Western diet.

Kuhnle *et al*., (2008) analyzed phytoestrogens isoflavone (biochanin A, daidzein, formononetin, genistein, and glycitein), lignan (matairesinol and secoisolariciresinol), and coumestrol content in 38 beverages, nuts, seeds, and oils.

Table 2.10: Phytoestrogen content in beverages, nuts, seeds, and oil*

				Isoflavones					Lignans		
Food	**Phytoestrogens**	**Isoflavones**	**Lignans**	**Diadzein**	**Genistein**	**Glycitein**	**Biochanin A**	**Formononetin**	**Secoisolaricisionol**	**Matai Resinol**	**Coumestrol**
Coffee											
Coffee, instant powder	**1833**	**913**	**920**	**153**	**594**	**162**	**-**	**4**	**862**	**58**	**-**
Coffee, infusion, average	**17**	**< 1**	**16**	**-**	**< 1**	**-**	**< 1**	**< 1**	**16**	**< 1**	**< 1**
Tea, strong (15g / lit), tea leaves	**12**	**< 1**	**11**	**< 1**	**< 1**	**-**	**< 1**	**< 1**	**9**	**2**	**< 1**
Tea, weak (10g/lit) from tea leaves	**8**	**< 1**	**8**	**< 1**	**< 1**	**-**	**< 1**	**< 1**	**6**	**2**	**< 1**
Tea, standard, from tea bags (4 bags/lit)	**7**	**< 1**	**7**	**< 1**	**< 1**	**< 1**	**< 1**	**< 1**	**4**	**2**	**< 1**
Alcoholic Beverages											
Beer, bitter, best/premium	**1**	**< 1**	**1**	**< 1**	**< 1**	**< 1**	**< 1**	**< 1**	**< 1**	**< 1**	**-**
Wine, red	**76**	**< 1**	**75**	**< 1**	**< 1**	**< 1**	**-**	**< 1**	**55**	**20**	**-**
Wine, white, dry	**14**	**< 1**	**14**	**< 1**	**< 1**	**< 1**	**< 1**	**< 1**	**9**	**5**	**< 1**
Cider, dry	**55**	**6**	**48**	**< 1**	**-**	**4**	**1**	**< 1**	**48**	**-**	**< 1**
Gin	**< 1**	**< 1**	**< 1**	**< 1**	**< 1**	**< 1**	**-**	**< 1**	**< 1**	**< 1**	**-**
Whisky	**5**	**< 1**	**4**	**< 1**	**< 1**	**< 1**	**-**	**< 1**	**4**	**< 1**	**< 1**

*Data are as micrograms per 100g of wet weight. A dash indicates the compound was not detected.

Table 2.10: Phytoestrogen content in beverages, nuts, seeds, and oil*(Contd.)

				Isoflavones					Lignans		
Food	Phytoestrogens	Isoflavones	Lignans	Diadzein	Genistein	Glyctein	Biochanin A	Formononetin	Secoisolaricisionol	Matairesinol	Coumestrol
Nuts & Seeds											
Almonds, Kernel only	112	27	84	< 1	1	< 1	25	< 1	84	-	< 1
Brazil nuts	867	105	781	6	85	< 1	13	< 1	770	12	< 1
Cashews, plain	182	12	170	-	2	1	7	2	165	5	-
Coconut, desiccated	26	3	23	< 1	2	< 1	-	< 1	19	4	-
Coconut, fresh	42	10	32	-	-	4	6	-	30	2	< 1
Hazel nuts	80	22	57	< 1	9	< 1	12	< 1	53	4	< 1
Peanuts, fresh	173	70	101	< 1	48	10	8	4	97	5	2
Peanuts, dry roasted	173	94	76	< 1	58	12	22	1	71	7	1
Peanuts, roasted, salted	427	154	273	4	70	56	21	3	256	17	-
Pine nuts	103	32	70	< 1	4	< 1	27	< 1	46	25	< 1
Pistachios, roasted & salted	62	33	29	-	2	2	27	2	22	7	< 1
Pumpkin seeds	539	18	520	< 1	5	2	7	3	510	11	< 1
Sunflower seeds	111	2	109	< 1	1	< 1	-	-	106	3	< 1
Walnuts	175	31	144	1	11	< 1	17	< 1	140	5	< 1
Oils											
Flaxseeds	23	10	13	-	-	2	6	1	13	-	< 1
Roasted pumpkin seeds	56	6	50	< 1	< 1	3	< 1	1	50	-	< 1
Rapeseeds	61	11	49	< 1	2	3	4	3	49	-	-

*Data are as micrograms per 100g of wet weight. A dash indicates the compound was not detected.

In all beverages analyzed, only small amounts of isoflavones were detected, with an exception of instant coffee powder, in which significant amounts of daidzein, genistein, and glycitein were found. In coffee and tea-but not chamomile tea-lignans were the main class of phytoestrogens with an average content of 12 μg/100 g. The phytoestrogen content in alcoholic beverages (except for gin and bitter) was significantly higher (45 μg/100 g) with a prominent difference between red (76 μg/100 g) and white wine (14 μg/100 g) and different types of beer (brown ale, 71 μg/100 g; lager, 68 μg/100 g; stout, 45 μg/100 g; bitter, 1 μg/100 g).
The phytoestrogens and in particular lignans content was much higher in nuts and seeds, in particular in Brazil nuts (887μg/100 g) and pumpkin seeds (539 μg/100 g); the main lignan detected was secoisolariciresinol.

Further, on comparing the results of Kuhnle *et al*., Thompson *et al* (2006) and Milder *et al* (2005); Kuhne *et al*. reported more lignans in beverages than reported by Thompson *et al*. (2006) and Milder *et al*. (2005), but Thompson *et al*. found significantly more isoflavones in both red and white wines. For most foods analyzed, the variation in total phytoestrogen content is less than 3-fold when compared with the data of Thompson *et al*. (2006). The phytoestrogen content in soybeans is known to vary more than 4-fold (Adlercreutz *et al*., 1995; Eldridge *et al*., 1983) depending on variety, harvesting, and processing, and in a recent study we showed a similar variability in a selection of other foods analyzed, including lignan-rich foods such as carrots and cabbage. (Kuhnle *et al*., 2008)
The variability observed could be due to different sources of food origin, sampling method, differences in the analytical method, different methods used for hydrolysis of phytoestrogen. (Kuhnle *et al*., 2008)

Clerk *et al*., (2002) conducted a study to analyze 13 phytoestrogens in the vegetarian duplicates diets which were collected in summer (16) and winter (19).Phytoestrogens were analysed by paired hydrolysed and non-hydrolysed assays. The mean total isoflavone concentration in diets, without hydrolysis, was 14 mg/kg in summer and 15 mg/kg in winter, corresponding to an overall hydrolysed aglycone concentration of 10 mg/kg.

Isoflavones were detected in 31 of the 35 diets. The isoflavone content of diets was up to 21 mg/ kg of daidzein, 43 mg/kg of genistein and 3 mg/kg of glycitein equivalents. The mean concentrations were: 3.1 mg/kg of daidzein, 6.1 mg/kg of genistein and 0.6 mg/kg of glycitein equivalents. The average combined aglycone daily intake figure was 10.5 mg/day. There were no statistical differences in the intake of any of the 12 individual isoflavones, aglycone equivalents or total isoflavones, between summer and winter diets.

In general, the total daidzein equivalents concentration (sum of the three glucose conjugates plus the parent aglycone) was in good agreement with the total measured hydrolysed daidzein concentration.

Agreement between the total genistein equivalent concentration and total hydrolysed genistein concentration was more variable, some hydrolysed samples were found to contain significantly more genistein than predicted.

The mean overall conjugation profile (in order of increasing molecular weight) was; aglycones 13%, glucose- conjugates 61%, acetylglucose-conjugates 5% and malonylglucose-conjugates 21%, with seasonal ratio averages of 8:62:3:27 in winter and 18:62:5:15 in summer diets. These profiles need to be viewed against the trend seen in primary soya products, where conjugation ratios of 1:52:9:38 for full fat soya flour, 6:77:2:15 for soya milk and 26:41:22:11 for a textured soya protein meal

were measured to assist in conjugation pattern recognition. In the diets an overall shift in conjugation was observed from the larger malonyl-glucose esters through the smaller acetyl-glucose and glucose conjugates to the free aglycones. This suggests that a significant portion of the isoflavone content of vegetarian diets may be derived from heavily processed ‘‘second generation’’ soya foods, where primary soya products such as soya flour, soya milk and textured vegetable protein are used as ingredients in composite foods that are subject to further processing.

To summarize, phytoestrogens are certainly worthy of more research though there remain many gaps in our knowledge. It is of note that studies have not shown any adverse effects from naturally occurring phytoestrogens, they are well tolerated and they remain a very popular supplement. The differences in reported databases of phytoestrogen content of foods are attributable to differences in variety of food, environmental factors, growth, harvesting and processing. (Kuhnle *et al*., 2008)

The research says the differences in the metabolism of phytochemical in the body complicate the interpretation of research studies and not able to drawn any recommendations.

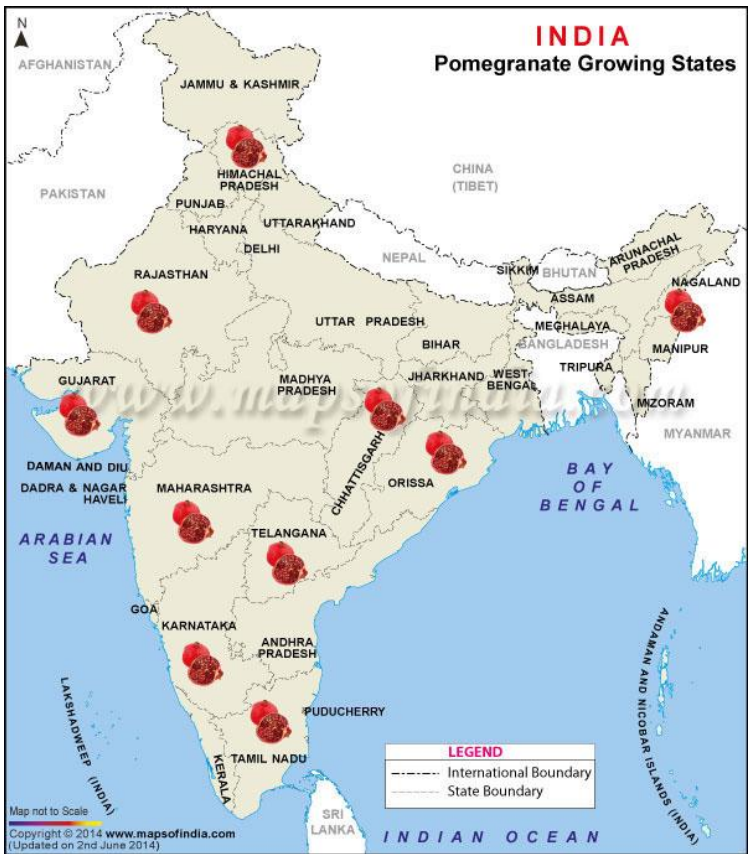
In developing countries like India, a large portion of community depends on the indigenous traditional medicine as a primary health care approach. Traditional medicine is an important part of healthcare. From variety of traditional herbs and foods which have potential in treatment of certain chronic diseases and play an important role in human health care, four of them (Elephant foot yam, Pomegranate, Flaxseeds, Fenugreek seeds) are brief in following.

Pomegranate:

Pomegranate has been valued as a symbol of health for centuries.

In ancient Greek mythology, pomegranates are known as the "fruit of the dead", the sustenance available in Hades for its residents. Hades himself, the master, benefitted amorously when six pomegranate seeds from his realm sealed for him the betrothal of the beautiful daughter of Zeus and Demeter, fair Persephone.

No less, in the ancient Hebrew tradition, pomegranates adorned the vestaments of the high priest, were extolled by Solomon in his song of Songs, the numbers of seeds were considered mystically equivalent to the number of virtues in even a simple person, and for the initiated, the pomegranate was understood to be symbolic of the female aspect of the Creator, i.e., the *Shekinah* (Wolfson,1988).



(source: www.mapsofindia.com, 2014)

Figure 2.4: Production of *Punica granatur* (pomegranate) in India

The Babylonians regarded the seeds as an agent of resurrection, the Persians as conferring invincibility on the battlefield and for ancient Chinese alchemical adepts, the bright red juice was mythopoetically regarded as a "soul concentrate," homologous to human blood, and capable of conferring on a person longevity or even immortality (Maadihassan, 1984; Lansky *et al*., 2000)

Ancient Egyptians used the extract to treat all types of maladies, including dysentery. Traditional Sri Lankan plant-based remedies included brewing a medicinal tea from the buds of pomegranate tree and imbibing it for treatment of either chronic diarrhea or bronchitis. The beautiful flowers of the tree were also used to relieve sore eyes.

It is also well known that the dried pomegranate seeds contain an oil, which has been shown to contain not only the steroidal estrogen estrone (Dean *et al*., 1971), but also the isoflavonic phytoestrogens genistein and daidzein and the phytoestrogenic coumesten, coumestrol (Moneam *et al*.,1988). When injected, a hydrolyzed concentrate of the oil produced increased uterine weight in immature rabbits, and cornification of vaginal epithelium in ovariectomized adult mice, both indicative of estrogenic action in vivo. (Lansky *et al*., 2000)

Ethnomedical explorations have documented pomegranate hull and/or root extract usageboth orally and intravaginally to prevent fertility (Gujral *et al*., 1960; Jochle, 1971; Razzack, 1980) and abortion (Ramirez *et al*., 1988) and to ameliorate assorted gynecological problems (Singh *et al*.,1980; Goh etal.,1984). Other traditional uses of these materials have included treatments for snakebite (Jain and Puri, 1984), diabetes (Singh, 1986), burns (Siang, 1983) and leprosy (Singh *et al*.,1980). The fresh fruit itself has been used as a refrigerant to lower fever (Arseculeratne *et al*.,1985; Lansky *et al*., 2000)

Besides the renowned abundance of flavonoids, anthocyanins, catechins and specific hydrolysable ellagic derivatives named punicalagins, pomegranates proved to be rich also in lignans. As a consequence of their significant presence, lignans may act in synergy with other healthy polyphenolics of the plant's phytocomplex and represent a potential contributor to the purported oestrogenic functional properties of pomegranate juice. Their abundance may constitute a relevant marker in quality definition of P. granatum fruit and its neutraceutical byproducts.

The lignans isolariciresinol, medioresinol, matairesinol, pinoresinol, secoisolariciresinol and syringaresinol were detected and quantified for the first time in pomegranate (Punica granatum L.) and in commercial pomegranate juices by means of GC–MS. The total lignan content in the different plant parts was estimated as follows: 36.1 ± 0.3 μg/g in seeds, 17.8 ± 0.2 μg/g in wood knots, 11.2 ± 0.2 μg/g in fruit pulp,3.3 ± 0.1 μg/g in endocarp. Secondary metabolite distribution varied greatly in the evaluated samples,with syringaresinol being the most abundant contributor in seeds (23.5 ± 0.4 μg/g) and pinoresinol in knots, pulp, endocarp and juice (8.9 ± 0.3, 7.4 ± 0.2, 3.3 ± 0.1 and 2.1 ± 0.2 μg/g, respectively). A survey on two concentrated juices and three commercial pomegranate beverages evidenced the presence of lignans in all of them, with values ranging from 0.4 ± 0.1 to 4.4 ± 0.1 μg/g. (Bonzanini *et al*., 2009)

Concentrated juices and commercial beverages made of pure pomegranate juice have been also evaluated, evidencing an inferior presence of secoisolaricresinol, pinoresinol and syringaresinol (Table 2.11). Both amounts of total lignan aglycones and the qualitative profile were however rather different between the tested samples. Natural

variability of plant sources (in terms of both phylogenetic and ontogenetic variability) and different industrial protocols due to dissimilar squeezing techniques and dilution rate may account for these fluctuations. A remarkable difference was noticed between concentrated and commercial fruit juices. It must be noticed, however, that most pomegranate juices available on the market are produced by dilution of concentrates, a step that is obviously detrimental in terms of lignan content. (Bonzanini *et al*., 2009)

Table 2.11: Amount of lignans (µg/g) in different pomegranate fruit juices, obtained by extraction under reflux in acetone-water

	CdJ[a] – Tunisia	CdJ – Wonderful	CJ[b] brand #1	CJ brand #2	CJ brand #3
Isolariciresinol	n.d	0.3 ± 0.1[d]	n.d	0.7 ± 0.1	n.d.
Secoisolariciresinol[c]	n.d	0.7 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	n.d.
Matairesinol	n.d	n.d.	n.d.	n.d.	n.d.
Pinoresinol	2.1 ± 0.2	1.5 ± 0.2	n.d.	0.9 ± 0.1	n.d.
Medioresinol	0.5 ± 0.2	n.d.	n.d.	n.d.	n.d.
Syringaresinol	1.8 ± 0.1	1.4 ± 0.1	n.d.	n.d.	0.6 ± 0.1
Total	**4.4 ± 0.**1	**3.9** ± 0.2	**0.4** ± 0.1	**2.5** ± 0.3	**0.6** ± 0.1

a Concentrated juice.
b Commercial juice.
c Obtained as a sum of secoisolariciresinol and anhydrous secoisolariciresinol.
dThe results reported are the mean of three independent extractions and three chromatographic analyses for each extraction.

Elephant Foot Yam

Among the tropical tuber crops, elephant foot yam (*Amorphophallus paeoniifolious)* is considered to be the highest income earner to thecultivator irrespective of the production system and temporal variations. It has both nutritional and medicinal value and is usually consumed as cooked vegetable. It must be washed and cooked thoroughly

to avoid it catching a throat, which feels like thorns covering the tonsil due to high amount of calcium oxalate raphides present in plant.

It is a crop of Southeast Asian origin, and grows in wild form in the Philippines, Malaysia, Indonesia and Southeast Asian countries. In India, it is traditionally cultivated on commercial scale in Andhra Pradesh, Gujarat, Tamil Nadu, West Bengal and Kerala states and slowly spreading to other states, like Bihar, Uttar Pradesh and so on. (Srinivas T and Ramnathan S, 2005)

Figure 2.5: *Amorphophallus paeoniifolious* plant and tuber

Elephant foot yam offers excellent scope for adoption in the tropical countries as a cash crop due to its production potential and popularity as a vegetable in various delicious cuisines. Many indigenous ayurvedic and unani medicinal preparations are also made using its tubers. The tubers are believed to have blood purifying characteristics and are used in medicines for the treatment of piles, asthma, dysentery and other abdominal disorders.

Yam is considered to be a healthy low fat food and is a rich source of essential fatty acids (Omega3fatty acids) which are known to increase the good cholesterol levels in the blood. Eating elephant yam helps in increasing the estrogen levels in women's bodies, thus helping in maintaining the hormonal balance. It is also high in vitamin B6 and has a high concentration of key minerals. Consuming vitamin B6 provides relief from premenstrual syndrome in women.

It is a natural product that is high in fiber. This property makes it a slimming food due to its reducing capacity of cholesterol levels and promotion of weight loss. As it is rich in dietary fibers, it may serve as a home for probiotic bacteria, helps to strengthen the digestion process and boost the immune system. These probiotic bacteria known for digestion of lignans, helps in PMS.

Elephant foot yam is rich in vitamin C, which helps to cure premature aging.

It is loaded with potassium, magnesium and phosphorous, as well as with trace minerals like selenium, zinc and copper. (Thumma S, 2015) These metals are responsible for sharp memory and concentration power. (Sony, January 2015)

The nutritional values of the edible portion of the elephant yam (*A. paeoniifolius*) are:

> Energy 330 kJ/100 g (approx), Water 7279%, Protein: 1.75.1%, Fat: 0.20.4%, Carbohydrate: 1824%, Fibre: 0.8 %, Calcium: 5056 mg/100 g, Iron: 0.61.4 mg/100 g, Phosphorus: 2053 mg/100 g, Vitamin A: 434 IU/100 g. (Thumma S, 2015)

The tuber of the elephant foot yam is edible and of interest for its antibacterial, antimycobacterial, antiviral, anti-inflammatory, analgesic, antidiabetic, blood pressure lowering effects. Various studies have been carried out to investigate the phytochemical properties of elephant foot yam.

Dey *et al* (2012) carried out a pharmacological review on an *Amorphophallus paeoniifolius*, where they mentioned various studies supporting the analgesic, anti-inflammatory, CNS activity, anti-microbial, anthelmintic, and hepatoprotective properties of elephant foot yam. They noted the benzodiazepine receptors may be responsible for the CNS depressant activity. The phyto constituents present in plant are mainly steroids and flavonoids which are involved in all the actions. They concluded that the elephant foot yam is safe and is having high medicinal value.

The toxicity study conducted by Dey *et al* (2009), found that the petroleum ether extract is safe to be used at a therapeutic dose of 250 mg/kg. The LD_{50} was found to be 2500 mg/kg in mice.

Arva *et al* (2012) studied anti-diabetic effect of Elephant foot yam in Streptozotocin-induced Diabetic rats. They compared control and diabetic (experimental) groups: starch-fed control/diabetic (SFC/SFD), 0.1% acetone extract fed control/diabetic ($AEFC_{0.1}$/$AEFD_{0.1}$), 0.25% acetone extract fed control/diabetic ($AEFC_{0.25}$/$AEFD_{0.25}$) and aminoguanidine fed control/diabetic (AFC/AFD). The parameters considered were water intake, diet intake, urine output, gain in body weight, urine sugar, fasting blood sugar (FBS) and glomerular filteration rate (GFR). They observed a

concentration-dependent amelioration of the diabetic status with respect to all dependent parameters. There was a 23% reduction in FBS in $AEFD_{0.1}$ group and 37% reduction in $AEFD_{0.25}$ group, whereas the AFD group showed a 45% reduction relative to the SFD group. The study concluded that the acetone extract of elephant foot yam is an effective anti-diabetic agent for streptozotocin-induced diabetic rats.

Various literatures support and mentioned the role of elephant foot yam in increasing estrogen levels in blood in women and also in relief from premenstrual syndrome in women. Yet evidenced based clinical studies are not found which explain the role of elephant foot yam during menopausal transition. Also the phytoestrogen components present in it, are not studied and documented in detail.

Fenugreek seeds

Trigonella foenum-graecum (L.) (Fabaceae), commonly known as Fenugreek is an annual herbaceous plant cultivated in the Mediterranean countries, Argentina, France, India, North Africa, Spain, Pakistan, Bangladesh, China and Nepal. In India, Rajasthan, Gujarat, Uttarakhand, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab are the major fenugreek producing states. It is known as *methi* in Marathi, Punjabi, Hindi, Urdu, Bengali and Nepali, as *menthiyam*, and *venthayam* in Tamil, "uluhaal" in Sinhala, Helba in Arabic, *menthya* in Kannada, uluwa in Malayalam, and *menthulu* inTelugu.It is used as an herb (dried or fresh leaves), as a spice (seeds), and as a vegetable (fresh leaves, sprouts, and microgreens).

It is an erect herb, mature to be about 2 feet tall. It White flowers blooms in the summer and develop into long slender green pods. Mature brown

pods have 20 tiny yellowaromatic seeds.(Fenugreek-Wikipedia, 2013; Shailajan S *et al*., 2011)

Figure 2.6: *Trigonella foenum-graecum* plant and seeds

The major constituents of fenugreek seeds have been identified as proteins 20 to 25 percent, dietary fiber (45 to 50 percent), mucilaginous soluble fiber (20 to 25 percent), fixed fatty acids and essential oils 6 to 8 percent and steroidal saponins 2 to 5 percent. In addition to these main components, some minor components like alkaloids (trigonelline, cholin, gentianine, carpaine etc), free unnatural amino acids (4-hydroxyisoleucine), and individual spirostanols and furastanols like diosgenin, gitogenin, yamogenin etc have also been identified, isolated and characterised as the principal components responsible for its varying biological effects. (Trivedi PD, 2007) Fenugreek has a distinctive sweet smell due to sotolon compound present in it. (Fenugreek-Wikipedia, 2013; Shailajan S *et al*., 2011)

Fenugreek has been used historically to treat stomach problems, bronchitis, arthritis and constipation. Fenugreek may also improve breast milk production in lactating women. (Rae U, 2011)

Most studies on *Trigonella foenum-graecum have focused on* antipyretic, anthelmentic, antileprotic, antibronchitic, carminative and anti diabetic

properties. (Mehrafarin A *et al*., 2011; Shailajan S *et al*., 2011; Toppo FA *et al*., 2009)

However, the other phytoestrogenic components present in fenugreek have not much explored.

One such study was conducted by Sreeja S in 2009 to investigate the effect of chloroform extracts of fenugreek seeds (FCE) in breast cancer cells for its estrogenic effect, and to assess its capacity as an alternative to hormone replacement therapy. The findings were:

FCE stimulated the proliferation of MCF-7 cells, showed binding to ER (IC_{50} = 185.6 ± 32.8 μg/ml) and acted as an agonist for ER mediated transcription via ERE. It also induced the expression of estrogen responsive gene *pS2* in MCF-7 cells. The study supports the *in-vitro* estrogenic activity of fenugreek seeds and recommends further *in-vitro* and *in-vivo* studies to demonstrate its suitability as an alternative to HRT.

The studies conducted in Egypt reported that fenugreek was the most common herb used to manage menstrual disorders. Fenugreek was considered as a cure for many symptoms and complaints including female troubles. Specifically, menstrual pain, menopausal troubles, lost periods, and to support healthy milk production. (Yassin SAT, 2012; El-Gilany *et al*., 2005; Assiut University Center for Environmental studies, 2002). So fenugreek may help in management of PMSby relieving pelvic congestion, breast tenderness, and weight gain by its diuretic effect. Also its antihistaminic effect can relive premenstrual tension. Its spasmolytic effect may relive premenstrual gastrointestinal spasms.

Natarajan, Dhananjayan (2007) explained the mode of action of Fenugreek in their experimental study about its spasmolytic and antihistaminic effect on spasm and GIT disturbances associated with dysmenorrhea. Its diuretic effect can also relive the pelvic congestions

and weight gain associated with dysmenorrhea. They also provided an evidence that fenugreek contains phytoestrogens - that are natural plant chemicals that mimic female hormones estrogen , which can in turn increase the building up of uterine endometrium and increase its thickness. Consequently, it can increase the menstrual flow.

Hakimi S *et al* (2014) studied the effect of Fenugreek seed on early menopausal symptoms.

They used quasi experimental design in the study; where 2 groups ofperimenopausal women were selected, each group contained 25 participants. Women in control group received 2 periods consist of

0.625 mg conjugated estrogen and 10 mg medroxy progesterone acetate. Women in experimental group received 6 g fenugreek seedpowder in granulated form for 8 weeks. The Greene menopausal scale was used to assess change in early menopausal symptomsat baseline and after 4, 8 weeks of treatment.

The score between two groups at baseline was not significantly different ($p=0.776$). After 4 and 8 weeks oftreatment on control group compared to Fenugreek group Greene score showed significant decrease ($p<0.001$, $p<0.001$).Use ofFenugreek seed for 4 and 8 weeks caused significant reduction of total Green menopausal score but this effect was less than HRT. They opined that furtherstudies using double blind placebo-controlled clinical trial are needed.

As the herbal supplements do not required any approval or any regulatory compliance, dose-range studies are hardly ever done. The dosages in use of such products are usually based on practical experience and/or a result of trial and error approach. In case of fenugreek, extra caution needs to be taken for dosage. For diabetes, some studies have used 10 to 15grams per day, either once daily with a meal or split into smaller doses with meals, to help limitthe rise in blood sugar that happens after meals. For

high cholesterol, studies have used alower fenugreek dose -- 0.6 to 2.5 grams twice a day with meals. It is not known if these dosesare safe or effective, since very little research has been done.

Flaxseeds

The *Linum usitatisimum (*L) also known as flaxseed is emerging as one of the key sources of phytochemicals. (Shazad *et al*., 2006; Amin T and Thakur M, 2014) It is considered as an excellent "nutritional package" due to presence of high quality proteins, soluble fibers, good amount of niacin and folic acid and zinc.

Flax is grown for its value either as an oil crop or as a fiber crop, with fiber linen derivedfrom the stem of fiber varieties and oil from the seed of linseed varieties. It has gainedrecent attention as a potential functional food: since it is an exceptionally rich source ofdietary lignan, possessing over 800-fold the amount in most other foods. Canada is theworld's largest producer of flaxseed (about 38% of the total production), where it is grownannually on approximately 1.3 million hectares of land and harvested primarily for its seedoil, then come China and India.

Functional foods and nutraceutical are becoming popular alternatives to pharmacological treatments by providing health benefits and/or reducing the risk of chronic diseases. Flaxseed is a rich source of three components with demonstrated cardio protective effects: dietary fiber, lignans (phytoestrogens) and the omega-3 fatty acid (alpha-linolenic acid).

Dietary flaxseed may also offer protection against ischemic heart disease by improving vascular relaxation responses and by inhibiting the incidence of ventricular fibrillation.

Figure 2.7: *Linum usitatisimum* plant and seeds

Because of its high fiber content and laxative effect, flaxseed is also used to treat constipation and hemorrhoids, and appear to help hormone imbalances, such as those that cause menopausal symptoms.

Crude fat content ranged from 37-43%, moisture 0.2-6.8%, carbohydrate 30-35%, crude protein 18-23%, and crude ash 3-4%. Flaxseed is also an important source of dietary fiber. The TDF(total dietary fiber) contents of the flaxseed samples were 28～31%, and the SDF(soluble dietary fiber) content of roasted flaxseeds was higher than that of raw flaxseeds. The major minerals found in flaxseed were calcium, potassium, magnesium, and phosphate. The flaxseeds were rich in γ-tocopherol with 234.3 mg/kg in raw brown flaxseed and 134.1 mg/kg in raw gold flaxseed, respectively. Roasted flaxseeds showed slightly lower vitamin and amino acid contents than those of the raw samples. The iodine, saponification, and acid values of brown flaxseed oil were 204.1 g/100 g, 193.6 mg/g, and 1.59 mg/g, and for gold flaxseed oil were 203.0 g/100 g, 189.9 mg/g, and 2.35 mg/g, respectively. α-Linolenic acid(ALA, C18:3n-3) was highly concentrated in the flaxseed oil, which constituted about 55.5-56.1% of total fatty acids. Thus, flaxseed oil is a good source of omega-3

fatty acids and beneficial for the heart. Flaxseed contains high levels of dietary fiber including lignans, as well as minerals and vitamins, which may have antioxidant actions and help protect against certain cancers. (Nam JS, 2010)

Thompson *et al.* (2004) reported the presence of secoisolariciresinol diglycoside in flaxseeds, which is changed to phytoestrogens by the ruminal microflora in compound stomach animals and by the microflora of the hind gut in rats and mice.

Ahmad *et al*., (2011) conducted a study to assess the effect of an aqueous methanolic extract of Flax seeds on serum estradiol, progesterone, total proteins and total cholesterol in immature rat. The results revealed serum estradiol, progesterone, total proteins and total cholesterol contents were higher ($P<0.05$) in rats given Flax seeds extract (orally @ 500mg/Kg body weight) and estradiol (40 µg/kg body weight) compared to controls. Estrogen-like activity of Flax seeds was also evident in the histological sections of ovaries. Ovaries of rats treated with extract of *Linum usitatissimum* (Flax seeds) revealed the presence of multiple mature Graafian follicles, together with corpora lutea.

8. MODE OF ACTION – ESTROGEN AND PHYTOESTROGEN

Estrogen influences growth, differentiation, maturation, and function of many different reproductive and non-reproductive tissues including those of central and peripheral nervous system. (Belcher & Zsarnovszky, 2001)

The major estrogens include estradiol, estrone and estriol.

The actions of estradiol (E2) at target tissues can be categorized as:

1) Long-term "genomic" actions that are mediated by intracellular estrogen receptor-induced changes in gene expression
2) Rapid actions that modulate a diverse array of intracellular signal transduction cascades

(Belcher & Zsarnovszky, 2001)

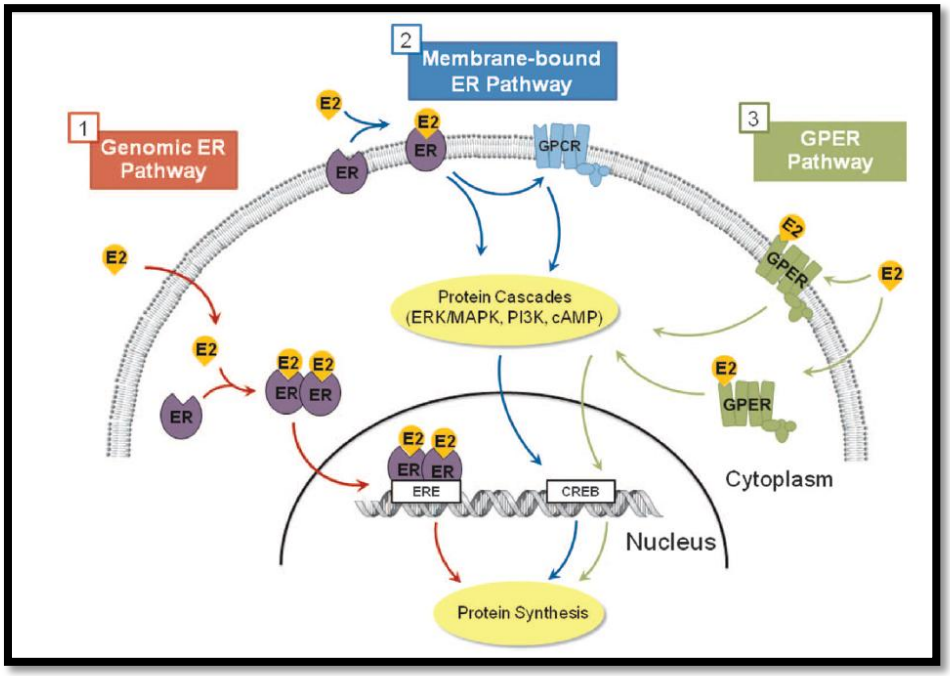


Figure 2.8: Estrogen signaling pathways: 1) Genomic ER pathway, 2) membrane bound ER pathway, 3) GPER pathway

Genomic Pathways:

Estrogen perform their function by interacting with Estrogen Receptors (ER).In mammals, two ER genes have been identified – ERα and ERβ, both having similar DNA- and ligand-binding properties, but distinct tissue distributions and functions. The ER belongs to the nuclear receptor category from three different sex steroid receptor categories. (Rodriguez, 2014)

After getting entry into the cell, they may act on either of the following way:

1) E2 bound ER can interact as homo- or hetero dimers with estrogen-responsive elements (ERE) (Figure 2.8, 1) or

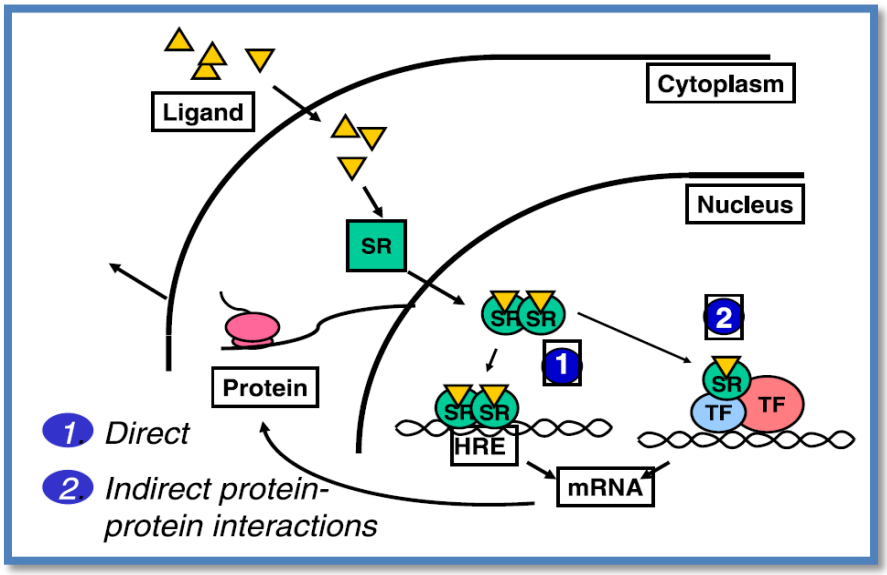


Figure2.9: Transcription action by liganded SRs. (SR – sterois receptors, e.g. Estrogen Receptors; TF – transcription factor; HRE – Hormone responsive element, e.g. Estrogen responsive element (ERE))

2) Associate with the AP1 (Activator protein 1) transcription factors c-Jun or c-Fos to modulate transcription of responsive genes. (Belcher & Zsarnovszky, 2001; Kuiper *et al*., 1996,1997; Paech *et al*., 1997)

Membrane bound pathway

Estradiol can rapidly influence cellular physiology in many different cell types of reproductive and non reproductive tissues thru the activation of a diverse array of intracellular signaling mechanisms. Estradiol rapidly activates adenylate cyclase, increase intracellular [Ca^{+2}], activate phospholipase C to generate inositol 1,4,5 – triphosphate and diacyl glycerol, stimulate nitric-oxide synthase to generate nitric-oxide and activate the Extracellular Regulated Kinases 1/2 (ERK1/2) mitogen-activated protein kinase (MAPK) pathway. (Belcher & Zsarnovszky, 2001)

Some rapid actions of estradiol are independent of intracellular ER. (Belcher & Zsarnovszky, 2001)

Non genomic effects of Estrogen via GPCRs

This is a G-protein coupled receptor and present in the cell membrane, nucleus, and endoplasmic reticulum. Its ability to modulate the anxiety-related behavior in rodents is reported. GPER pathway facilitates the rapid non-genomic effects of estradiol by activating intracellular cascades (Figure 2.10).

These complex modulations of multiple intracellular signaling systems can then have both acute, non genomic effects or chronically modify gene expression.

Each cell or target of sex steroid action may have a different complement of membrane receptors and responses to ligands to mediate cell-specific effects in normal physiology and altered complements in disease states.

In vascular cells, estrogen may interact with plasma-bound GPCRs to induce acute effects on intracellular signalingthrough cAMP and can be blocked by pertussis toxin.

This signaling can modify Src, which phosphorylates the EGF receptor (EGFR) and releases metalloproteinases, which trigger the release of heparin-bound EGF ligand to active EGF to augment the tyrosine receptor kinase EGFR.

These steps can be blocked with protein phosphatase 2 or CRM-197.

Ligand-stimulated EGFR interacts with the docking proteins of Sos and Shc to eventuate in the activation of the Ras/Raf/MEK/ERK signaling system.

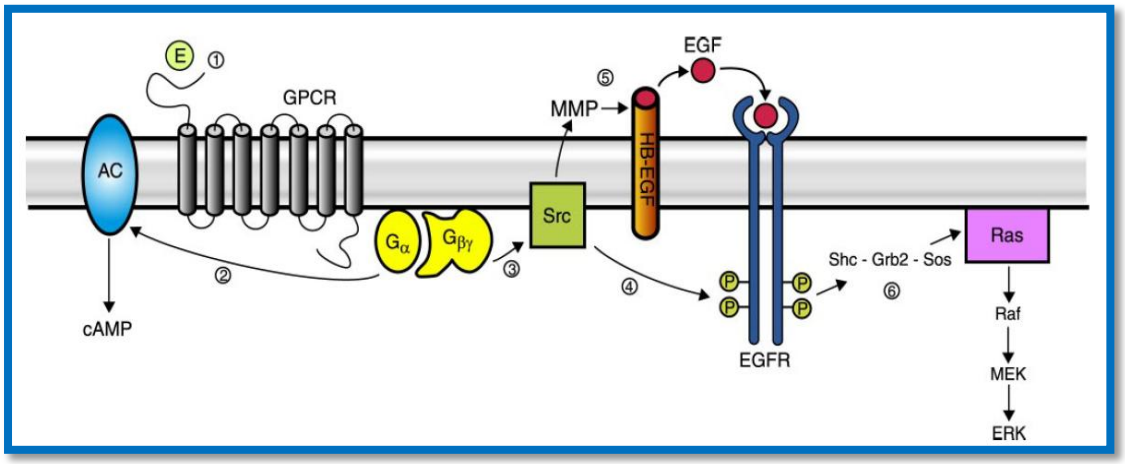


Figure 2.10: Nongenomic effects of estrogen action via G protein-coupled receptors (GPCRs)

(E2 – estradiol, MMP – metalloproteinase, HB-EGF – heparin-bound EGF, Sos – GTP exchange factor, Grb2 – adaptor, Shc – adaptor, Ras – small G protein, Raf – Ser/Thr protein kinase, PP2- protein phosphates 2)

Phytoestrogen – actions in the Hypothalamus –Pituitary –Gonad axis

Gonadotropin releasing hormone (GnRH) functions as the final common hypothalamic signal peptide to regulate reproduction.

Zhao and Mu (2010) have quoted various studies to demonstrate the effect of various phytoestrogenic components on GnRH activity.
Christoffel *et al* (2006) found that the favanone 8-prenylnaringenin exhibited an E2-mimic negative feedback to inhibit the activity of the hypothalamic GnRH pulse generator and thereby serum LH levels in rat.

Bowe J *et al* (2003) reported that coumestrol exerted a direct inhibitory effect on GnRH gene expression in the GnRH neuron, and then elicited an inhibitory regulation on the reproductive system via binding to ERβ. While Jacob *et al* (2001) demonstrated that coumestrol inhibited GnRH effects on LH release by antagonizing the neuroendocrine action of estrogen thru ERα. These two findings suggesting that the estrogenic activity of phytoestrogen is mediated via different mechanism in the brain.
McGarvey *et al* (2001) and Hughes *et al* (1991) reported that the GnRH-inhibited effect of coumestrol and selective endocrine activity of genistein led to reducing LH release at the level of rat pituitary.high dose administration of dietary equol markedly enhanced serum LH level in the ovariectomised rat (Rachon D, 2007) and intra cerebrroventricular of ganister increased the percentage of LHβ – subunit gene expressing cells in the anoestrous ewe pituitary via activation of cellular ERα. (Polkowska *et al*., 2004)

The studies on phytoestrogen modulating gonadotropin generation and secretion in the pituitary exhibited some contradictory patterns.

FSH is another kind of gonadotropin released from the pituitary.

Polkowska *et al* (2004) reported that Genistein had no effect either on FSH cellular immunoreactivity or on the FSHβ gene expression possibly due to no ERα existence on FSH-gonadotrophs in ewe. The recent evidence of high-dose and long term treatment of dietary puerarian mirica (a herb containing phytoestrogen) might produce a clear reduction in the monkey urinary FSH levels, suggested the potential regulatory effect of phytoestrogen on FSH release. (Trisomboon *et al*., 2007)

The studies on steroidogenesis indicated the phytoestrogen effects might be mediated indirectly via gonadotropin regulation or directly in the ovary.

Phytoestrogen – supports osteogenesis and prevents adipogenesis

For various phytoestrogenic components like genistein, daidzein, resveratrol, Rg1, kaempferol, coumestrol, and their involvement in differentiation of mesenchymal stem cells have been illustrated.

Besides ER signaling mimicking the action of E2, phytoestrogens have been shown to activate alternative pathways like Wnt and Sirt 1 signaling. Along with this, other multiple intracellular signaling cascades are involved by which phytoestrogen works either directly (solid arrows) or indirectly (dashed arrows) (Figure 2.11). These signaling ultimately leads to the activation of osteogenic transcription whereas repression of genes promoting adipogenic differentiation. (Schilling *et al*., 2013)

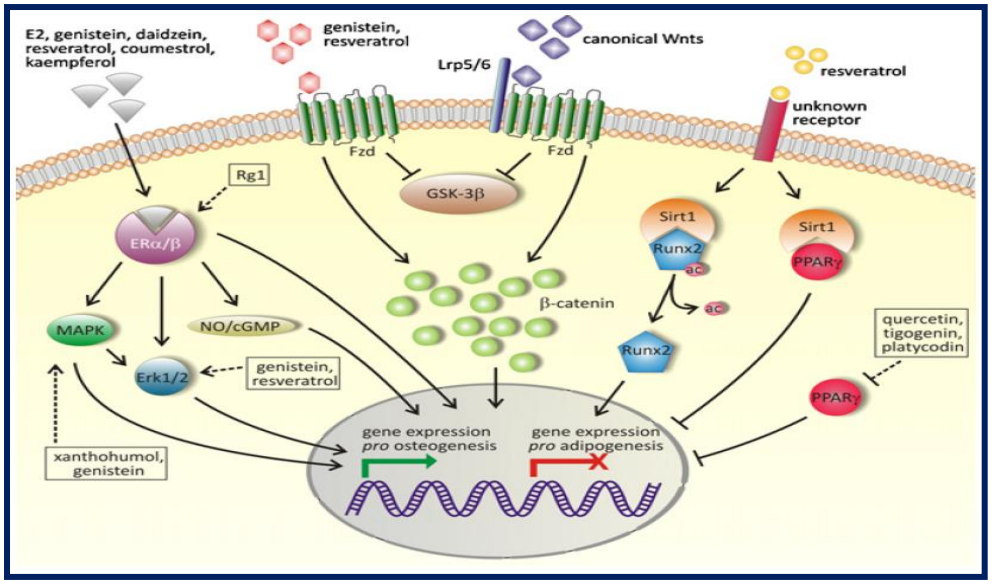


Figure 2.11: Signaling pathways for phytoestrogen activity in mesenchymal stem cells (MSCs)

Kuiper *et al* (1997) reported that as genistein is ER-dependent, it acts on ERβ for the enhancement of osteogenic differentiation and ERα to suppress adipogenesis. The binding affinity of genistein has been considered to be higher for ERβ than ERα at the biochemical level. The outcome of genistein might dependent on the distribution of receptors on tissue.

Ise *et al*., (2005) did the expression profile of the estrogen responsive gene in response to phytoestrogens, isoflavone (genistein, daidzein, glycitein, biochanin A and ipriflavone), flavones (chrysin, lutteolin and apigenin), flavonols (kaempferol and quercetin), and a coumestan (coumestrol) using a customized DNA microarray. A total of 172 estrogen responsive geneswere monitored with a customized DNA microarray and their expression profiles for the above phytoestrogens were compared with that for 17β-estradiol (E_2) using correlation coefficients, or R values, after a correlation analysis by linear regression. From 172 genes, 108 genes showed upregulation and 64 genes showed

downregulation by E_2 treatment. Phytoestrogen were analyzed at a concentration of 10µM for treating the cell cultured for 72 hr.

Relationship of gene expression profiles between estrogen and phytoestrogens revealed isoflavones to be more effective interms of estrogen activity, followed by coumestrol in compare to less effective flavones and flavonols.

Responses of the genes related to signal transduction were categorized as p53-related genes (CDKN1A, TP53I11 and CDC14), Akt2-related genes (PRKCD, BRCA1, TRIB3 andAPPL), MAPK-related genes (RSK and SH3BP5), Ras superfamily genes (RAP1GA1, RHOC and ARHGDIA), AP-1 family and related genes (FRA2, ATF3, FOS, JUN and RIP140). They were classified into the genes up-regulated (CDKN1A CDC14, TRIB3, APPL, RSK, SH3BP5, RAP1-GA1, RIP140, FOS, ATF3 and FRA2) or down regulated (TP53I11, PRKCD, BRCA1, RHOC and ARHGDIA) on estrogen treatment. Among them, a total of six (three up and three down regulated) genes were analyzed by real-time quantitative RT-PCR. All genes examined here showed similar responses to treatments with E2, genistein, daizein and glycitein, and between DNA microarray and RT-PCR assays.

Such genetic and genomic evidences for estrogenic effects of phytoestrogen support the growing attention and use of them to improve the health quality of life.

9. PHYTOESTROGEN – METHODS FOR IDENTIFICATION AND QUANTIFICATION

Phytoestrogens offer various health benefits;since last few years various analytical methods are employed and developed for identification and quantification of different phytoestrogenic components in various food/plants, to understand its bioavailability and any toxic effects.

These methods can be divided into two main categories:

1) Based on the chromatographic separation: High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Capillary Electrophoresis (CE) and

2) Those not based on separation: Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MAL-DI-TOF-MS), UV and Infrared Spectroscopy and Immunoassay.

Two different reviews were carried out in 2002 by Wang *et al*., (in USA) and Wilkinson *et al*., (In UK)to compare the different methods available for phytoestrogen estimation in terms of their specificity, sensitivity, advantages, disadvantages, limitations and application in potential areas. Basic differences amongst different methods were showed in Table2.12.

Table2.12: Comparison of analytical methods for analysis of Phytoestrogen

Technique	Sensitivity	Specificity	Pros	Cons
GC–MS	50 fmol	High	High resolution	Complex work up
			Good for unknowns	Difficult chemistry
HPLC				
–UV (and DAD)	2 pmol	Moderate,	Good for soy food and conjugates	Low sensitivity
		better with DAD		Less specific
–Fluorescence	200 fmol	Good	Sensitive	Limited to fluorescent analytes
–ED (and array)	20 fmol	Better with detection array	Suitable for biological samples	Cannot determine novel compounds
–MS	1–500 fmol	High	Ease of use and sensitive	Limited chromatographic resolution
CE				
–UV (DAD)	50 fmol	Moderate,	High separation resolution	Limited injected sample volume
		better with DAD	Excellence mass sensitivity	Poor concentration sensitivity
–Fluorescence	1–5 fmol	Moderate	Sensitive	Limited fluorescent analytes
–ED	1–2 fmol	Moderate	Sensitive	Limited specificity
–MS	100 amol	High	Sensitive	Difficult interface
				Low resolution
UV and IR spectroscopy	NA	Fair	High throughput	Lack of specificity
MALDI-MS	100 fmol	High	High throughput	Lack of quantitation
Immunoassay	1–100 fmol	Good	High throughput	Cross reactivity

The range of detection limits reported varies widely between individual laboratories, but the best reported sensitivity they observed was as follows: immunoassay>HPLC–mass spectrometry=HPLC–multichannel electrochemical detection (coularray)>GC–single ion monitoring-mass spectrometry>HPLC–UV diode array>HPLC–single channel electrochemical detection.

In Korea, the study was carried out for identification of steroid hormones in pomegranate seeds (Punica granatum) using HPLC and GC – mass spectrometry. They observed that in case of HPLC, an isocratic elution method using 35% aqueous acetonitrile solution at 1.0 ml/min with photodiode-array (PDA) detection at 225 nm and 254 006Em was found to optimally separate and identify the steroid hormones from the pomegranate samples with a run time of less than 30 min. The GC/MS method was carried out having detection on a HP-1 (30 m length, 0.32 mm I.D.) with helium as carrier gas under the oven temperature control as follows: start 220 °C for 5 min, raising 5 °C per min, final 280 °C for 10 min. The study concluded that the HPLC and GC methods were successfully applied to the identification of steroid hormones in pomegranate samples. (Choi *et al*., 2005)

10. EFFECTS OF FOOD SUPPLEMENT ON SERUM GONADOTROPINS (FSH, LH), ESTRADIOL AND MRS

Dietary phytoestrogens are emerging as a valid alternative to estrogens in the treatment of menopause-related diseases, such as the climacteric syndrome, cardiovascular diseases, osteoporosis, dementia. (Mario *et al*., 2005)

The symptoms like hot flushes, night sweats, and vaginal dryness indicate the need for estrogen supplementation. Such women should try to have a good diet, take good daily supplements for vitamin and get plenty of exercise. (safemenopausesoultions.com)

Isoflavones and lignans also exhibit antioxidant properties. Especially Isoflavones exhibit cholesterol-lowering effects that may be protective against heart disease. Soy products rich in isoflavones and margarines with added phytosterols are in use in the diets of people with hypercholesterolemia. (Perspective: Phytochemicals and herbal supplements in health and disease)

Firenzuoli F and Gori L (2001) recommended a dose of 100-200 mg daily of soy isoflavones for treatment of menopause related depression and hot flushes and found about 70% positive clinical results in 226 patients.

Atkinson *et al*., (2005) in their study showed a positive effect of a red-clover derived isoflavone supplementation on bone density of post menopausal women compared with placebo. In this double blind, randomized, placebo-controlled trail n=177 post-menopausal women completed the study and intervention group was provided a daily dose of 26 mg biochanin A, 16 mg formononetin, 1 mg genistein and 0.5 mg daidzein for 1 year. Loss of lumbar spine bone mineral content and bone

mineral density was significantly (p=0.04 and p=0.03 respectively) lower in the women taking the isoflavone supplementation than in those taking the placebo. There were no significant treatment effects on hip bone mineral content or bone mineral density, markers of bone resorption, or body composition, but bone formation markers were significantly increased (p=0.04 and p=0.01 for bone specific alkaline phosphatase and N-propeptide of collagen type 1, respectively) in the intervention group compared with placebo in postmenopausal women.

Few researchers have suggested that the isoflavone extraction process may remove some component of the food that has synergistic effects in combination with isoflavones or that isoflavones may become inactivated during the isolation and purification process. (Atkinson *et al*., 2005)

Few Egyptian studies have reported that fenugreek was the most common herb used for the treatment of menstrual disorders. Fenugreek may help in management of PMS (premenstrual syndrome) by relieving pelvic congestion, breast tenderness, and weight gain by its diuretic effect. Its antihistaminic effect can relieve premenstrual tension. Its spasmolytic effect may relieve premenstrual gastrointestinal spasms. (Shadia ATY, 2012; El-Gilany *et al*., 2005; Assuit University Bulletin, 2002)

Japanese women (n=1106) aged between 35-54 years (all were premenopausal at the start of the study) were studied in 6 year prospective study on the effect of soy intake on hot flushes (Nagata *et al*, 2001a). A food frequency questionnaire (FFQ) was completed at the start of the study period and women were questioned on the occurrence of hot flushes at the end of the study period. Women (n=101) reported moderate or severe hot flushes. The incidence of hot flushes was inversely associated with consumption of soy products in terms of total soy product

intake (OR= 0.47, 95% CI 0.28-0.79, p=0.005) and isoflavone intake (mg/day) (OR= 0.42, 95% CI 0.25-0.72, p=0.002).

A group of Spanish postmenopausal women (n=190) were studied in a multicentre, prospective nonrandomized trial of the effects of an isoflavones dietary supplement on menopausal symptoms (Albert *et al*, 2002). Each subject received 35 mg isoflavones/day over four months and menopausal symptoms including sleep disorder, anxiety, depression, vaginal dryness, loss of libido and bone pain were assessed. Incidences of hot flushes were decreased in 81% of participants ($p<0.05$). All the other parameters studied also showed a statistically significant decrease. The study did not include a placebo group.

A study by Somekawa *et al* (2001) evaluated the effect of dietary isoflavones on menopausal symptoms of women in Japan. The women were assigned to two groups according to years since menopause (n=269 early postmenopausal, < 5 years since menopause; n=209 late postmenopausal, > 5 years since menopause). Each group was also subdivided according to isoflavone intake. Subjects reported weekly, monthly and yearly consumption of soy products. Menopausal symptom scores of palpitations and backache or aching joints were lower in early postmenopausal women with a higher isoflavone intake ($p< 0.05$). The severity of other menopausal symptoms tended to be less in the higher intake group although the differences were not significant. No differences in menopausal symptoms were reported between women in the late postmenopausal group.

A randomised, placebo controlled study by Brzezinski *et al* (1997) reported that following 12 weeks of intervention with a soy and flaxseed rich diet, both peri- and postmenopausal women (n=145) experienced a reduction in the severity of hot flushes (p=0.004) and vaginal dryness

(p=0.005). Similarly, Albertazzi *et al* (1998, 1999) reported that following 12 weeks of supplementation with 60 g soy protein (76 mg isoflavone aglucone/day), postmenopausal women (n=104) experienced a reduction ($p<0.01$) in moderate to severe hot flushes. There was no difference in vaginal maturation index. In addition, a double-blind, cross-over study in perimenopausal women (n=51) found that women consuming a soy protein isolate (34 mg isoflavones) twice daily showed improvements in the severity of menopausal symptoms and hypoestrogenic symptoms compared with those receiving the supplement once daily

A double-blind randomised placebo-controlled trial in postmenopausal women (n=177) given a soy isoflavone supplement (50 mg total isoflavones/day) for 12 weeks found a reduction in hot flush severity with the soy supplement (p=0.01) compared to the placebo. There was no change in endometrial thickness nor vaginal maturation index or vaginal pH with either treatment (Upmalis *et al*, 2000). Similar results were obtained in a double-blind randomised placebo-controlled trial in postmenopausal women (n=39) receiving 400 mg/day soy extract (50 mg total isoflavones/day) by Scambia *et al* (2000). Women receiving soy had a reduction in the number of hot flushes ($p<0.01$) and the severity of hot flushes and night sweats ($p< 0.001$). There were no oestrogenic changes in vaginal cytology, endometrial thickness or uterine artery pulsatility index.

In a double-blind placebo controlled study, Brazilian postmenopausal women (aged 45-55 years) were randomly assigned to daily treatments of an isoflavone supplement (33.3 mg isoflavones, n=40) or placebo (n=40) (Han *et al*, 2002). Menopausal symptoms were examined at baseline and

after 4 months of treatment. Isoflavone treatment significantly decreased menopausal symptoms ($p < 0.01$) versus control as measured by the Menopausal Kupperman Index.

Van der Weijer & Barentsen (2002) reported a 44% reduction (p_ 0.01) in the incidence of hot flushes in a double blind placebo controlled trial of postmenopausal women (n=30) ingesting isoflavones (80 mg/day) for 12 weeks. The Green climacteric scale score also decreased (13%) in the isoflavone treatment group compared with controls. The researchers included a 4 week blind placebo phase prior to the treatment phase to eliminate the placebo response.

In a study by Jeri (2002), a 48% reduction ($p < 0.001$) in hot flushes was found in a placebo-controlled double blind study of postmenopausal Peruvian women (n=30) ingesting isoflavones (40 mg/day) for 16 weeks. Studies of children who had been fed soybased formula as infants and who were followed through adolescence (Klein, 1998) and young adulthood (Strom *et al*., 2001) found no adverse reproductive or endocrine effects. *In vitro* and animal studies suggest that soy isoflavones may have immunologic and thyroid effects (Doerge and Sheehan, 2002; Sirtori *et al*., 2005). The Center for the Evaluation of Risks to Human Reproduction (CERHR) of the National Toxicology Program reviewed developmental and reproductive toxicity of both soy formula and genistein and concluded that available data were inadequate to determine whether soy formula has developmental or reproductive toxicity (Rozman *et al*., 2006*a*). The expert review panel expressed negligible concern for adverse effects in the general population consuming dietary sources of genistein (Rozman *et al*., 2006*b*)

11. KNOWLEDGE AND PERCEPTION OF PEOPLE REGARDING MENOPAUSE AND ITS MANAGEMENT

The menopausal transition is one of the most important transitions that women experienceduring lifetime. Knowledge of menopause may influence the management of this transition. It is considered as a key predictor ofHRT use. Expectations, experience, confirmation, regaining of balance and control,and freedom are suggested to affect how women experience the transition processes.Othertransitions such as social changes related to both family and work are usually ongoing at thesame time. The transition shouldnot be seen as a process that is the same for all women.

The attitudes of women to menopause arestrongly influenced by social, cultural and economicsettings in which they live and may also reflect thedifferences in modes of treatment for or perceptions of itssymptoms.Attitude towards menopause varybetween cultures and individuals.

A cross sectional study was conducted at Jinnah MedicalCollege Hospital, Karachi on 102postmenopausal women (Mean age=55.1 ± 10.1 years). Regarding their awareness of menopause, 97(95%) women had previously heard of menopause and 29.4% respondents had someknowledge of menopausal symptoms. Mostof these women had heard about it from elders in familyand friends. Only 4 (3.92%)respondents were aware of long term implications ofmenopause and only 2 (1.96%) knewabout Hormone replacement therapy from their health professionals. Majority ofwomen considered menopause as a positive (47%) orneutral change (39.2%) and indicated that it did not affecttheir relationship with their spouse and children.

Majority of women (94%) did not perceive menopause to be amedical condition but a natural transition. Thirty-six(35.29%) respondents were not sexually active. Decreaselibido and frequency was reported by 33 (32.3%)respondents while 25(24.5%) reported no change. Out of102, only four (3.92%) respondents wereaware of treatment of menopause. Only one respondentrealized that HRT could relieve menopausal symptomsand prevent long term health risks, while the rest had noknowledge of this aspect. Anotherreason for not having the knowledge on HRT was thatwomen were not advised by their health care provider touse HRT. (Malik HS, 2008)

Another study was conducted in Pakistan by Nusrat *et al* (2008) to determine the knowledge and attitude of women (n=727) towards menopause. The findings were 78.79% women had little knowledgeabout menopause, while 15.8% women knew about effects and symptom of menopause. 78.79% women considered menopause as a natural process, while 21.2 % perceived it as a disease, 83.42% women were happy about cessation of menses and they did not want to have menses again, while 16.57% women wanted to have menses again.36.84% women were bothered by menopausal symptoms but only 31.86% has consulted doctor.

Currently Shaheen *et al*., (2015) also documented that 65% women were without awareness regarding menopause and HRT.

The conclusion from the above studies, majority of women in Pakistan were unaware of menopausal symptoms and its health effects. Most of them considered it as a natural process of aging, though bothered by symptoms but did not go for consultation due to lack of awareness and poverty.

A community based cross-sectional house to house survey was conducted at Anjarakandy, India to know the symptomatology and perception of

reasons for menopause. The findings revealed none of the ladies had ever heard of any HRT but 70% had heard of calcium supplements which could be used in old age. 22.4% of ladies thought that the main reason for menopause was due to God. The subjects were interrogated again since these are explanations given by lay people and cannot be considered entirely incorrect. 9.3% did not know any specific reason for menopause. The others (69%) knew that the reason was due to physiological hormonal changes that occur in the female which leads to menopause. 11.2% did not feel any need to clean the external genitalia since they thought that menses had stopped by now. 52.3% used to clean it with plain water. Only 36.5% knew the exact way to clean the external genitalia. 22.4% did not feel any need to visit a doctor for the complaints since they thought it was not possible to treat these symptoms. The study concluded that disseminating health education for postmenopausal women is of prime importance in Indian scenario. (Broker *et al*., 2013)