

The study was designed and divided into four phases. The phase I constituted mapping of prevalence of menopause related symptoms in women aged 30-60 years of Vadodara, Gujarat. Phase II comprised the identification and quantification of phytoestrogen rich foods namely fenugreek seeds, pomegranate seeds, flaxseeds and elephant foot yam available in local market. Phase III was designed to assess the impact of quantified phytoestrogen supplementation at MRS and hormone levels. Since the supplementation was of natural food, it is easier to incorporate them in a routine diet on a daily basis without any hesitation. The phase IV was the dissemination of findings of phase II and III to the women from control group, to maintain an ethical aspect of the study.

### **Highlights of Phase I**

- All subjects (n=1000) were categorized as per STRAW (Stages of reproductive Aging Workshop) classification which revealed, 58.7% (587) had normal menstrual cycle pattern, 14.6% (146) was passing through perimenopause phase and 26.7% turned up to their post-menopause phase .
- The mean age of the study population was  $42 \pm 5.1$ ; where mean age of premenopausal group was  $40.07 \pm 3.79$ , perimenopausal group was  $41.72 \pm 4.28$  and post menopausal group was  $46.4 \pm 5.32$ .
- Their education profile revealed, 30-36% had completed primary or secondary level schooling and only 3% had completed their graduation, though majority of the women (79%) were working as a maid, salesgirl, peon, gruhadyuog, etc. The distribution of work (profession) is same across all the menopausal phases ( $p=0.212$ ). The general work situations did not bring any specific impact on reproductive health in study population.

- The younger population was more educated than the elders; this indicates that the current generation is more aware and concerned with respect to their education ( $p < 0.001$ ) which may bring impact on healthy lifestyle.
- Looking to their family history, 47% had  $\leq 2$  children and 45% had 3-4 children (Table 3, Fig. 3) and half of the women, 53.6% had experienced pregnancy  $\geq 3$  times (Table 3, Fig. 4). The parity numbers were high amongst elderly ( $p < 0.001$ ).
- The self reporting medical history were also collected from the women, which revealed 2.5% suffered from thyroid disorder, 25.3% suffered from blood pressure and amongst all, only 14.4% were on medication.
- 80.8% women were aware and had the knowledge about menopause, but very few knew about what is hormone replacement therapy (0.9%), how the menopause is coupled with thyroid hormones (0.6%) or bone health (1.2%).
- It was a miserable feeling to understand that, almost all the women had never heard about phytoestrogen and only 31.5% knew about what is calcium.
- Symptoms for MRS:  
The women ( $n=1000$ ) reported the pain in hands or legs was the most prevalent (73.6%), followed by anxiety (67.4%), and physical and mental exhaustion (66.8%). The other symptoms like dryness of vagina (48.5%), heart discomfort (45%), mood swings (35.5%) and irritability (32.8%) were reported by nearly half of the population. The other symptoms like sleep disturbances (24.5%), hot flashes (22%), and bladder problems (9.3%) were reported by less number of women.
- The severity of symptoms was increased for hot flashes, mood swings, anxiety, physical and mental exhaustion, pain in hands and legs, swelling, weight

fluctuations, constipation and visual problems during transition from perimenopause to post menopause.

- Amongst other symptoms related with menopause, hair loss was reported by 82.8% women, irregular menses by 41.2%, swelling by 17.3%, weight fluctuation by 16.3%, constipation by 13.9%, visual problem by 13.1% and only 2.1% women were reported with nails cracking. Among these hair loss and constipation experienced were at moderate frequency, rest all experiences were on mild frequency.
- The mean of total MRS was found to be stepping up during transition from premenopause ( $4.60 \pm 3.07$ ) to perimenopause ( $6.53 \pm 3.93$ ) and showed again a step down during transition from perimenopause ( $6.53 \pm 3.93$ ) to post menopause ( $5.78 \pm 3.03$ ). A similar pattern was observed for mean of all three dimensions i.e., Psychological ( $2.20 \pm 1.73$  to  $3.05 \pm 1.90$  and  $3.05 \pm 1.90$  to  $2.81 \pm 1.71$ ), Somato-vegetative ( $1.64 \pm 1.24$  to  $2.40 \pm 1.7$  and  $2.40 \pm 1.7$  to  $2.07 \pm 1.31$ ) and Urogenital ( $0.77 \pm 0.88$  to  $1.08 \pm 1.09$  and  $1.08 \pm 1.09$  to  $0.89 \pm 0.90$ ).
- Between the three subscales of MRS, the mean of the Psychological domain ( $2.59 \pm 1.91$ ) was higher followed by somatic symptoms ( $2.03 \pm 1.54$ ) and the least by the Urogenital symptoms ( $0.92 \pm 0.99$ ). A similar trend was observed for all three subscales during all stages of reproductive life.
- Amongst three sub scale, psychological symptoms were more profoundly affect the women's health, irrespective of menopausal phase.

## Highlights of Phase II

- The HPLC limit of detection was different for each analyte: for isoflavones (daidzein and genistein) 1µg/g and for lignans (secoisolariciresinol, matairesinol) and coumestrol it was 2.5µg/g.
- HPLC analysis of fenugreek seeds revealed the presence of isoflavones: daidzein-18.2ppm; genistein-11.8ppm and lignans: secoisolariceresinol-283.6ppm in non hydrolyzed extract. The presence of isoflavones: daidzein-100.9ppm; genistein-56.1ppm; lignans: secoisolariceresinol-1893ppm and coumestrol -170ppm in hydrolyzed extract of fenugreek seeds.
- The non hydrolyzed extract of pomegranate confirmed the presence of isoflavones: daidzein- 7.82ppm and in hydrolyzed extract isoflavones: daidzein-31.23ppm; genistein-30.46ppm; lignans: metairesinol-562.34ppm.
- The non hydrolyzed extract of flaxseeds did not confirm the presence of any interested analytes and in hydrolyzed extract isoflavones: daidzein-3.27ppm; genistein-29.36ppm; lignans: secoisolariceresinol-1067.4ppm.
- The non hydrolyzed extract of yam confirmed the presence of isoflavones: genistein-24.30ppm and in hydrolyzed extract isoflavones: daidzein-21.62ppm; genistein-150.76ppm; lignans: secoisolariceresinol-1216.28ppm.
- There is an improvement in quantification of phytoestrogens following alkaline hydrolysis.
- Amongst all food samples fenugreek seeds ranked highest for total phytoestrogen content (2.22 mg/g), followed by elephant foot yam (1.38 mg/g), flaxseeds (1.1 mg/g) and the least was reported for pomegranate seeds (0.62 mg/g).

### Highlights of Phase III

- The mean age of EG1 was  $40\pm3.3$ , EG2 was  $40.7\pm3.1$  and CG was  $40.6\pm3.5$ . The distribution of women was same across three groups, there was no any significant difference amongst women of three groups for age ( $p=0.499$ ).
- Mean $\pm$ SD for weight in EG1, EG2 and CG were  $62\pm7.4$ ,  $61.3\pm7.5$ , and  $63.2\pm7.4$  respectively before intervention; which were reduced significantly to  $60.6\pm6.9$  ( $p<0.001$ ) in EG1,  $60.2\pm7.4$  ( $p<0.001$ ) in EG2 and non-significantly to  $63.1\pm7.5$  ( $p=0.6$ ) in CG.
- For BMI, EG1 showed a significant reduction from  $25.9\pm3.4$  to  $25.3\pm3.2$  ( $p<0.001$ ) and EG2 from  $25.5\pm2.8$  to  $24.8\pm3.2$  ( $p<0.01$ ). No change was observed for BMI in control group.
- Waist circumference was significantly reduced in all three groups; in EG1 – from  $89.67\pm4.7$  to  $88.65\pm5$  ( $p<0.01$ ), in EG2 – from  $90.80\pm4.5$  to  $90.12\pm4.4$  ( $p<0.001$ ) and in CG – from  $90.5\pm4.5$  to  $90.06\pm4.8$  ( $p<0.001$ ).
- The nutritional status of women based on their BMI revealed: in EG1 - 19 (39.6%) women were normal, 27 (52.1%) were overweight and 4 (8.3%) were obese, similarly in EG2 19 (38.8%) were normal, 29 (59.2%) were overweight and 1 (2%) were obese; and in control group 18 (37.5%) were normal, 27 (56.2%) were overweight and 3(6.2%) were obese at baseline
- The supplementation results showed the significant improvement in all symptoms accounted for MRS in experimental groups; the sleep problems, hot flashes, bladder problems, anxiety, pain in hands and legs were improved after nutrition and physical health education.

- There was no significant difference in mean MRS at baseline amongst all three groups, while after supplementation there was significant difference in mean MRS ( $p<0.001$ ). Between the groups analysis revealed a significant difference in mean MRS between EG1 - CG ( $p<0.001$ ) and EG2 – CG ( $p<0.001$ ) while no significant difference between two experimental groups ( $p=0.508$ ).
- The pomegranate supplementation showed significant improvement in Hb, serum FSH, LH and Estradiol level ( $p<0.001$ ).
- The yam supplementation showed significant improvement in Hb ( $p<0.05$ ) and serum FSH, LH and Estradiol level ( $p<0.001$ ).
- The control also showed significant improvement in serum FSH ( $p<0.01$ ) and LH ( $p<0.001$ ).
- The post hoc analysis revealed there was a significant difference in improvement for Hb ( $p<0.05$ ) and serum estradiol ( $p<0.01$ ) between Experimental groups and control group. For serum estradiol a significant difference was also observed between EG2 and CG ( $p<0.001$ ).
- For thyroid hormone profile (T3, T4, and TSH), the intervention did not show any significant changes from baseline values in all study groups, except the mean TSH was significantly increased in EG2 (from  $3.51\pm2.68$  to  $3.76\pm2.43$ ).
- The knowledge and perception level of women were poor irrespective of study groups; and physical and nutrition health education were bring significant improvement in their knowledge and perception level.
- Similarly, after an intervention, the consumption frequency of phytoestrogen, iodine and iron rich foods were increased from

baseline. The responses were not differing amongst experimental and control groups – at baseline or after the intervention.

- The study supports the acceptability and effectiveness of elephant foot yam and pomegranate for alleviating menopausal symptoms and to improve the serum FSH, LH and estradiol level to a lesser extent.