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METHODOLOGY

The research methodology pertaining to the study on "**Effect of Nutrition Intervention on Symptoms of PMS (PMS) among Women of Reproductive Age (20-45 Years)"** is presented under the following phases.

PHASE-I: Identification of participants for intervention studies

A. Selection of area
B. Selection and screening of participants having PMS
C. Formulation oftools to conduct the study

PHASE -II:Mapping of participants for nutrition intervention

A. Conduct of the survey
B. Assessment of the nutritional status of the selected participants
C. Assessment of the psychological profile of the selected participants
D. Recording the symptoms of PMS using Premenstrual Daily Symptom Diary and
E. Assessment of knowledge related to PMS

PHASE - III: Formulation, sensory evaluation and nutritional analysis of health mix cookies for dietary intervention

A. Selection of ingredients and formulation of health mix
B. Sensory and nutritional evaluation of health mix
C. Preparation of cookies with the best scored health mix
D. Analysis of the physical qualities, nutrient content, microbial count and cost effectiveness of the health mix cookies

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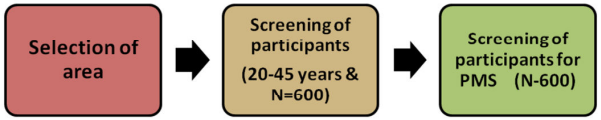
A. Impact of dietary intervention on the nutritional status and symptoms of PMS among the selected participants
B. Impact of nutrition and health education on the knowledge of PMS among the selected participants

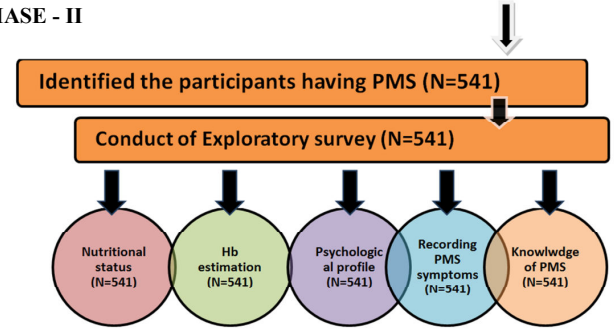
PHASE - V: Data Analysis and Interpretation

RESEARCH DESIGN

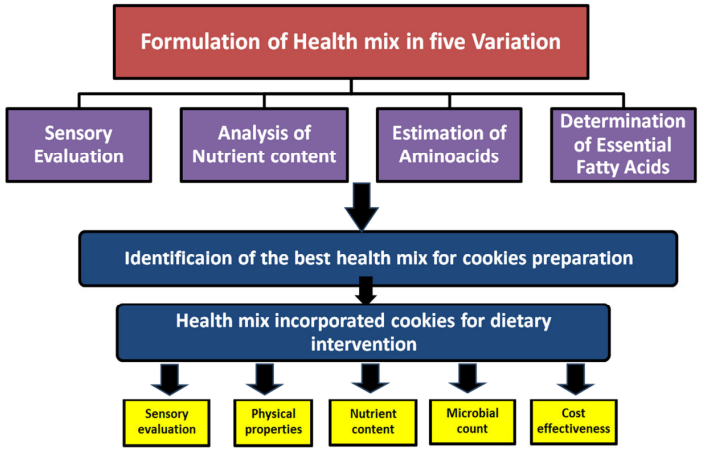
PHASE - I



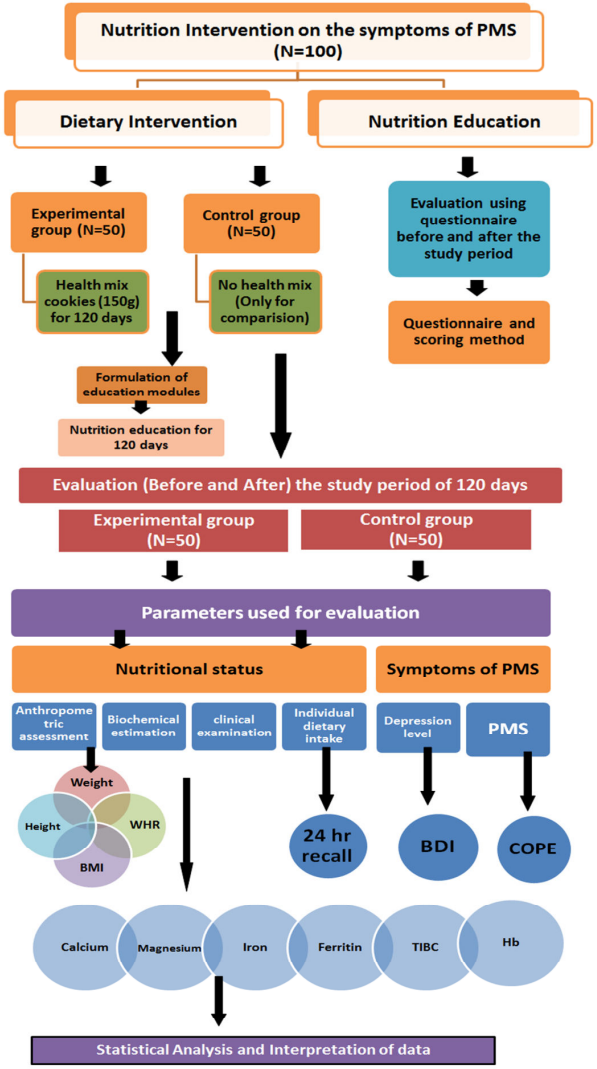
PHASE - II



PHASE - III



PHASE - IV



PHASE – I

Identification of participants for intervention studies

A. Selection of area

The target group of reproductive age women (20-45 Years) was selected from the prominent residential areas of Sulur, Coimbatore District. Coimbatore is an expanding city with a total population of 34, 58,045 and a female population of 17, 28,748 with a female sex ratio of 964 (Census of India, 2011). Sulur is a taluk area located at 11^0 2' 0" North, 77^0 8' 0" East in Coimbatore Urban District of Tamil Nadu (Figure-2). This area was selected on the basis of easy accessibility, availability of adequate number of participants and the cooperation rendered towards the study by the participants, since the assessment of PMS was recorded for two to three months. Sulur has a total female population of 1, 21,157 (Census of India, 2011). Total female population (15-49 years) in Sulur town was 6061.

Figure-2 Suleur area map

B. Selection and screening of participants having PMS

Premenstrual Syndrome (PMS) refers to a cluster of physical, mood and cognitive symptoms that occur during the luteal phase (from 14 to 28 days of menstrual cycle) of menstrual cycle and subsides with the onset of menstruation. Nearly 80 per cent of the women of reproductive age, suffer from premenstrual physical and emotional change that occurs between ovulation and first day of menstrual bleeding, stop after bleeding and are not present for at least seven days after the menstrual period (ACOG, 2014).Up to 70-90 per cent of reproductive age women have one or more signs of physical discomfort or emotional symptoms in the menstrual phase i.e. the luteal phase of menstrual cycle. About 20-40 per cent of the menstruating women have PMS that are bothersome. A small

number up to eight per cent experienced more severe symptoms which lead to substantial distress or functional impairment and are referred to as PMDD (Halbreich *et al.,* 2013). According to Ussher and Perz (2013) PMS affects women in their age of 20s-30s. Hence, the investigator selected adult women of reproductive age group (20-45 years) as participants for the present study. Table-III highlights the Criteria for selection of participants for Phase-I

Table-III

Criteria for selection of participants

Inclusion criteria	Exclusion criteria
• Adult women of reproductive age (20-45 years) • Willing to participate and extend their co-operation for the study • Who are literates • Ready to give written consent for the intervention	• Adult women above the age of 45 years and less than 20 years • Pregnant and lactating mothers • Who have undergone hysterectomy • Who are illiterates • Who have not given the written consent • Who are severely anaemic

On the basis of the inclusion criteria, a quick screening of PMS was carried out among a representative sample of 600 reproductive age women using American College of Obsteristics and Gynaecology (ACOG) diagnostic criteria (2000). Based on the ACOG Diagnostic Criteria (Appendix-I), those who have PMSwere selected for the study. Convenience sampling refers to the fraction of population being investigated which is selected neither by probability nor by judgement but byconvenience (Gupta, 2016).Based on the formula given below, the sample size was calculated and it was 595.

$$N = \frac{Z^2 P (1-P)}{(d)^2}$$

where N= sample size; P= expected prevalence or proportion; d =precision

Hence the number of participants selected for the study was 600 and ACOG diagnostic criteria for PMS was used to find out the prevalence ofPMS. A total of 541 adult women of reproductive age group (20-45 years) had PMS. The selected participants were given orientation regarding the protocol of the study and also explained modalities

and purpose of the study. They were also informed about the dietary intervention and nutrition education and the follow-up to be carried out for four months for the assessment of nutritional and health status.Prior to the actual conduct of the study, a good rapport was established among the selected participants and their family members through proper counselling. The purpose and procedure involved in the study were clearly explained to the selected participants and their family members were also effectively motivated through an interactive session by the investigator to extend their full co-operation for successful conduct of the study.

C. **Formulation of tools to conduct the study**

A Questionnaire schedule was prepared to collect demographic profile related to family background, age, educational, occupational, economic and marital status, dietary pattern, medical history mainly related to the reproductive health and knowledge on the causes, prevalence, symptoms, problems and treatment of PMS and anxiety level of the participants (Appendix-II).

The specially designed questionnaire was validated by medical and nutrition experts for its contents and the necessary changes were carried out on the basis of the suggestions given by the experts. Tamil and English version of the questionnaires were tested by parallel form questionnaire method among ten participants for the reliability of the questionnaire and the Tamil version was similar to English questionnaire with a score of r=0.926.

All the selected participants were clearly instructed to record the daily symptoms, at the same time itself in the form given to them. This was supervised by the investigator regularly by checking the entries made by the selected participants in the Premenstrual daily symptom diary (PMDD) through periodical visits and this was systematically followed through two menstrual cycles (three months) in order to obtain accurate and reliable data related to PMS.

The study design was registered in the Clinical Trial Registry of India (CTRI), ICMR. CTRI registration number is **REF/2013/005727.** To conduct nutrition intervention, the methodology of the present study was presented beforethe Institutional Human Ethical

Committee (IHEC) of Avinashilingam University and the approval number **AUW/IHEC-13-14/FHP-01** was obtained**.**

PHASE – II

Mapping of participantsfor nutrition intervention

A. Conduct of the survey

Demographic profile, dietary pattern, the personal and family medical history were collected from the selected participants (N=541) using the specially designed questionnaire (Appendix-II). Background information such as age, sex, type and number of members in the family, educational qualification, marital status, occupation and income level were collected.

Dietary pattern such as type of food items, inclusion of foods, consumption of coffee, tea, health drinks and other beverages were collected. The details of family medical history, frequency of health problems including reproductive health problems and the complications were gathered and recorded carefully.

B. Assessment ofnutritional status of the selected participants

Assessment of nutritional status of the selected participants included medical, social and dietary history, anthropometric data, biochemical data, clinical evaluation and review of drugs used (Weigley *et al,* 2014). In the present study, nutritional status of the selected participants was assessed through anthropometric measurements, clinical examination, biochemical estimation and dietary intake.

a. Anthropometric measurements

Anthropometry is the universally acceptable, inexpensive and non-invasive procedure. It is the measurement of the body's physical dimensions. The physical dimensions are used to develop an understanding of individual's nutritional status. The anthropometric data including height, weight, BMI and waist/ hip ratio of the selected participants (N=541) were recorded carefully to obtain reliable data.

Plate - 1 Recording anthropometric measurements

i. **Height:** Height is a stable measurement of growth as opposed by body weight. Weight reflects only the present health status of an individual whereas, height indicates the events in the past also (Park, 2017). For recording height of the selected participants, non-stretchable measuring tape was fixed to the wall and the participants were asked to stand erectly on the flat floor with feet parallel and with heels, buttocks, shoulders and back of the head touching the wall. The head was held comfortably erect and arms hanging at the sides in a natural manner. A head piece (scale) was lowered, crushing the hair and making contact with the top of the head and height was recorded to the nearest 0.5 cm. This process was repeated thrice and the consistent reading was obtained (Plate-1). Height of the selected participants was recorded in centimetre to the nearest millimetre (ICMR, 2015).

ii. **Weight**: Weight is the most widely used simplest reproducible anthropometric measurement for the evaluation of nutritional status of the population. It is a measure of total mass and hence, it is sensitive to the changes in body fluids, fats, muscle mass, skeleton and other organs (Gopalan and Chatterjee, 2010). Weight gives the present nutritional status of an individual. Weight was recorded in the morning after evacuation of bladder with minimum clothing, while standing in upright position (Rey *et al*, 2008).

The investigator used a sturdy portable digital weighing balance to record the weight of participants. Weight was recorded accurately to the nearest 0.1 kg. and precautions suggested were followed while taking the weights. The electronic digital weighing machine was checked periodically using standard weights while recording the weight of the selected participants.

iii. **Body Mass Index:** Several indices and ratios were derived from anthropometric measurements. The most commonly used, well known indicator of body fatness is the Body Mass Index. According to WHO, Body Mass Index (BMI) is a simple index of height and weight that is commonly used to classify the different grades of malnutrition. BMI is calculated using the formula

$$BMI = \frac{Weight(kg)}{Height(m^2)}$$

After the computation of BMI, selected participants were classified according to the norms given by ICMR (2011), as mentioned in the Table-IV**.**

Table- IV

Grades of Malnutrition

BMI classification	Grade of malnutrition
Underweight	<18.50
Severe thinness	<16.00
Moderate thinness	16.00 - 16.99
Mild thinness	17.00 - 18.49
Normal range	18.50 - 24.99
Overweight	≥25.00
Pre-obese	25.00 - 29.99
Obese	≥30.00
Mild Obese class I	30.00 - 32.49
Moderate Obese Class I	32.50 - 34.99
Mild Obese class II	35.00 - 37.49
Moderate Obese Class I	37.50 - 39.99
Obese class III	>40

(Source: NIN Methodologies for fitness assessment, ICMR, 2011)

BMI is a measure of the body weight relative to height that is associated with body fat and health risk. As a measure of relative weight, BMI is easy to obtain. It is an acceptable proxy for thinness and fatness, and has been directly related to health risks and death rates in many populations (Nishida *et al.,* 2004). BMI was computed carefully for the participants.

iv. Waist/Hip Ratio

Waist-Hip Ratio (WHR) is used as the best indicator along with the BMI values to measure the health status of an individual and also measure the risk of health problems.Scientific data of the research revealed that people with ‘apple shaped’ bodies face more health risks than those who are with ‘pear shaped’ bodies. WHR used as a measurement of obesity, is a possible indicator of other serious health conditions in recent

past, with the increasing incidence of obesity, considering the incidence of abdominal adiposity in diet related chronic diseases, waist-hip circumferences were used to assess the abdominal adiposity in selected participants (WHO, 2008).

The Waist-Hip ratio (WHR) is the ratio of the circumference of waist to that of hip. The waist circumference should be measured at the midpoint between the lower margin of the last palatable rib and the top of the iliac crest, using a stretch-resistant measuring tape. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor. For both measurements, the selected participants were asked to stand with feet close together, arms at the side and body weight evenly distributed and with little clothing. The selected participants were made to relax, and the measurements were taken at the end of a normal expiration. Each measurement was repeated thrice. Measurements were within one cm of accuracy, the average was calculated and if the difference between the two measurements exceeded one cm, the two measurements were repeated.

Waist Hip Ratio (WHR) was calculated using the formula

$$WHR = \frac{\text{Waist circumference(cm)}}{\text{Hip circumference(cm)}}$$

According to the guidelines (ICMR, 2011), the cut-offs for WHR is 0.90 for Indian man and 0.80 for Indian woman.

b. Biochemical estimation

Biochemical estimation is the most sensitive indicator of the health status of an individual. Compared to other methods, biochemical estimation provides the most objective and quantitative data on nutritional status. The usefulness of biochemical tests is to provide indications of nutrient long before clinical manifestations and signs appear (FSAU, 2004).

Haemoglobin estimation was carried out by cyanomethaemoglobin method for all the participants (N= 541). Twenty micro litres of finger prick blood samples by skin puncture technique using sterile lancet needles were collected into standard micropipettes (Plate-3) and transferred to Whatman No.1 Filter Paper, having identification particulars of blood sample and spread over an area of 1-1.5 cm. diameter. Papers were dried in shade

and placed in a cover. Each twentieth sample was collected in duplicate to check variation. The collected samples were used for haemoglobin estimation.

C. Assessment of the psychological profile of the selected participants

Health is defined as the condition of complete physical, mental and social well-being and not merely free from illness or infirmity (WHO, 2010). Based on the references, the present study indicates that the presence of anxiety, low self esteem or depression has a great impact on PMS symptoms. Psychological status of the selected participants was evaluated using the anxiety level, self esteem and depression levels.

a. Assessment of anxiety level –It is measured using State Trait Inventory and state trait inventory-I, STI and STI-I form (Spielberger and Sydeman, 1994), which differentiates between temporary or emotional state anxiety versus long standing personality trait anxiety in adults. The State-Trait Anxiety Inventory (STI) is a psychological inventory based on a 4-point Likert scale and consists of 40 questions on a self-report basis. The STI-I measures two types of anxiety - state anxiety or anxiety about an event, and trait anxiety or anxiety level as a personal characteristic. Higher scores were positively correlated with higher level of anxiety. The average maximum score was 4. UsingState anxiety (S-anxiety) and Trait anxiety (T-anxiety) the anxiety level of the selected participants of 541 was carefully recorded and assessed.

b. **Assessment of self-esteem** – Self-esteem was done based on Rosenberger'sInventory for self-esteem. It contains four point Likert scale with positive and negative statements. A 10-item scale that measures global self-worth by measuring both positive and negative feelings about the self. The scale is believed to be uni-dimensional. All items were answered using a 4-point Likert scale format ranging from strongly agree to strongly disagree (Rosenberg, 1965). The scale was administered to the selected participants (N=541) and the relevant data was collected.

D. Recording the symptoms of PMS

Premenstrual Daily Symptom Diary was used to find out the symptoms of PMS. It is a table with list of 21 common symptoms of PMS and graduated with 30 columns for

entry of symptom presence as numbers (1- no symptom, 2-mild symptom, 3-moderate symptom, 4 –severe symptom). The selected participants (N=541) were instructed to make an entry at the end of each day to note the different types of symptoms. Average score of all symptoms marked only in the luteal phase of the menstrual cycle for three consecutive monthswere considered as symptoms of PMS.

E. Assessment of knowledge related to PMS

The questionnaire consists of 10 questions of multiple choice type with one correct answer, carries one mark for each question and are related to meaning, causes, prevalence, age group affected, complications and treatment.

Grading the marks was done based on the formula

Good < average score + sigma

Average = (average score - sigma) - (average score + sigma)

Poor > average score – sigma (Bhatacharya , 2006)

The average score was arrived and from that the marks, good and poor werefixed.

PHASE - III

Formulation, sensory evaluation and nutritional analysis of health mix cookies for dietary intervention

Food supplementation is one of the most effective methods of promoting health and preventing or combating health problems, to reach some or all population.Supplementary foods were formulated on the basis of low cost, locally available foods, familiar to home maker and easy for preparation. These aspects helped to develop a suitable health mix to meet the nutritional support for the prevention of the signs and symptoms of PMS. Keeping all the points in mind, the investigator formulated macro and micro nutrient rich health mix for dietary intervention.The formulated health mix was valuable for its Calcium and Magnesium content apart from the macronutrients contribution.

A. Selection of the ingredients and formulation ofthe health mix

List of Calciumand Magnesiumrich food items were selected to formulate the health mix for dietary intervention. Table-IIIand Figure-3 depicts the food items used in the formulation of health mix.

Table- V

Food items used in the formulation of health mix

Ingredients	Botanical Name	Active Constituents	Action	Responses
Sprouted ragi	*Eleusine coracana*	Iron and Calcium	Improving blood Iron and Calcium level	Reduces the symptoms of PMS like cramps, fatigue
Rice flake	*Orysa sativa*	Iron	Readily absorbed when taken with Vitamin C	Reduces fatigue
Sprouted horse gram	*Macrotylom auniflorum*	Rich in Iron, Calcium Molybdenum, Polyphenols	High antioxidant property, rich in EFA and EAA	Polyphenols help to reduce weight gain, fatigue, cramps
Roasted Bengal gram	*Cicer ariertinum*	Rich in Protein and Iron	Readily digested and enhances Iron absorption	Reduces the symptoms of PMS
Soya bean	*Glycine max*	Rich EFA, EAAs, Poly phenols, Protein	Maintains adequate protein levels	Reduces overall symptoms of PMS
Gingelly seed	*Seasmum indicum*	Rich in EFA , Iron and Calcium	Improving blood Iron and Calcium levels	Reduces symptoms of PMS like cramps and fatigue
Jaggery	*Caryota urens*	Iron	Improving blood Iron and Calcium Level	Reduces symptoms of PMS like cramps and fatigue

Figure – 3.Ingredients used in the formulation of health mix

i. Pre-treatment of the ingredients

Ragi and horse gram were germinated to enhance the nutrient content. Then it was sun dried and roasted. The other ingredients were slightly roasted. The quantity of ragi was gradually increased from 20 to 60 grams to increase Calcium content of the health mix and proportionately the quantity of rice flake content was reduced from 40 to10 grams. All the other ingredients were kept constant except jaggery and gingelly seed which were slightly altered. Horse gram, roasted Bengal gram dhal and soya were kept in constant quantity to avoid digestive problems due to regular consumption of health mix in the form of cookies during dietary intervention. The ingredients were mixed in various combinations and ground into fine flour for the preparation of health mix. Quantity of ingredients used in each of the variations is given in Table –VI.

Table-VI

Ingredients used in the formulation of health mix

Ingredients	Variations (g)				
	I	II	III	IV	V
Sprouted ragi	20	30	40	50	55
Rice flake	40	30	20	10	15
Jaggery	20	10	20	20	10
Gingelly seed	5	15	5	5	5
Sprouted horse gram	5	5	5	5	5
Roasted Bengal gram	5	5	5	5	5
Soya	5	5	5	5	5
Total	100	100	100	100	100

The steps involved in the formulation of the health mix and cookies are given in Figure -4.

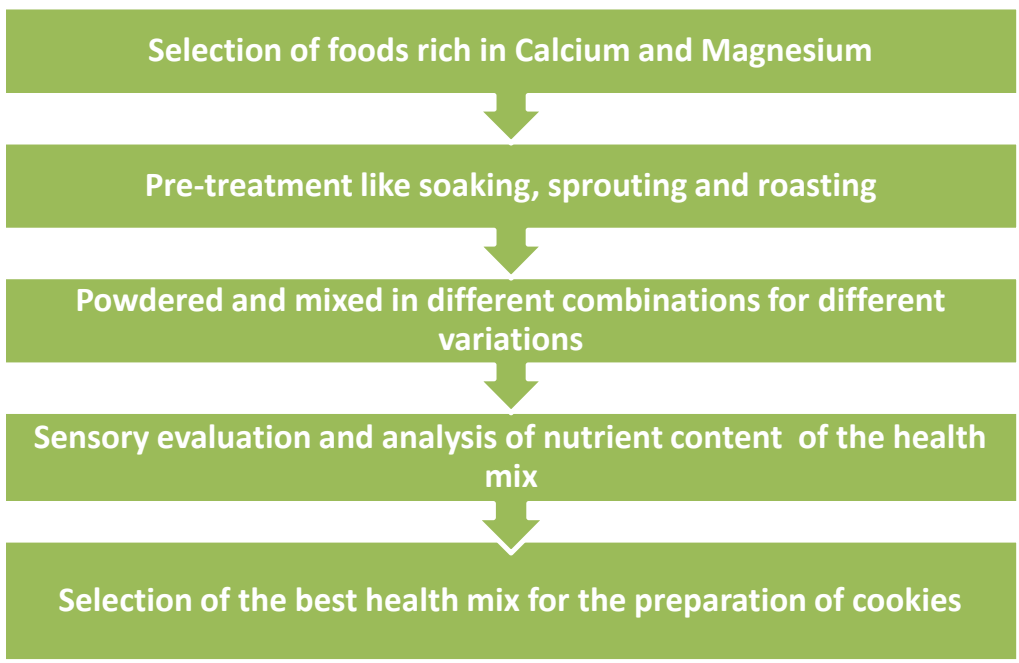


Figure- 4 Steps involved in the formulation of health mix

B. Sensory and nutritional evaluation of the health mix

Sensory evaluation is a multi disciplinary science that uses human panellist's senses of sight, smell, taste, touch and hearing to measure the sensory characteristics and acceptability of the products. Thus, quality of food is judged in terms of appearance, colour, taste, texture and doneness which are considered as key factors in successful product development (Chandrasekar, 2002). Steps involved in the sensory evaluation of the health mix were discussed in the following paras.

i. Selection of taste panel members

A taste panel is a group of assessors chosen to participate in a sensory test. To avoid errors due to physical, psychological, envIronmental and individual characteristics, a panel of assessors is used rather than a single assessor. Twenty semi skilled personnel were selected on the basis of their health, co-operation, willingness and knowledge of sensory analysis and also the ability to discriminate the various criteria for sensory evaluation.

ii. Formulation of the score card

Scoring is a form of rating of the prepared food using a numerical scale where the numbers form an interval or ratio scale was demonstrated mathematical relationship to each other (Piggot, 1988). A seven point hedonic scale was formulated for the acceptance of appearance, colour, taste, flavours and texture and grades were given according to the degree of acceptance by the selected taste panel members (Appendix-IV).

iii. Formulation and evaluation of health mix

Fifteen variations selected for acceptability trial were prepared at different point of time and presented to the panel members for sensory evaluation. Five variations which obtained high acceptability scores were chosen for the nutrient analysis. The five variations were once again given to the panel members; invite all the panel members at a time to score for the organoleptic qualities of the five variations. So as to prevent a biased result the remarks were recorded. Acceptability trials were carried (for the five variations) thrice to obtain more

reliable results and were considered for further standardization of recipes to prepare cookies. The health mix was prepared using nutrient rich ingredients and their nutritive value, sensory attributes, amino acid profile and essential fatty acid profile were analysed.

iv. Sensory evaluation

Sensory evaluation was carried out using 7 Point Hedonic Scale and presented in Table-VII.

Table-VII

Sensory evaluation scores of the health mixes

Sensory attributes	Mean scores of different Variations				
	I	II	III	IV	V
Appearance	5.46	6.80	5.78	6.66	4.71
Colour	6.0	6.0	5.84	5.75	4.84
Flavor	5.16	6.16	5.16	5.9	4.15
Taste	6.0	5.98	5.96	3.83	4.93
Texture	6.2	6.6	6.0	3.95	4.95
Overall acceptability	**28.62**	**32.58**	**28.64**	**26.09**	**23.58**

Sensory evaluation of the health mix cookies is presented in Table-V. Among the various variety of health mixes, Variation II secured the highest score for all sensory attributes except for taste which was secured second.

v. Analysis of nutrient content, amino acids and essential fatty acids profile

Five variations of health mixes were considered for the nutrient analysis, using Standard methods (NIN, 2011). Table-VIII presents the nutritive value of the health mix.

Table-VIII

Nutritive value of formulated health mix***

Nutrient	Variations(100g)				
	I	II	III	IV	V
Energy (Kcal)	271.5	296.5	282.6	261.4	286.2
Protein (g)	27.82	33.04	20.05	26.10	18.25
Fat (g)	5.087	4.223	3.986	4.361	3.989
Calcium (mg)	613.3	707.2	360.5	703.8	702.3
Magnesium (mg)	673.3	881.3	630.2	666.8	792
Iron (mg)	9.0	7.18	6.01	6.1	9.01
Vitamin –A (µg)	667	337	602	804.5	905
Thiamine (mg)	0.575	0.756	0.854	0.934	1.02
Riboflavin (mg)	1.255	0.845	0.599	2.464	1.02
Niacin (mg)	4.49	3.801	2.733	3.026	5.251
Vitamin-C (mg)	13.07	10.77	17.877	10.25	10.927

***Procedure given in Appendix

The analysed nutrient content of the formulated health mixes in five variations is presented in Table- VIII. It was noted that Variation –II had the highest levels of Calcium, Magnesium and protein content and considered for the preparation of cookies.

vi. Estimation of Amino acid and Essential Fatty acid

Essential amino acids play a major role in maintaining health status. They are involved in the synthesis of serotonin that helps in reducing emotional problems in the luteal phase. The amino acids and essential fatty acids profile of the five variations is presented in Table-IX. Amino acid profile of the five variations of the health mixes shows that the Variation II had highest score for alanine, isoleucine, phenyl alanine, histidine, leucine, threonine, tryptophan and valine. Methionine was scored third in Variation-II. From the literature study, it is also evident to note that the essential fatty acids like linoleic acid and gamma linoleic acid are helpful in reducing the PMS symptoms. These amino acids are highest in Variation II. Hence the amino acid profile of the Variation II was suitable for the preparation of cookies used in the intervention study.

Table-IX

Amino acidsand essential fatty acids profile of the formulated health mixes***

AA/EFA	Variations(100g)				
	I	II	III	IV	V
Amino acids (g/100 g)					
Alanine	1.223	1.787	0.9034	8.754	1.04
Isoleucine	0.784	1.02	0.6762	0.7343	0.992
Phenyl alanine	0.345	0.763	0.313	0.5453	0.445
Histidine	0.196	0.234	0.184	0.1676	0.229
Leucine	0.113	0.193	0.201	0.2034	0.203
Methionine	0.443	0.374	0.313	0.1893	0.543
Threonine	0.194	0.378	0.154	0.1435	0.215
Tryptophan	0.201	0.354	0.173	0.1734	0.235
Valine	0.198	0.304	0.191	0.408	0.304
Essential fatty acids					
Alpha linoleic acid (**g/100g**)	0.7834	0.9453	0.7724	0.8734	0.7743
Morotic acid(g/100g)	0.7434	0.8945	0.6734	0.5045	0.7394
Ecosa pentanoic acid (mg/100g)	145.7	156.0	141.2	152.5	98.4
Decosa hexanoic acid (mg/100g)	72.5	78.5	68.2	74.5	60.5

***Procedure given in Appendix

Based on the nutrient content, aminoacids and essential fatty acids profile, Variation-II was selected for the preparation of cookies which was used for dietary intervention.

C. Preparation of cookies with the best scored health mix

Cookies were prepared using the health mix (Variation-II)and other ingredients used in cookies preparation and are tabulated in Table-X.

Table- X

Ingredients used for the preparation of cookies

Ingredients	Quantity (g)
Health mix	90
Jaggery	5
Groundnut oil+ coconut oil	2.5+2.5
Total	100

In the standard cookies, the basic ingredients were wheat flour 50g, sugar 25g, fat 25g, egg 10g and baking powder 1g and prepared using the standard procedure (Mc Watters et. al. 2003). Whereas in the health mix cookies, wheat flour was replaced by the formulated health mix (90 g), jaggery (5 g) and a mixture of coconut oil and groundnut oil (2.5+2.5 g).

Each cookie weighed 18.62 grams and eight cookies were given to each participant (150 g) to meet the micronutrient requirement of the selected participants. Four cookies were given at mid morning and four at mid afternoon daily. Distribution schedule was planned and implemented effectively by the investigator.

D. Analysis of physical qualities, nutrient content, microbial count and cost effectiveness of the healthmix cookies

a. Physical evaluation

The physical characteristics like weight, width, diameter, thickness and spread factor were determined. The physical characteristics were measured according to the methods described by Mc Watters *etal.,* (2003). The physical measurements were recorded for the standard and formulated health mix cookies (Plate-2).

i. Weight (W): Weight was determined using electronic weighing balance.

ii. Diameter (D): Diameter of cookies was measured by placing six cookies horizontally edge to edge and rotated at 90 degree angle for duplicated reading.

iii. Thickness (T): The thickness of cookies was measured by placing six cookies one on the top of other and the duplicate reading was recorded.

iv. Spread ‘ratio (SR): The spread ratio is defined as a ratio of diameter and thickness was calculated according to the formula SR= (Diameter/Thickness x CF) x 10

Plate-2Physical measurements of the cookies

b. Nutrient content

Variation II of health mix was used for the preparation of cookies and analysis of the nutrient content. Standard cookies were also prepared and considered for comparison and analysis of the nutrient content. Analysis of different nutrients in cookies was carried out using the standard methods suggested by ICMR-NIN (2015)

c. Microbial count

Microbial count of the health mix cookies was done in the microbiology lab. The result of the score was tabulated. The microbial count gave valuable information on the keeping quality of the cookies, which were prepared without using preservatives.

d. Cost effectiveness

The ingredients used in the health mix cookies were seasonal, locally available and low cost items. Hence the cost of the cookies was comparatively lower than the standard cookies.

PHASE – IV

Impact of nutrition interventionon the symptoms of PMS

A number of 100 participants consisting of experimental group (N= 50) and the control group (N= 50) having moderate level of PMS, were selected for the study by purposive sampling method. The written consent was collected from the selected participants of experimental (N=50) and control group (N=50). The impact of nutrition intervention (dietary intervention and nutrition and health education) on the nutritional status and the symptoms of PMS and on knowledge of the selected participants is presented under the following headings.

A. Impact of dietary intervention on nutritional status and symptoms of PMS among the selected participants

Health mix in the form of cookies (150g consists of eight pieces) was distributed in airtight containers once in a week who were gathered periodically in the same place for a period of 120 days. The cookies were given to each participantin the experimental group (N=50) and instructed to consume four cookies in the mid morning and four in the mid afternoon regularly and were also instructed not to take coffee or tea or carbonated beverages before and after the intake of the health mix cookies. The study participants were encouraged to drink fresh fruit juices or fruits rich in Vitamin C. Dietary intervention were carried out for a period of 120 days.

Impact of dietary intervention on nutritional status and symptoms of PMS was evaluated. For the present study, nutritional anthropometry, biochemical estimation, clinical examination and individual dietary intake by 24 hour recall method and were used to assess the nutritional status of the selected participants. Impact of dietary intervention on the symptoms of PMS was also assessed by standard tool of calendar of premenstrual event (COPE) and depression level by Beck depression index (BDI).

a. **Impact of dietary intervention on nutritional status of the selected Participants**

i. Anthropometric measurements

Anthropometric measurements like height, weight, BMI, and WHR were carried out for the selected participants (N=100) before and after the intervention study.

ii. Biochemical estimation:

Biochemical parameters like blood haemoglobin level, serum Calcium, Magnesium, Iron, ferritin and Total Iron binding capacity (TIBC) were conducted. Two ml of the blood sample was collected from 100 selected participants who were involved in the dietary intervention and nutrition education (Plate-3).

- **Blood haemoglobin**

  Blood haemoglobin level was estimated by cyanomethaemoglobin method.

- **Serum Calcium**

  Calcium supplementation reported that 48 per cent of selected participants receiving 1000 mg of Calcium showed that there was a reduction in the severity of symptoms of PMS compared to placebo (Johnson, 2004). A reduction in

Calciumlevel causes disturbances like muscular cramps and prevented by suitable Calcium supplements (Parker, 2007). With this View Serum Calcium level was assessed using colometric method.

- **Serum Magnesium**

  Serum Magnesium concentration has been shown to vary cyclically in women of reproductive age group. The level of Magnesium in erythrocytes and leukocytes of women with PMS were found to be lower than those women without PMS, whereas in plasma Magnesium level, this trend was not noticed (Muneyyirci-Delale, *et al.,* 2001).Magnesium is also involved in the activity of serotonin and other neurotransmitters, as well as in vascular contraction, neuromuscular function and cell membrane stability. These might be possible pathways by which it influences PMS (Tonick and Muneyyirci-Delale, 2016). Serum Magnesium level was assessed gravimetrically.

Plate – 3 Collection of blood for intervention study

- **Serum Iron**

  According to Bertone (2008) limited studies were done on the relationship of Iron with PMS symptoms. But it was noted that the intake of non-heme Iron reduced the PMS risk whereas heme Iron from animal sources did not have the same effect of reducing the risk of PMS symptoms. For its health benefit, serum Iron content was analyzed by Wong's method.

- **Serum ferritinand Total Iron binding capacity (TIBC)** was estimated to know the serum Iron profile of the selected participants using colorimetric method.

  Blood parameters (serum Calcium, Magnesium, Iron, TIBC, serim ferritin) were analyzed using standard procedures mentioned in NIN Laboratory Manual (2011).

iii. Clinical examination

Clinical examination relies on the examination of physical signs of the body that are symptomatic of nutritional disorders (Jelliffe and Jelliffe, 1991). The objective of clinical examination is to assess the levels of health of individuals or of groups in relation to the food consumption pattern. It is the simplest and the most practical method of ascertaining the nutritional status of an individual (Park, 2017). Clinical sign is the assessment of nutritional status that results from lack of both nutritional and non-nutritional factors. Signs and symptoms should be investigated and combined with anthropometrical, dietary evaluation and biochemical estimation for accurate analysis and interpretation of the data (FSAU, 2004). Clinical examination was carried out using Clinical assessment schedule of Jelliffe and Jelliffe (1991) to all the selected participants with the help of general physician (Appendix-II).

iv. Individual dietary intake

Twenty four hour dietary recall method was used to gather information on their food intake. In this method, the selected participants were asked to recollect the quality and quantity of foods consumed and was expressed in terms of grams during the last 24 hours. The amounts of various nutrients were calculated using Food composition tables. This was done for the 100 selected participants in experimental and control groups.

Plate – 4.Distribution of the formulated health mix cookies to the selected participants

b. Impact of dietary intervention on symptoms of PMS of the selected participants

The impact of dietary intervention on the symptoms of PMS was determined by using the depression level and PMS symptoms expression.

i. Assessment of depression level

It was done using Beck Depression Index (BDI). The Beck Depression Scale consists of 21 items to assess the intensity of depression in clinical and normal patients. Each item is a list of four statements arranged in increasing severity about a particular symptom of depression. The BDI was administered to find out the depression level of the selected participantsin the experimental and control groups, befor and after the study period of 120 days.

ii. Assessment of PMS symptoms

Calendar of Premenstrual Events (COPE) was used to gather accurate details on the PMS symptoms. It is a self assessment standard tool used for assessment of PMS in many studies. This also rated symptoms from 1-4 (0- No symptom, 1-mild symptom, 2-moderate symptom, 3-severe symptom). The COPE is a reliable instrument for identifying fluctuations in behavioural and physical symptoms during the luteal phase of menstrual cycle and PMS symptoms can be reliably conceptualized within four factors. Symptom expression may increase in response to daily self-monitoring.It is made up of a calendar of 32 days with column of 23 symptoms (physical, social and

emotional) and rows with provision for entry of date, weight and bleeding days. There are provisions for entry of any other symptoms not in the list to be entered by the selected participants and also use of medication. (Appendix- III). The selected participants were clearly explained about the filling of COPE tool and their doubts were clarified. The entries made by the participants in the study groups were also noted regularly. Regular visits of the investigator helped to confirm the recording of the symptoms experienced byselected participants and to indentify the premenstrual changes in participants. The recording of COPE was carried out for two successive menstrual cycles so as to confirm the premenstrual changes missed in one period may be entered in the next cycle before the dietary intervention.

During the dietary intervention period, COPE was not administered to find out the symptoms of PMS among the participants in the study groups. After the study period of four months, the same tool was implemented to assess the PMS symptoms among the participants in the study groups.

B. Impact of nutrition education on the knowledge of PMS among the selected participants

Nutrition education and diet counselling was given to 100 selected participants inthe study group of experimental and control group.

a. Imparting nutrition and health education to the participants

Nutrition and health education is defined as "a process that informs, motivates and helps people to adopt and maintain healthy practices and life style, advocate envIronmental changes as needed to facilitate this goal and conducts professional training and research. Nutrition education is a major intervention for the prevention of malnutrition, promotion of health and improving quality of life (Park, 2017). According to Evans (2006) social marketing and education are the vital strategies to bring about changes in feeding behaviour of the population and enhance their quality of life. With this back drop nutrition education was given to the selected participants adopting the following steps. Nutrition and health education for the 100 selected participants was given periodically for 120 days.

Plate – 5. Nutrition and health education on PMS

b. Preparation of the nutrition and health education module

The investigator prepared an educational module covering all the aspects of PMS (Appendix-V). Modules for nutrition and health education were developed using relevant scientific literature with suitable pictures related to PMS. The developed modules consisted of brief introduction and meaning of PMS, prevalence, causes, signs symptoms, complications, prognosis and diagnosis, modes of treatment, role of nutrients on PMS symptoms, dietary and other non-pharmacological approaches to minimise the PMS symptoms and the role of adequate exercise and lifestyle on prevention of PMS symptoms. Hand book in English and Tamil (Appendix-VIa, VIb),power point presentation and nutrition educationposter (Appendix-VIIa, VII b), and and handouts (Appendix-VIII) were prepared covering all the above mentioned aspects. The time allotment for each topic was planned systematically and evaluated carefully.

c. Conduct of Nutrition and Health Education

Nutrition and health education was given to the selected participants in experimental and control groups for 120 days. Totally ten topics were framed for nutrition and health education. Each topic consisted of 15 minutes lecture on the topic followed by group discussion along with Power point presentation and exhibition (Plate-4). Posters were used as supportive aids as the common mode of education to the participants. The investigator gave lecture with power point presentation once in a week for each topic and covered all aspects within the study period.

i. Topics for Nutrition and Health Education

The topics covered for education followed by discussion were introduction and meaning of PMS, prevalence, causes, signs symptoms, complications, prognosis and diagnosis, modes of treatment, role of nutrients on symptoms ofPMS, dietary and other non-pharmacological approaches to minimise the symptoms of PMS and the role of adequate exercise and lifestyle on prevention of PMS symptoms. In group discussion, the quarries of selected participants were clearly clarified. The developed educational modules were distributed to the selected participants during the study period.

ii. Formulation of the questionnaire

A specially designed short structured questionnaire was developed and pre-tested to collect information on knowledge about PMS among the participants. All the

questions were multiple-choice types with only one correct answer. Totally twenty questions were given related to the ten aspects of educational modules. All the questions had equal weightage of scores. Each correct answer was given one mark. Total scoring for the questionnaire was twenty.

iii. Administration of PMS knowledge questionnaire

As the first step in nutrition and health education, a specially framed questionnaire with 20 questions on various aspects of PMS was administered to the selected participants (N=100) in the experimental as well as control group (Plate-II). Then at the end of the study period, the same questionnaire was administered to evaluate the effect of nutrition and health education on the prevention or minimizing the signs and symptoms and the consequences of PMS among the selected participants in the experimental and control groups. Application of statistical analysis revealed the impact of nutrition education on the knowledge of the selected participants in the study groups.

PHASE – V

Analysis and interpretation of the data

After the data collection, it is essential to organize the discrete data in a systematic manner to obtain the desired results and interpretation scientifically. The collected data was systematically consolidated and statistically analysed for arriving at the result of the effect of different intervention on reducing the severity of the symptoms of PMS. Statistical methods play a major role in transforming a data into information. Statistical data, once collected, must be arranged purposively, in order to bring out the important points clearly. Therefore the manner in which statistical data is presented is the most important aspect for the effective documentation and recommendation (Park, 2017). Descriptive statistics like range, percentage and mean were used for exploring the demographic characteristics, BMI levels, prevalence of PMS and anaemia, knowledge levels of the study groups. Inferential statistics like correlation and chi-square were applied to find the association and differences between the variables, effect of dietary intervention and nutrition and health education on the symptoms of PMS. The detailed discussions are presented in chapter IV – Results and Discussion.