Methodology

Polycystic Ovarian Syndrome (PCOS) is a prevalent endocrine condition impacting women of reproductive age. It is marked by increased testosterone levels, irregular menstruation cycles, and the existence of ovarian cysts. Common symptoms include weight gain, excessive hair growth on the face and back, infertility, mood changes, and depression. In light of these issues, the present study was conducted. This chapter describes the research techniques used to investigate the effect of nutrition intervention programmes on the nutritional and health status and nutrition knowledge of young adult women (18-21 years old) diagnosed with Polycystic Ovary Syndrome (PCOS) in Thrissur, Kerala, India under the following headings.

3.1 Phase I: Screening of young adult women (18-21 years) to identify PCOS

3.1.1 Selection of area

3.1.2 Selection of subjects

3.1.3 Formulation of the tools

3.1.4 Conduct the survey

3.1.5 Screening and identification of PCOS subjects

3.2 Phase II: Mapping of subjects for nutrition intervention programme

3.2.1 Pre Assessment of Nutritional status using ABCD techniques

3.2.2 Exercise pattern and Stress level

3.2.3 Menstrual Hygiene

3.2.4 Self-administered Polycystic Ovarian Syndrome Questionnaire

3.2.5 Knowledge related to PCOS

3.3 Phase III: Formulation and Evaluation of Nutrient Dense Health Mix Powder (NDHMP) and Nutrition Education Modules

3.3.1 Nutrient Dense Health Mix Powder (NDHMP)

3.3.1.1 Selection of the ingredients to formulate NDHMP

3.3.1.2 Sensory evaluation of the NDHMP

3.3.1.3 Estimation of Nutrient content of the NDHMP

3.3.1.4 Analysis of Physicochemical, Microbiological and Toxicity of the Formulated NDHMP

3.3.1.5 Calculation of Cost-effectiveness of the NDHMP

3.3.2 Nutrition Education Modules

3.3.2.1 Development and validation of Nutrition Education Modules of PCOS

3.3.2.2 Creation of WhatsApp groups and YouTube channel for Nutrition Education intervention

3.4 Phase IV: Nutrition Intervention Programmes on Symptoms of PCOS

3.4.1 Grouping of the Participants

3.4.2 Nutrition Education intervention for the study participants

3.4.3 Aerobic Exercise Training for the study participants

3.4.4 Nutrient Dense Health Mix Powder Supplementation for dietary intervention programme

3.5 Phase V: Effect of Nutrition intervention programmes on nutritional knowledge, nutrition and health status and on symptoms of PCOS

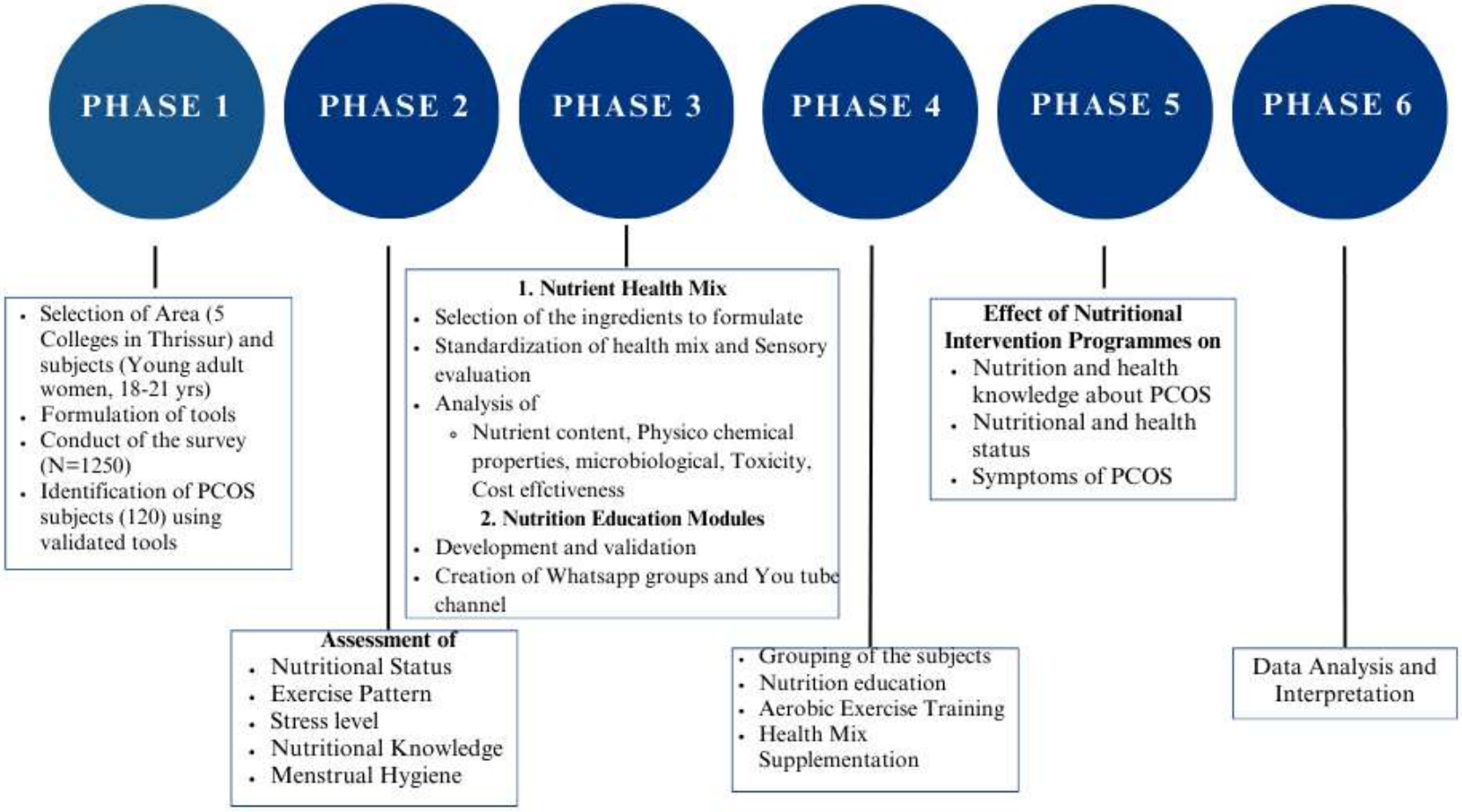
3.5.1 Effect of nutrition education on the nutritional knowledge of PCOS among the selected participants

3.5.2 Effect of nutrition interventions on the nutritional and health status of the selected participants

3.5.3 Effect of nutrition intervention programmes on the Symptoms of PCOS among the selected participants

3.6 Phase VI: Data analysis and interpretation

RESEARCH DESIGN



3.1 Phase I: Screening of young adult women (18-21 years) to identify PCOS

3.1.1 Selection of Area

Five different women's colleges from Thrissur district, Kerala, were selected for conducting the study in order to make certain that inclusion of a diverse and representative sample. Thrissur is known as Kerala's cultural capital and a major centre for education (Sreekumar, 2018). It was a suitable area to understand how nutrition interventions make an impact on young adult women with Polycystic Ovary Syndrome (PCOS). The district has been a great part of social reform movements that have struggled for women's right to an education, which has helped Kerala have such extraordinary female literacy rates (Tharamangalam, 1998).

Figure 3.1 Thrissur Area Map

We have selected a mix of government, aided and Autonomous colleges to get more accurate details on socioeconomic position and educational levels. Colleges which have a larger number of female students aged between 18-21 years were the first choice to improve the possibility of identifying eligible subjects with PCOS. Students favoured colleges that were interested in becoming involved, made it easy for them to get to campus, and helped with logistics. First, we have located the qualified women's colleges, and we contacted the authorities to acquire relevant approvals. After that, from those colleges we selected five colleges based on their wish to participate in the study, possible subject availability and practical concerns. Vimala College, St Mary's College, St Joseph's College, Little Flower College and Carmel College were selected for the present study. Variety in the chosen institutions was significant for making the study's results more generalizable and for farsighted how dietary changes affected people from varied socioeconomic and educational backgrounds.

3.1.2 Selection of Subjects

The prevalence of polycystic ovary syndrome (PCOS) in India varies widely, ranging from 3.7 per cent to 22.5 per cent depending on the population studied and diagnostic criteria used (Bharali *et al.*, 2022 and Ganie *et al.*, 2019). However, most estimates fall between 3.7 per cent to 10 per cent using the NIH and AES criteria. A few studies have looked at PCOS prevalence and perceptions in Kerala, India. A pilot study at Karuna Medical College, Palakkad found an 18 per cent PCOS prevalence in young women, with higher rates in urban vs rural areas (Harrini, 2020). One 2015 study noted that in India, one out of five women are found to have PCOS, with numbers increasing every year. In summary, while the exact PCOS prevalence in India is unclear, most estimates range from 3.7 per cent to 22.5 per cent of reproductive-aged women. More research is needed, especially large-scale community-based studies in Kerala, to determine the true burden and identify barriers to treatment in this population.

In this study, we selected young adult women aged 18- 21. Table 3.1 details the criteria for selecting subjects for Phase 1.

Table 3.1 Criteria for selection of subjects

Inclusion Criteria	Exclusion Criteria
• Young adult Women of reproductive age (18-21 years) and attended menarche	• Adult women above 21 years and less than 18 years old who have not yet attended menarche
• Willing to participate and extend their full cooperation for the study	• Married, pregnant and lactating women
• Adult women who can operate digital mode	• Adult women having health issues
• Willing to give written consent	• Those who are non-co-operative and not willing to give written consent

Based on inclusion criteria, the data collection was carried out among the 1250 young adult women in the age group of 18-21 years. The following simple formula was (Harrini, 2020) used for the sample size calculation and sample size was calculated as 1235.

$$n = \frac{z^2 P(1\text{-}P)}{d^2}$$

Where n= Sample Size,
Z = Z statistics for a level of confidence
P = expected prevalence or proportion
(In proportion of one; if 20%, P=0.2 and
d = precision
(In proportion of one; if 5%, d= 0.05)

3.1.3 Formulation of the tools

A specially designed questionnaire was created to gather information about the selected subjects demographic profiles and dietary and lifestyle patterns. Hirsutism Scoring Sheet (Kahraman, 2021), the Risk Assessment Questionnaire (Selvaraj, 2020) and Food Frequency Table were distributed among the study subjects to identify the prevalence of PCOS, eating behaviour, dietary pattern and to select the subjects for nutrition intervention programs. Medical background information, menstrual hygiene, and knowledge and attitudes on the causes, prevalence, symptoms, complications, and treatments related to PCOS among the selected subjects were gathered for further study analysis.

Various experts in the field of medicine and nutrition verified the quality of the questionnaire and required changes were made, based on their recommendations to validate the questionnaire. Thirty subjects completed tests on the dependability of the Malayalam and English versions of the questionnaires and validated perfectly.

The study design was registered in the Clinical Trial Registry of India (CTRI), ICMR and CTRI registration number is CTRI/2024/04/065879. The methodology for this study was submitted to the Institutional Human Ethical Committees (IHEC) of Vimala College and Avinashilingam University to undertake nutrition intervention programmes. The VC/REC/23-24-1 and IHEC/23-24/FSN-05/09-02-2024 approval numbers were acquired, respectively from the above-mentioned institutions.

3.1.4 Conduct of the survey

Survey information was gathered on age, education, marital status, income brackets, medical and reproductive health issues, menstrual irregularities and hirsutism and also family history of diseases like diabetes mellitus, hypertension, and so on. Following permission from Vimala College's research ethics committee, data on food consumption, dietary pattern, food frequency pattern, and 24-hour recollection, knowledge and attitude on the causes, prevalence, symptoms, complications, and different modes of treatments related to PCOS were also gathered from the selected subjects (N= 1250) using a validated questionnaire. Using the Kuppuswamy Socioeconomic Scale (2019), the socioeconomic profile of the selected subjects was examined.

Prior to administering the survey, the investigator established a positive rapport with the selected study subjects. The investigator transparently communicated the purposes, objectives, and potential benefits of this present study to the selected subjects. Willingness of the subjects to participate in the present study was obtained by both written and verbal consent. The investigator used a specially designed interview schedule to collect reliable and accurate data.

3.1.5 Screening and identification of PCOS subjects

The Rotterdam criteria (2003) are a set of guidelines used to diagnose polycystic ovary syndrome (PCOS), one of the most prevalent endocrine disorders among women of reproductive age. Developed in 2003 by a consensus group of experts from the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine. According to the Rotterdam criteria, a woman can be diagnosed with PCOS if she meets at least two of the following three criteria: (1) oligo-ovulation or anovulation, (2) clinical or biochemical signs of hyperandrogenism, and (3) the presence of polycystic ovaries on ultrasound.

Table 3.2 Rotterdam 2003 criteria to diagnose PCOS

Criteria	Description
Oligo-ovulation or Anovulation	Infrequent or absent ovulation, as evidenced by irregular menstrual cycles or amenorrhea.
Clinical or Biochemical Signs of Hyperandrogenism	Clinical signs of excess androgen levels, such as hirsutism, acne, or male-pattern hair loss, OR biochemical evidence of elevated androgen levels (e.g., high testosterone or androstenedione levels).
Polycystic Ovaries on Ultrasound	Presence of Polycystic Ovaries on Ultrasound examination, characterized by an increased number of follicles (≥12 follicles measuring 2-9 mm in diameter) and/or an ovarian volume greater than 10mL

The hirsutism level and risk of PCOS among the study subjects were evaluated to determine the prevalence of Polycystic Ovary Syndrome (PCOS) among the selected young adult women using standardised assessment tools.

By validated Hirsutism Scoring Sheet the extent and severity of excess hair growth associated with PCOS, was measured. The scores range from 8 or less (no risk) to 26 -36 (high risk). Likewise various risk factors associated with PCOS evaluated by the Risk Assessment Questionnaire. The scores range from 15 or less (no risk) to 50 - 60 (high risk). By administering this standardized assessment, it is easy to quantify the hirsutism level and overall PCOS risk for each of the selected subjects.

The study subjects were classified into four risk categories based on their scores, which were obtained from the Hirsutism Scoring Sheet and the Risk Assessment Questionnaire. That is No risk, Low risk, Medium risk and High risk for Polycystic Ovary Syndrome (PCOS). The classification method observed at the combined results of both evaluation tools, taking into account how bad the hirsutism was and whether or

not the person had any of the risk factors that are linked to PCOS. This method made it possible to fully assess each subject's risk profile.

Table 3.3 Grading of PCOS Risk Assessment Scores

Assessment tool	Scores			
	No Risk	Low Risk	Medium Risk	High Risk
Hirsutism Scoring Sheet	<8	8-15	16-25	26-36
Risk Assessment Questionnaire	<15	15-29	30-49	50-60

3.2 Phase II: Mapping of subjects for the nutritional intervention programme

Following phase I, Phase II was planned for mapping the subjects for nutrition intervention programmes. 120 subjects who showed more visible symptoms were selected from the whole subjects. Nutritional status, activity patterns, stress levels, menstrual hygiene, menstrual irregularities, and knowledge of nutrition were also collected in this phase.

3.2.1 Pre Assessment of Nutritional status using ABCD techniques

Proper nutritional status is important for staying healthy and avoiding nutritional excesses or shortfalls that can lead to a number of health problems (Swinburn *et al*., 2019). The ABCD technique, an acronym for Anthropometric, Biochemical, Clinical, and Dietary evaluation, is a commonly used method for assessing the nutritional status of the target population.

Anthropometric Measurements

Anthropometric measurements are essential tools for assessing nutritional status, growth, and development. Common anthropometric indicators include weight, height, BMI, Waist and Hip circumference, arm circumference, skinfold thickness, and head circumference. These measurements are simple, non-invasive, and inexpensive methods. Anthropometric data provides insights into under nutrition, over nutrition, and micronutrient deficiencies when interpreted using appropriate reference standards. The

measurements help identify individuals or populations at risk of malnutrition. Anthropometry guides interventions aimed at improving nutritional status and overall health in clinical settings and large-scale surveys (Rumbo *et al*, 2021)

A stadiometer was used to measure height and the selected subjects (N=1250) were asked to stand straight, place their feet flat, and position their head in the Frankfort horizontal plane. A calibrated digital or beam balance scale was used to record the subject's weight, and they were asked to wear light clothing and no shoes. A non-stretchable tape was used to measure the subject's waist circumference at the midpoint between the last palpable rib and the top of the iliac crest, and the subject stood upright and the measurements were taken to the nearest 0.1 cm or 0.1 kg, respectively (The International Fetal and Newborn Growth Consortium, 2012).

Figure 3.2 Recording of Anthropometric Measurements

Body Mass Index (BMI) is calculated by dividing a person's weight in kilograms by their height in meters squared (kg/m²). The BMI ranges reflect the increased risk of diseases and mortality associated with higher body fat levels. BMI serves as a reasonably accurate indicator of adiposity. The main purpose of BMI is to identify potential health risks, as higher BMI values are linked to greater risks of conditions like cardiovascular disease, hypertension, osteoarthritis, some cancers, and diabetes.

Following the BMI calculation, the selected subjects (N= 1250) were categorized based on WHO (2020) guidelines and indicated in Table 3.4.

Table 3.4 Grades of Malnutrition*

BMI (kg/m^2)	Interpretation
<18.5	Underweight
18.5 – 24.9	Normal weight
25.0 – 29.9	Overweight
30.0 – 34.9	Obese Class I
35.0 -39.9	Obese Class II
≥40.0	Obese Class III

*WHO 2020

Biochemical Estimation

Biochemical estimation plays a vital role in the evaluation and management of polycystic ovary syndrome (PCOS), a complex endocrine disorder marked by hormonal imbalances, insulin resistance, and metabolic dysregulation. (Trent and Gordon, 2020). In PCOS, several biochemical markers play a significant role in assessing the associated nutritional imbalances and metabolic disturbances. The haemoglobin level, random Blood Sugar, and Cholesterol level of the 120 selected study subjects were estimated in this study. It was done with the help of a biochemist and a general physician from the Bhavana Clinic, which was situated in the study area.

In PCOS patients, anaemia may be made worse by menstrual irregularities and nutritional deficiencies. So the evaluation of Haemoglobin will help to know the real

condition of the subject. 12-16g/dL is the normal haemoglobin level for women. According to WHO (2011), monitoring the haemoglobin level is essential because there is a chance for fatigue and other PCOS related symptoms. Cholesterol levels, including total cholesterol, LDL (low-density lipoprotein), and HDL (high-density lipoprotein), are important markers in PCOS. Desirable levels are <200 mg/dL for total cholesterol, <100 mg/dL for LDL, and >50 mg/dL for HDL.

According to Amisi and co-workers (2022), for assessing and managing metabolic issues in subjects with Polycystic Ovary Syndrome (PCOS), random Blood Sugar levels are important. Metabolic issues include insulin resistance and impaired glucose tolerance. The risk of hyperadrogenism and type 2 diabetes get worsens with elevated RBS levels in PCOS patients. RBS levels are a more useful and convenient way to check blood sugar levels at any time, which makes them an important part of managing metabolic problems connected to PCOS (ADA, 2023).

Clinical Examination

Figure 3.3 Biochemical Estimation with the support of Medical Personals

To detect Polycystic Ovary Syndrome (PCOS), doctors do a full medical history to look for problems with menstruation, focusing on hirsutism and acne, which are two common indications of hyperandrogenism (Younis *et al*., 2024). For assessing the severity of hirsutism, which is significantly characterised by excessive hair growth in androgen-dependent areas, a physical examination is crucial. It also evaluates the presence of acne, a stressful symptom of PCOS (Sprintzer *et al.*, 2022)

3.2.1.3.1 Hirsutism: Modified Ferriman-Gallwey scoring system (mF-G score) helped participants for self-assessment of degree of hirsutism (Ferriman & Gallwey, 1961; Hatch *et al*., 1981). Detailed instructions, illustrations and explanations were given to the participants to make sure accurate self-evaluation. The mF-G score evaluates terminal hair growth in nine androgen-sensitive parts of the body: the upper lip, chin, chest, upper abdomen, lower abdomen, upper back, lower back, upper arm, and thigh. Each area is allotted a score ranging from 0 (indicating no terminal hair growth) to 4 (indicating extensive terminal hair growth), resulting in a maximum possible score of 36. A score of ≥8 was considered revealing of hirsutism.

Acne: The Global Acne Grading System (GAGS) was developed by Doshi *et al.*(1997). This tool is used for assessing the severity of the acne. This validated tool assessed acne lesions in six areas: the forehead, right cheek, left cheek, nose, chin, and chest/upper back. Each area was graded separately on a 4-point scale (0-3), based on the types and extents of comedones, papules, pustules, and nodules present.

Dietary Intake

24-hour recall method and Food Frequency table were used to evaluate the dietary intake of the selected subjects. Selected subjects filled out the 24-hour recall and FFQ independently. The Dietcal software was used to analyse the 24-hour recall, which helps to estimate the intake of energy, macronutrients and micronutrients on the recalled day. Usual dietary pattern focused on the 10 food groups was provided by FFQ.

Physical Examination

In the present study, ultrasound scans were conducted on 40 per cent of the participants within the study group (N=33) to evaluate ovarian morphology and detect cysts, which are characteristic indicators of PCOS. Locating many cysts with an

ultrasound is one of the most dependable ways to diagnose this problem, which helps doctors come up with good treatment approaches. However, it is important to note that while ultrasound findings can support a diagnosis, they cannot exclusively confirm PCOS, as clinical and biochemical evaluations are also essential for a complete diagnosis.

3.2.2 Exercise pattern and Stress level

Exercise pattern, stress level and their link with polycystic ovary syndrome of the participants were evaluated by Perceived Stress scale and Godin Leisure Time Exercise Questionnaire. The degree of stressful conditions over the past month for the subjects was evaluated by Perceived Stress scale (PSS) (Harris *et al.,* 2023). Levels of mild, moderate and strenuous leisure exercise of the participants were determined by Godin Leisure Time Exercise Questionnaire (GLTEQ) (Godin, 2011). These two tools were self-administered, which gave them more comfort to fill out. As the recent studies explored (Constantini *et al*., 2018; Greenwood *et al*., 2016), physical inactivity have been linked to an increased PCOS risk. It confirms the relationship between PSS and GLTEQ scores and PCOS status.

3.2.3 Menstrual hygiene

The Menstrual Practices Questionnaire, which was developed by Hennegan *et al.* (2020), was used to evaluate the menstrual hygiene practices of the 120 selected subjects. This tool is also self-administered. Multiple sections of the questionnaire collected the data on menstrual absorbent materials used, frequency of changing materials, bathing and cleansing practices, disposal methods for used materials and other societal restrictions related to menstruation. Analysis of MPQ helps researchers to understand the menstrual hygiene practices and identify the areas requiring educational sections.

3.2.4 Self-Administered Polycystic Ovarian Syndrome Questionnaire

Cronin and co-workers (1998) developed a Self-Administered Polycystic Ovarian Syndrome Questionnaire (SAPOCQ) which aids in assessing the presence and symptoms of polycystic ovarian syndrome. This tool was used by the researcher for this present study. Based on the Rotterdam Criteria, a 26-item self-report measure that

identifies PCOS was included in the SAPOCQ. Menstrual irregularity, clinical symptoms of hyperandrogenism such as hirsutism and acne and polycystic ovaries on ultrasound are evaluated by SAPOCQ. The 120 selected subjects each completed the SAPOCQ on their own, which gave them a total score that showed how likely they were to have PCOS. Scores that were higher than a validated cut-off value were seen as positive screenings, which led to more clinical assessment.

3.2.5 Knowledge related to PCOS

In this present study, the researcher evaluated the knowledge and awareness of the selected study subjects by using a specially developed 30-item questionnaire. The questions regarding the definition, symptoms, causes, diagnostic criteria, associated health risks, and management strategies of PCOS were included in the developed questionnaire. The questionnaire was self-administered, and a correct response was marked with a score of 1 for each question. The understanding of basic concepts, clinical features, and implications of participants on PCOS was evaluated by this questionnaire. The analysis of the questionnaire helps to gather information for educational interventions that need to be included in the education module.

3.3 Phase III: Formulation and Evaluation of Nutrient Dense Health Mix Powder (NDHMP) and Nutrition Education Modules

This study aimed to develop and evaluate a nutrient-dense health mix powder and accompanying nutrition education modules for the nutrition intervention programmes for improving the health status of the young adult women having PCOS conditions.

3.3.1 Nutrient Dense Health Mix Powder (NDHMP)

3.3.1.1 Selection of the ingredients to formulate NDHMP

Ingredients used in the nutrient mix are rich in micronutrients - calcium, phosphorus, selenium, zinc, magnesium, Vitamin B complex and fibre that are helpful in improving PCOS health outcome. Combination of these ingredients support female reproductive system and maintain hormonal level normally.

Table 3.5 Ingredients in the Health Mix Powder

FOOD INGREDIENTS	BOTANICAL NAME	GOOD SOURCE	HEALTH BENEFITS
Sesame Seeds 	*Sesamum indicum*	Magnesium and Calcium	• Increase progesterone production in women • Improve insulin sensitivity • Help to alleviate stress • Improve Iron and Calcium levels
Jaggery 	*Saccharum officinarum*	Iron	• Support the healthy blood cells • Reduce tiredness • Improve muscular function
Ragi 	*Eleusine coracana*	Calcium Iron	• Help in controlling cholesterol and blood sugar level
Whole oats 	*Avena sativa*	Dietary fibre Zinc Magnesium	• Anti-inflammatory effects • Help in weight loss • Improve immunity
Flax seeds 	*Linum usitatissiumum*	Lignan secoisolaricireinol diglucoside	• Enhance menstrual frequency by significantly decreasing ovarian volume and the number of follicles in the ovaries.
Pea nuts 	*Arachis hypogaea*	Calcium B vitamins Magnesium	• Delay digestion and feel satiety • Stabilises blood sugar levels • Prevent free radical damage

Sunflower seed 	*Helianthus annus*	Vitamin E Selenium Magnesium	• Stimulate the production of progesterone hormones • Regulate other PCOS symptoms like inflammation, mood swings etc.
Pumpkin seed 	*Cucurbita maxima*	ω-3 fatty acids beta-sitosterol	• Manage elevated insulin and cholesterol levels. • Reduce excessive androgen levels. • Address PCOS symptoms such as hirsutism, acne, and weight gain.

Using an electronic blender, all the ingredients were ground into a fine powder after being roasted at 120°C for 15 minutes. The quantity of Ragi was gradually increased from 30 to 40 grams, and whole oats were gradually reduced from 20 to 10 grams. The other ingredients remained constant in quantity. The substances were combined in three different variations to formulate the health mix. The amount of ingredients used in each variation is listed in Table 3.6. The steps involved in the formulation of the NDHMP are given in the figure 3.4.

Table 3.6 Ingredients used in Three Variations of NDHMP

Ingredients	**Variations (g)**		
	I	**II**	**III**
Ragi	30	40	35
Whole Oats	20	10	15
Flax seed	10	10	10
Peanuts	10	10	10
Sunflower seeds	8	8	8
Sesame	8	8	8
Pumpkin seed	6	6	6
Jaggery	8	8	8
Total	**100**	**100**	**100**

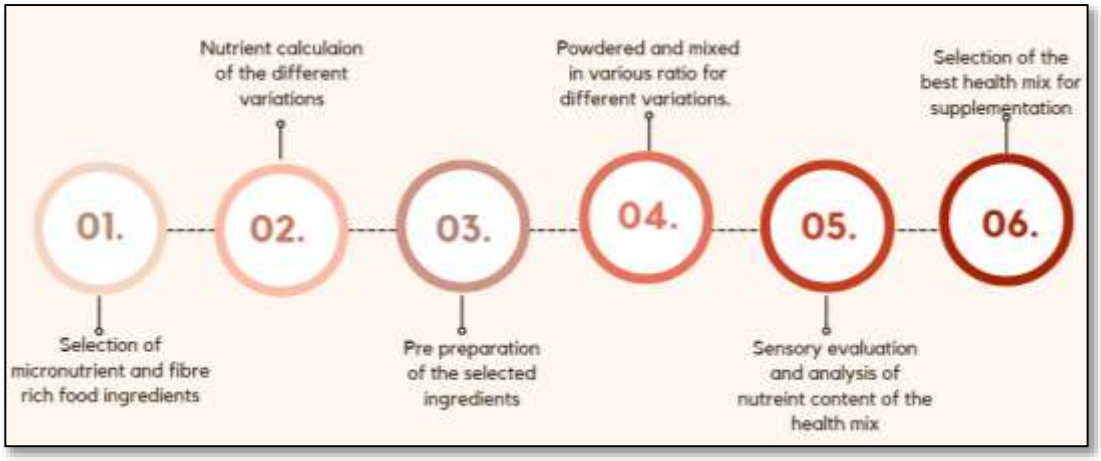


Figure 3.4 Steps involved in the formulation of NDHMP

3.3.1.2 Sensory Evaluation of the NDHMP

The five senses of sight, smell, taste, touch, and hearing are used by human panellists during sensory evaluation, a multidisciplinary science, to assess the products' sensory qualities and acceptability. Thus, food quality is assessed based on its appearance, colour, flavour, texture, and doneness, which are the essential elements of successful product development (Petrescu *et al*., 2019). The following paragraphs detail the steps involved in the sensory evaluation of the health mix.

i. Selection of panel members

A selection of assessors for a sensory test is known as an evaluation panel. Instead of using a single assessor, a panel of assessors is employed to reduce errors in the physical, psychological, environmental, and other factors caused by an individual. Thirty semi-skilled individuals were chosen based on their health, cooperation, willingness, understanding of sensory analysis, and ability to distinguish between several sensory evaluations criteria.

ii. Formulation of the scorecard

A method of grading cooked food is scoring, which uses a numerical scale using numbers mathematically related to one another to create an interval or ratio scale (Ackbarali and Maharaj, 2014). The acceptance of appearance, colour, taste, flavour, and texture was measured using a five-point hedonic scale, and grades were assigned

based on how much the panel members approved of each factor of the product in three variations.

iii. Formulation and evaluation of the NDHMP

The panel members evaluated the sensory quality of the 3 variations that were chosen for the acceptance testing at various points in their preparation. One variant with high acceptance scores were selected for the nutrient analysis, sensory attributes, microbial count analysis and assessment of presence of heavy metals. The comments were recorded to avoid a biased outcome. Three acceptance trials were conducted (for the three variations) to get more accurate findings, and were taken into consideration for further standardizing.

iv. Sensory evaluation

Table 3.7 presents the scores of sensory evaluation, using the five-point hedonic scale. Among the three variations of health mixes, variation III secured the highest score for all sensory attributes, with a score of 22.6, followed by Variation II (18.5) and Variation I (16.6)

Table 3.7 Mean Scores of Different Variations

Sensory attributes	Mean scores of different Variations		
	Variation I	**Variation II**	**Variation III**
Appearance	4.2	3.9	4.6
Flavour	3.5	3.7	4.4
Taste	2.4	3.5	4.6
Texture	3.7	3.8	4.3
Overall Acceptability	2.8	3.6	4.7
Overall score	**16.6**	**18.5**	**22.6**

Figure 3.5 Weighing and Packaging of NDHMP

3.3.1.3 Estimation of Nutrient content of the NDHMP

Variation III scored the highest overall score was considered for the analysis of nutrient content, total microbial count and toxicity level. The nutrient composition of the formulated Nutrient Dense Health mix powder, designed to improve polycystic ovary syndrome (PCOS), was comprehensively analysed using standard analytical techniques suggested by the ICMR-NIN Laboratory Manual (2020) to ensure its adequacy and conformity with PCOS-specific nutritional requirements. The procedures used for the analysis are listed below (Table 3.8). The rigorous nutrient profiling ensured the developed health mix powder met the desired specifications, provided sufficient amounts of nutrients implicated in improving insulin resistance, hormonal

imbalances, and oxidative stress associated with PCOS, and supported dietary interventions for managing PCOS symptoms and complications effectively.

Table 3.8 Methods/Procedures adopted for Nutrient analysis

Nutrient	Procedure
Energy (KCal)	Calculation Method
Fat (g)	AOAC 18th Edition / FSSAI
Carbohydrate (g)	Calculation method
Protein (g)	FSSAI Manual of Methods
Fibre (g)	AOAC/BIS
Calcium (mg)	AOAC/BIS/FSSAI
Magnesium (mg)	AOAC/BIS/FSSAI
Iron (mg)	AOAC/FSSAI
Zinc (mg)	AOAC
Selenium (mcg)	FSSAI
Beta-carotene (mcg)	Biochemical Methods/AOAC
Thiamine (Vit B1) (mg)	AOAC/FSSAI
Riboflavin (Vit B2) (mg)	AOAC/FSSAI
Niacin (Vit B3) (mg)	AOAC/FSSAI
Phosphorus (mg)	AOAC/BIS/FSSAI
Potassium (mg)	AOAC 18th Edn/ FSSAI

3.3.1.4 Analysis of Physicochemical and Microbiological profile and presence of Heavy metals in the formulated NDHMP

After formulating the health mix powder, a comprehensive series of analyses were conducted to evaluate its physicochemical properties, microbiological safety, and presence of heavy metals. The physicochemical characterization assessed appearance, colour, odor and pH which influence the powder's stability, acceptability, handling, and processing characteristics. Microbiological testing involved enumerating total bacterial count, and total fungal count, as well as screening for potential pathogens to ensure the product met food safety standards. The toxicological analyses screened for the presence of heavy metals like lead, mercury, arsenic, cadmium, and nickel to identify and

quantify any potential toxic contaminants, ensuring the safety of the formulated health mix powder for human consumption and suitability for large-scale production and distribution as part of the nutrition intervention program.

3.3.1.5 Calculation of Cost-effectiveness of the NDHMP

Cost-effectiveness analysis evaluated the economic viability and scalability potential of the formulated health mix powder. It accounted for costs related to raw ingredients, production (equipment, utilities, labour, and overheads), packaging, and distribution. These expenses were weighed against the nutritional value and projected therapeutic benefits based on the powder's nutrient profile. Sensitivity analysis explored the variations in ingredient prices, production efficiencies, and health impact estimates that influenced cost-effectiveness. This analysis enabled to assess the affordability, identifying cost optimization opportunities, and informing strategies to maximize cost-effectiveness for real-world implementation of the nutrition intervention programme.

3.3.2 Nutrition Education Modules

Nutrition education programme is crucial in managing Polycystic Ovary Syndrome (PCOS) and its associated metabolic complications, such as insulin resistance and increased risk of type 2 diabetes. A comprehensive program should empower women with knowledge about the balanced, nutrient-dense diet, emphasizing healthy eating pattern, portion control, reducing the quantity of refined carbohydrates and high-glycemic foods, incorporating insulin-sensitive foods, managing cravings, meal planning, healthy cooking techniques, adapting regular physical activity pattern and stress management.

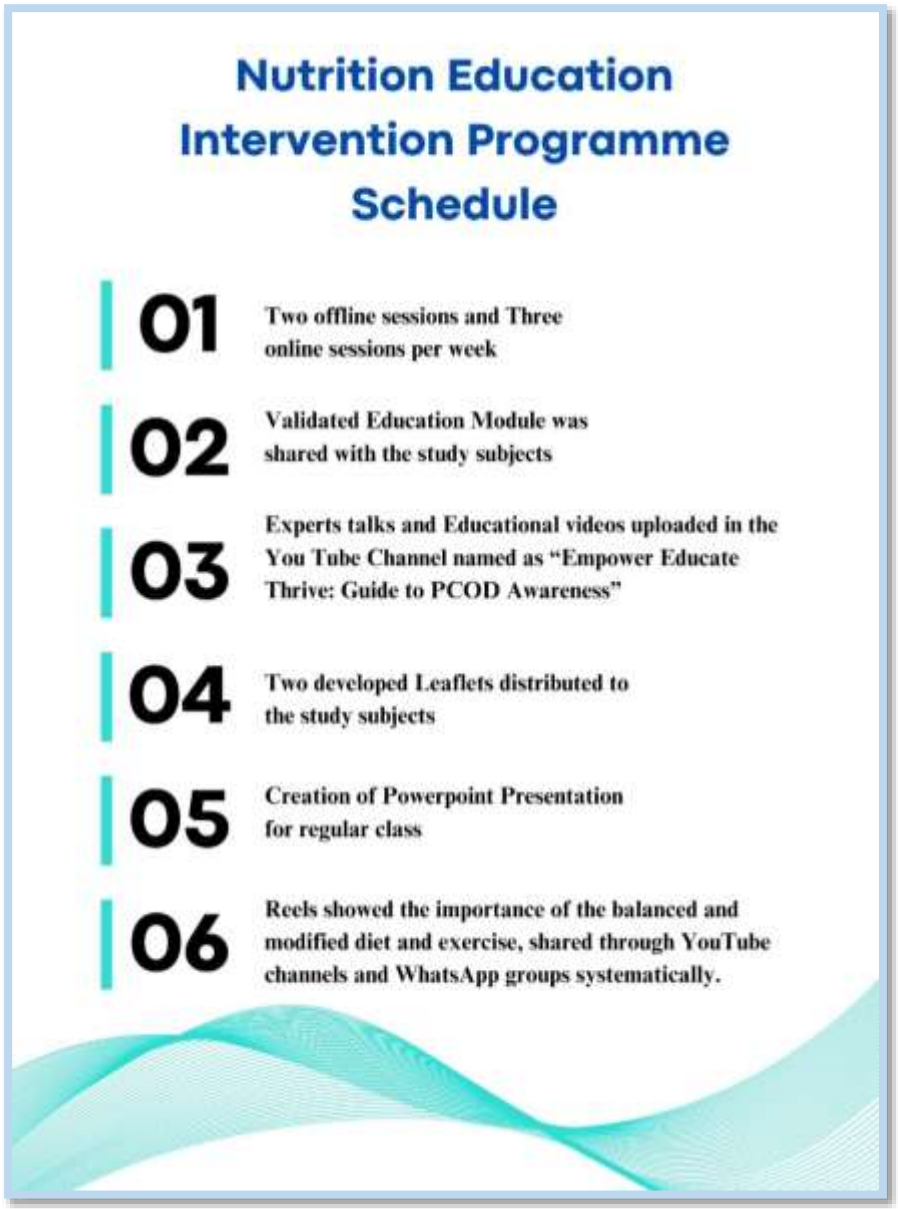


Figure 3.6 Nutrition Education Intervention Programme Schedule

Nutrition education schedule outlined in the image (Figure 3.6) presents a comprehensive and multi-faceted approach for imparting knowledge about healthy eating habits and overall wellness. The program combine offline and online sessions, providing flexibility and accessibility to the selected participants in the study groups. Additionally, it incorporates various educational resources such as validated modules, expert talks, educational videos, leaflets, power point presentations, and social media

reels. This diverse range of materials and delivery methods catered to different learning preferences and ensured that the information is conveyed effectively. The schedule showed a commitment to engaging study subjects through various platforms, enhancing their overall understanding of the importance of diet and exercise in maintaining a healthy lifestyle.

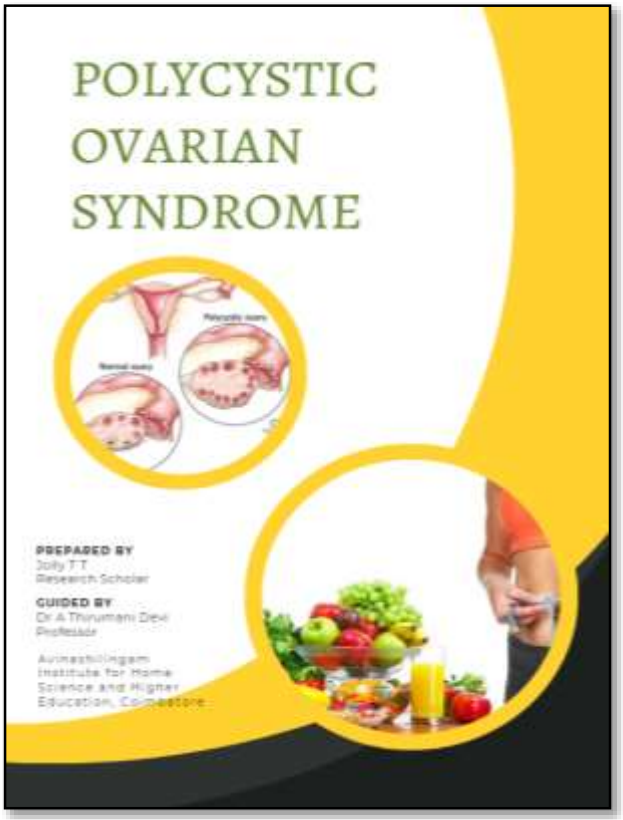
3.3.2.1 Development and validation of Nutrition Education Module of PCOS

A comprehensive PCOS Education Module submitted to the organised development and validation process. The process began with the assessment of needs through the literature review to identify the educational needs. An expert panel, which includes professors, gynaecologists, scientists and dieticians, designed the content, format and delivery methods incorporating evidence-based information on PCOS management. Brochures and booklets were made to help in the delivery of nutrition education intervention. It was proposed that the modules go through a thorough validation process that included having specialists review the content for correctness and relevancy and having cognitive interviews with the target group to assess how clear and acceptable they were. We changed things because of what you said. Then, the module was tried on a small group of people to see if it was possible, acceptable, and maybe even useful. It was then improved even more.

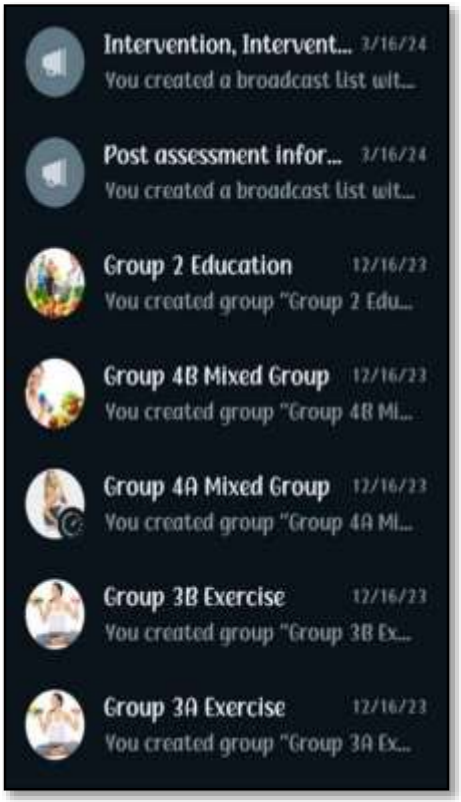
3.3.2.2 Creation of WhatsApp groups and a YouTube channel for Nutrition Education Intervention

A YouTube channel named "Empower Educate Thrive: Guide to PCOS Awareness" was created as part of this study to propagate essential knowledge about Polycystic Ovary Syndrome (PCOS). The role of a balanced diet and regular exercise in regulating PCOS was highlighted in the informative expert talks, educational videos and reels, and the channel served as an all-inclusive resource. For easy communication with the selected subjects, WhatsApp groups were also formed that facilitate discussions, provide support and share tips and resources.

Cover Page of YouTube Channel



Nutrition Education Booklet



WhatsApp Groups

Figure 3.7 Nutrition Education Programme Modules

3.4 Phase IV: Nutrition Intervention Programmes on Symptoms of PCOS

3.4.1 Grouping of the Participants

Young adult women with Polycystic Ovary Syndrome (PCOS) attended in full 90-day dietary intervention programs. There were four different groups. There were 30 subjects in each group. The first group was the control group and did not take part in any of the nutrition intervention programs.

The second group benefited from nutrition and health education and was provided a series of educational sessions in offline and online sessions. An in-depth knowledge regarding reproductive health, especially the metabolic complications of PCOS, was provided in the education sessions. Also, the importance of lifestyle modifications and principles of healthy eating was highlighted in the education sessions.

The third group engaged in a structured exercise programme supervised by the researcher along with the nutrition education sessions. Exercise session combined aerobic exercise, resistance training and flexibility exercises in order to improve insulin sensitivity, encourage weight loss and reduce androgen levels. With gradual progression in intensity and duration, exercise sessions were conducted five days per week.

Along with attending the nutrition education sessions and participating in the exercise program, fourth group participants were involved in a dietary intervention programme. They were provided with a health mix powder supplement which is having ability to improve insulin sensitivity, reduce oxidative stress and regulate hormonal imbalances associated with PCOS. Also, they were provided with diet counselling that helped them to design their diet and detailed the modifications according to their needs to develop a PCOS diet. Supplementation was thoroughly monitored throughout the intervention period of 90 days (ICMR's Guidelines for Biomedical Research involving Human Participants, 2017).

3.4.2 Nutrition Education intervention for the study participants

There were two offline sessions and three online sessions per week in the nutrition education intervention programme. Participants were gathered in classrooms

for interactive offline sessions. Power point presentations and nutrition education videos were applied to deliver knowledge on several aspects of PCOS. Pathophysiology, diagnostic criteria, lifestyle modification, dietary recommendations and stress management of PCOS were discussed during the sessions.

Google Meet facility was used for the online sessions during evening hours that allowed participants to attend from their homes after college hours. These online sessions provided a platform for discussion about the topics that we detailed in offline sessions. Doubts regarding the content of nutrition and health education was also clarified during online sessions. Duration of each session was 45 minutes followed by a 15-minute discussion.

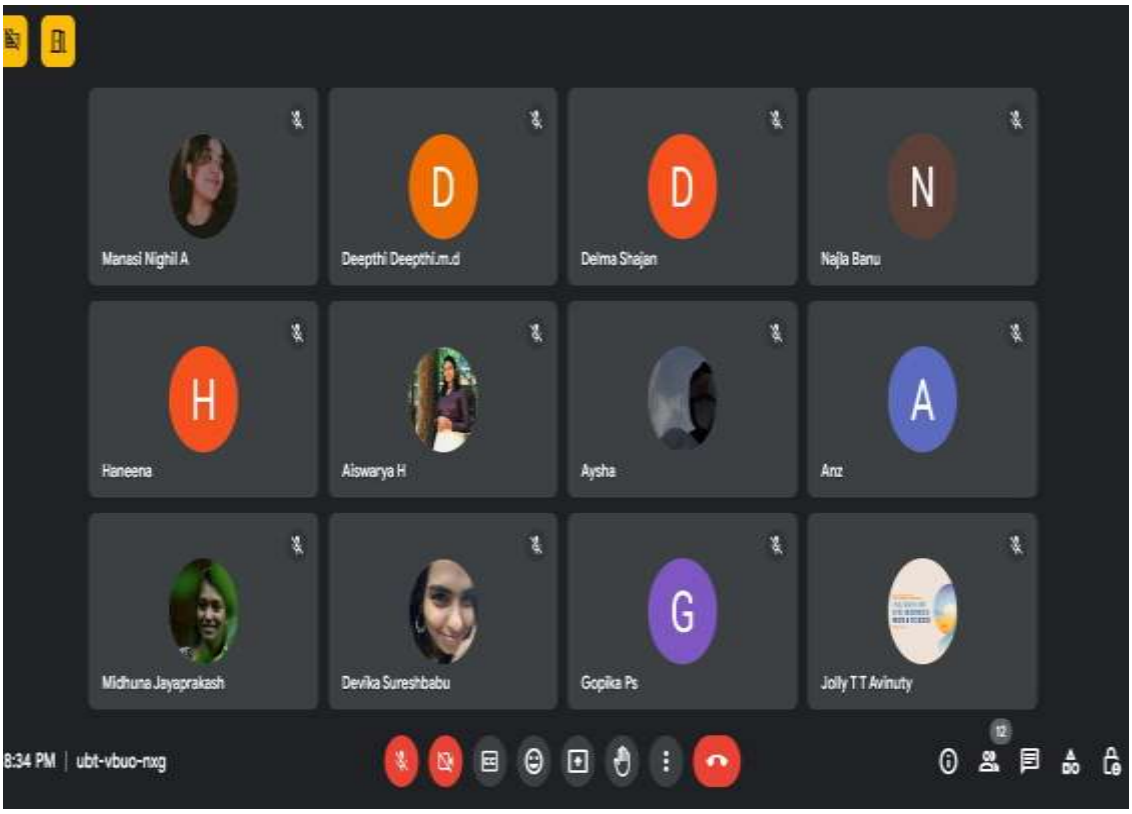


Figure 3.8 Nutrition Education by Google Meet

Varied teaching approaches, like PowerPoint presentations, films, interactive conversations, and both online and offline sessions, were used to meet the needs of different learners and make sure that information was shared effectively. Participants could choose between in-person and online sessions, which gave them more options and made it easier for them to get to the meetings. The pre-intervention evaluation made sure that the education was appropriate to their unique requirements.

Figure 3.9 Nutrition Education in offline

3.4.3 Aerobic Exercise Training for the study participants

A structured aerobic exercise training programme was added to the nutrition intervention programme to improve the status of PCOS of the selected participants. After the college hours from 3.00 PM to 3.30 PM, the exercise training was conducted on the basis of five days per week. The college indoor stadium was selected for the exercise training programme, which provided a manageable environment for exercise routine. A booklet containing detailed information about the exercise, its benefits and proper techniques was given to the selected participants before starting the exercise program.

Considering the special needs and limitations of the PCOS participants, the exercise plan was carefully designed by experts in the field. The sessions were led by the researcher and along with the participants actively attended the sessions. This kind of training made sure the proper guidance, motivation and supervision throughout the exercise sessions.

Figure 3.10 Aerobic Exercise Training

3.4.4 Nutrient Dense Health Mix Powder Supplementation for Dietary Intervention Programme

As part of a dietary intervention programme, a health mix powder was specially developed, including the ingredients which improve the symptoms of PCOS participants. Two packets of the 35-gram health mix powder were consumed by the participants per day for the period of 90 days. Soon after the aerobic exercise, they were instructed to consume one packet of health mix powder at the college indoor stadium. The other packet was recommended to be consumed at their house in the morning.

The researcher also helped the fourth group of patients with their diets. They got meal plans and personalised dietary advice based on their specific nutritional needs and support to make their PCOS condition better. Along with the nutritional intervention, it also helped them to improve their health status.

Persistent stress can worsen the symptoms in PCOS. During the diet counselling sessions for the management of PCOS, the role of stress control was highlighted. Stress-reducing dietary habits, such as including low glycemic index meals, were introduced to the participants during these sessions. Consumption of low glycemic food will maintain the stable blood sugar level and reduce the hormonal swings caused by stress. Anti-inflammatory foods were also advised to the subjects that mitigate inflammation linked to stress. Fatty fish and leafy greens are examples of anti-inflammatory foods. The role of lifestyle changes is emphasised, and asked to practice mindful techniques like meditation and yoga and exposure to sunlight. These factors can reduce stress levels and enhance emotional well-being. Importance of sufficient sleep also emphasised as crucial for hormone control. Usually, inadequate sleep increases stress levels and increases symptoms of PCOS.

We kept track of how well the people in the fourth group followed the dietary supplement schedule and meal recommendations by having them fill out self-reported diaries and check in with them on a regular basis. Depending on how each participant answered and any side effects or issues they reported, the dosage, formulation, or diet plans were changed.

In summary, fourth group participants were provided with a combined nutrition intervention programme, including nutrition education, exercise training, along with dietary supplementation that supports metabolic regulation, hormonal balance and overall symptom management of PCOS. The following tables show the validated Diet plans for Underweight, over weight and Normal BMI study subjects.

Table 3.9 Diet Plan for Underweight PCOS Subjects

Time	1800 Calorie
6.00 am Early Morning	Tea/Coffee – 1 Cup Skimmed milk 75 ml
8.00 am – 9.00 am Breakfast	Idli/ Dosa/ Appam/ Idiappam – 3nos Or Puttu- 3 small pieces/ Upma- 1 cup Sambar/ Dal Curry/ Bengal Gram Curry – ½ cup Egg- 1 no
10.00 am -11.00 am Mid-morning	Lemon Juice/ Buttermilk/ Vegetable soup/ Vegetable Salad/ Fruits – 100-150 g
1.00 pm – 2.00 pm Lunch	Rice – 1 ½ cup Or Chappati/ Dosa – 3 nos Sambar/ Dal – ½ cup Fish/ Chicken – 1 piece (50 g) Or Soya/ Pea – 50 g Cooked vegetables – 1 cup Salad – 1 cup, Rasam/ Buttermilk – 1 cup Raitha, Curd, Pachadi -1/2 cup
4.00 pm – 5.00 pm Tea time	Tea/ Coffee – 1 cup (Skimmed milk 75 ml) Biscuits – 4 nos Or steam-cooked snacks – 1 no
7.30 pm – 8.30 pm Supper	Rice – 1 ½ cup Or Chappati/ Dosa – 3 nos Fish/ Chicken – 1 piece (50 g) Or Soya/ pea – 50 g Cooked vegetables – 1 cup Salad – 1 cup
9.30 pm	Skimmed milk – 150 ml
Oil needed for one day - 4 teaspoon	

Table 3. 10 Diet Plan for PCOS Subjects with Normal BMI

Time	1500 Calorie
6.00 am Early Morning	Tea/Coffee – 1 Cup Skimmed milk 75 ml
8.00 am – 9.00 am Breakfast	Idli/ Dosa/ Appam/ Idiappam – 3nos Or Puttu- 3 small pieces/ Upma- 1½ cup Sambar/ Dal Curry/ Bengal Gram Curry – ½ cup Or Egg curry – ½ cup (Egg – 1 no)
10.00 am -11.00 am Mid-morning	Lemon Juice/ Buttermilk/ Vegetable soup/ Vegetable Salad/ Fruits – 100 g
1.00 pm – 2.00 pm Lunch	Rice – 1 ½ cup Or Chappati/ Dosa – 2 nos Sambar/ Dal – ½ cup Fish/ Chicken – 1 piece (50 g) Or Soya/ Pea – 50 g Cooked vegetables – 1 cup Salad – 1 cup, Rasam/ Buttermilk – 1 cup Raitha, Curd, Pachadi - ½ cup
4.00 pm – 5.00 pm Tea time	Tea/ Coffee – 1 cup (Skimmed milk 75 ml) Biscuits – 2 nos/ Veg Sandwich– 1 piece
7.30 pm – 8.30 pm Supper	Rice – 1 cup Or Chappati/ Dosa – 2 nos Fish/ Chicken – 1 piece (50 g) Or Soya/ pea – ½ cup (50 g) Cooked vegetables – 1 cup Salad – 1 cup
9.30 pm	Skimmed milk – 150 ml
Oil needed for one day – 3 teaspoon	

Table 3.11 Diet Plan for Overweight PCOS Subjects

Time	1200 Calorie
6.00 am Early Morning	Tea/Coffee – 1 Cup Skimmed milk 75 ml
8.00 am – 9.00 am Breakfast	Idli/ Dosa/ Appam/ Idiappam – 2 nos Or Puttu- 2 small pieces/ Upma- 1 cup Sambar/ Dal Curry/ Bengal Gram Curry – ½ cup Or Egg curry – ½ cup (Egg – 1 no)
10.00 am -11.00 am Mid-morning	Lemon Juice/ Buttermilk/ Vegetable soup/ Vegetable Salad/ Fruits – 100 g
1.00 pm – 2.00 pm Lunch	Rice – 1 cup Or Chapatti – 2 nos Sambar/ Dal – ½ cup Fish/ Chicken – 1 piece (50 g) Or Soya/ Pea – 50 g Cooked vegetables – 1 cup Salad – 1 cup, Rasam/ Buttermilk – 1 cup
4.00 pm – 5.00 pm Tea time	Tea/ Coffee – 1 cup (Skimmed milk 75 ml)
7.30 pm – 8.30 pm Supper	Rice – 1 cup Or Chappati/ Dosa – 2 nos Fish/ Chicken – 1 piece (50 g) Or Soya/ pea – ½ cup (50 g) Cooked vegetables – 1 cup Salad – 1 cup
9.30 pm	Skimmed milk – 150 ml
Oil needed for one day – 2 teaspoon	

3.5 Phase V: Effect of nutrition Intervention programmes on Nutritional knowledge, nutrition and health status and symptoms of PCOS

3.5.1 Effect of nutrition education on the nutritional knowledge of PCOS among the selected participants

The study aimed to improve nutritional knowledge about Polycystic Ovary Syndrome (PCOS) among young adult women through the nutrition education intervention. The questionnaire was administered as a pre-assessment before the nutrition intervention programme for 90 days, and the same questionnaire was used as a post-intervention assessment to evaluate changes in PCOS knowledge of the selected participants in the four study groups. Data analysis involved calculating total scores and individual item scores for the pre-and post-intervention questionnaires, and analysing the changes in overall nutrition and health knowledge scores and specific knowledge domains using appropriate statistical tests. Areas where nutrition knowledge gaps persisted or misconceptions remained after the nutrition intervention were identified for further targeted nutrition education or reinforcement.

3.5.2 Effect of nutrition interventions on the nutritional and health status of the selected participants

The present study assessed the effect of nutrition intervention programmes on PCOS participants' nutritional and health status in four different study groups. ABCD (Anthropometric Biochemical Clinical and Dietary) technique was employed to assess the nutritional and health status before and after the nutrition intervention programme. Haemoglobin, cholesterol and random blood sugar levels as biochemical parameters were also assessed both before and after the intervention.

3.5.3 Effect of nutrition intervention programmes on the Symptoms of PCOS among selected participants

The severity of PCOS symptoms for each participant in the four study groups was assessed using the Cronin Self-Assessment Questionnaire both before and after the nutrition intervention programmes. Post-intervention, data analysis focused on determining changes in PCOS symptom severity across the four groups by analyzing the pre- and post-intervention Cronin Self-Assessment Scores. Statistical analysis of the

changes in symptom scores enabled identification of the most effective intervention(s) for alleviating PCOS symptoms among the selected participants.

3.6 Phase VI: Data analysis and interpretation

To achieve the intended results and a scientific interpretation, the discrete data must be arranged in a methodical manner following data collection. The gathered information was methodically combined and subjected to statistical analysis in order to determine how various interventions affected the degree of PCOS symptoms. A significant part of turning data into information is done using statistical approaches. Once gathered, statistical data needs to be purposefully organised to highlight the key findings. SPSS (Version 21) was used for the statistical analysis of the data. The study groups' nutritional knowledge level, BMI level, and demographic features were all examined using descriptive statistics, including mean, range, and percentage. To determine the relationship and variations between the variables, the effect of nutrition and health education, as well as dietary intervention, on PCOS symptoms, inferential statistics such as ANOVA, paired t-tests, correlation analysis, and regression models were applied. The detailed discussions are presented in Chapter IV, Results and Discussion.