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## **Effectiveness of micronutrient rich supplement on the symptoms of polycystic ovarian syndrome among reproductive age women**

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**Abstract**---The incidence of PCOS the most common endocrinopathy has an alarming rise the recent years. The aim of the study is to evaluate the effectiveness of Nutrition intervention on reducing the Symptoms of PCOS in comparison to Nutrition education and Medication alone. The study was an Intervention among 93 women after systematic grouping of the subjects into three groups. The supplementation group received 30g of Nutritional supplement daily for 3 months, experimental group II received nutrition Education alone for three months and Control group was received allopathic medication. The mean age of the participants was 29.31±7.1. Nutrition intervention resulted in a significant reduction in weight, BMI, TSF body fat , waist circumference and hip circumference, total cholesterol levels , cholesterol to HDL ratio and total testosterone levels ( $p < 0.001$ ). Between the groups the mean change in testosterone ( $M = 0.145$ ,  $SD = 0.238$ ) showed significant difference with education group ( $M = 0.0023$ ,  $SD = 0.0646$ ), difference was remarkable between Intervention and control group ( $M = -0.0147$ ,  $SD = 0.1167$ ). The difference in total cholesterol, cholesterol to HDL ratio, waist circumference, and hip circumference indicated significant difference between the intervention and control group but no difference between intervention and education group. The mean change in BMI, and body fat percentage significantly differed between intervention and education group, intervention and control group. Regularity of association of intervention with menstrual cycle revealed that the Nutrition intervention had 9.1 times and Nutrition education group had 4.3 times higher chance of getting regular periods than those on nutrition education and control respectively. There was a significant considerable decrease in the mean value of Stress ( $p = 0.000$ ) among

all the groups .In the nutrition intervention group acne score physical activity level showed considerable increase compared to other groups

**Keywords**---Polycystic ovarian syndrome, Nutrition Intervention, anthropometric parameters, biochemical parameters.

## Introduction

Polycystic Ovarian Syndrome is a difficult to define heterogeneous disorder usually characterised by biochemical abnormality such as elevated lipid profile and androgen levels in blood, menstrual cycle irregularities, lack of ovulation, enlarged ovaries with numerous cysts and infertility. Insulin increases the production of androgens by directly affecting the ovarian theca cells (Ovalle and Aziz 2002).Higher than normal androgen level in women can prevent the ovaries from releasing egg during each menstrual cycle, and can cause extra hair growth and acne. Sedentary lifestyle and improper dietary habits are often noticed among the women with PCO syndrome. Intake of food of low nutritional value and high calorie further leading to insulin resistance along with increasing obesity (Asemi 2015).According to recent studies PCOS was diagnosed among 9.38% of patients consulted the gynaecologist in a hospital based in South India. Women in the age group of 13-20 years showed a greater prevalence (39.25%) followed by 21-30 years as 27.1 percent and 41-50 years as 11.2 percentage (Cheema et al, 2019).As per the studies done by Gowri and Venkata Ramana 2020 Women with high level of education under low economic status, adverse living and working conditions, stress and family life style modifications are the main associated factors for PCOS.The common features usually include excessive weight gain, oligomenorrhea/Amenorrhoea ,Increased triglyceride and insulin levels in blood, acne, hirsutism, etc. It is also associated with menstrual disorders and infertility usually occurring due to chronic anovulation. PCOS is responsible for about 75 per cent of anovulatory infertility in women during their reproductive years (Teede *et.al*;2018).Lifestyle management is followed as the first-line treatment in PCOS which helps to improve hormonal disturbances and to prevent future reproductive and metabolic complications (Moran, *et.al*; 2017).Previous studies have inscribed the role of micronutrients in reducing insulin resistance and its role in improving the symptoms (Ni Y 2015).Abdominal obesity, hypertension, impaired glucose tolerance usually observed with low serum levels of Vitamin D (Zaeemzadeh 2021). Some of the previous studies have also imposed strict restriction of calories and was found that calories alone will not significantly improve biochemical and anthropometric parameters even along with physical activity (Gann 2003). The role of a calorie restricted balanced diet and healthy lifestyle is important .Hence the present study focusing on structured dietary intervention along with a micronutrient rich formula on reducing the anthropometric, biochemical and clinical symptoms is very relevant. Antioxidant intake in the diet, including  $\beta$ -carotene, zinc, selenium, Vitamin E and Vitamin C protects against free radicals there by preventing the adverse effects such as oxidative stress, damage of cellular membranes (Ruder 2009). Schaefer 2019 in the recent studies have proved the role of micro nutrients in fertility and its adverse impact on female fertility if consumed in inadequate amounts.Folate, vitamins B6, B12, vitamin D, and iron all have roles in mechanisms that could

affect fertility. Folate and Vitamin A important for the quality of the oocyte, fertilisation and implantation. The present research is undertaken to determine the role of micronutrient rich indigenous supplement and a structured diet plan in improving the metabolic, biochemical and clinical symptoms of PCOS and to compare with group who were given nutrition education and medication alone. (Szczuko 2021)

## Objectives

Assess the Effectiveness of Nutrition Intervention in terms of anthropometric measurements, biochemical parameters and clinical symptoms of PCOS. Comparison of symptoms of PCOS between the Nutrition intervention, Nutrition Education and the Medication group.

## Selection of Research Tools and Methods

The study was conducted in the Outpatient Department of Gynaecology and Dietetics of Believers Church Medical College Hospital, located in Thiruvalla, Kerala, India. Women of reproductive age group of 20-45 years consulted in the Gynaecology and Dietetics outpatient department between January 2019 and June 2021 were screened using the validated screening tool Rotterdam criteria (2003). The Rotterdam criteria for selection of Subjects for the study is represented in table I.

Table I Selection of subjects for the study based on Rotterdam 2003 criteria

<b>To define PCOS the subject should have any two of the three features</b>
1. Clinical features of Oligomenorrhea Irregular menstrual cycle - Absence of Menstruation for more than 35 days - 182 days, Amenorrhea (Absence of menstruation for more than 182 days)
2. Ultra sound scan with at least 12 follicles of 2-9mm in diameter with a pearl like appearance arranged in the ovarian stroma, Ovarian volume >10mm <sup>3</sup>
3. Clinical or biochemical evidence of hyperandrogenism (Hirsutism, acne, androgenic alopecia or elevated serum androgen)

Research tools were formulated on the basis of the objectives. For collection of data, interview schedule using specially designed questionnaire was used. In the interview schedule, demographic profile in terms of age, marital status, educational qualification, occupation, monthly income and dietary pattern were included. A comprehensive questionnaire consisting demographic, dietary and lifestyle pattern, reproductive, menstrual history and food frequency questionnaire, was filled during face to face interview both in paper and the "google docs" forms for easiness of analysis by the investigators. The questions on scoring of symptoms of Polycystic Ovary Syndrome was adapted from International evidence based guideline for assessment and management of Polycystic Ovarian Syndrome (Teede, et al, 2018). Modified Ferriman-Gallwey (FG) score was used for screening and quantitative evaluation of clinical hyperandrogenism using nine parts (upper lip, chin, chest, upper and lower abdomen, thighs, upper and lower back and upper arm). Hair growth was rated

from 0 (no growth of terminal hair) to 4 (extensive hair growth) in each of the nine body areas. A score  $\geq 8$  was indicative of androgen excess. Patients were also screened for oily skin and acne suggestive of clinical features of androgen. Anthropometric measurements such as height (cm), weight (kg) were measured using standard procedures. Waist circumference in cm (at waist level minimum circumference), hip circumference in cm (maximum circumference below the level of umbilicus) was measured. Obesity was assessed according to Asian Classification of BMI, calculated as  $BMI = \text{weight (Kg)} / \text{height (m)}^2$ , Classified as  $< 18.5 \text{ kg/m}^2$  Underweight,  $18.5\text{-}22.9 \text{ kg/m}^2$  Normal,  $23.0\text{-}24.9 \text{ kg/m}^2$  Over weight and  $\geq 25 \text{ kg/m}^2$  Obese. Body fat distribution was assessed by measurements of the waist to hip ratio (WHR). A WHR  $< 0.80$  was considered normal. Random blood glucose and lipid profile was done to diagnose impaired glucose tolerance, dyslipidaemia and metabolic syndrome, normal values taken were  $RBS < 140 \text{ mg/dl}$   $TC < 200 \text{ mg/dl}$ ,  $HDL > 50 \text{ mg/dl}$ ,  $LDL < 130 \text{ mg/dl}$ ,  $TG < 150 \text{ mg/dl}$  and  $VLDL < 50 \text{ mg/dl}$ . Additionally, blood pressure was measured in patients in sitting position,  $\geq 140/85$  was considered as hypertension. Total testosterone values were analysed as a part of hormonal assay. Testosterone levels  $> 0.59 \text{ ng/mmol}$  was considered as high. Ultrasonography results were also analysed. The study design was registered in the Clinical Trial Registry of India (CTRI), ICMR and received registration number as **CTRI/2021/09/036850**. The Research proposal to conduct the nutrition intervention was presented in the Institutional Human Ethical Committee (IHEC) of Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore and obtained approval with the Registration number of **AUW/IHEC-1920/FSN/FHP-01** and also Ethical clearance from Believers Church Medical College Hospital Thiruvalla, Kerala with approval number **IEC/2020/02/126**.

### **Formulation and Evaluation of Nutrient dense Health mix powder and Nutrition Education for PCOS subjects**

Well planned diet is always a cornerstone in treatment of any lifestyle disorders. Food supplementation is one of the reliable method to make the diet well planned to fulfil the requirement. Nutritional supplement was prepared by using the commonly available ingredients in the local market, low cost seasonal and easy to prepare ones. The micronutrient rich supplement is expected to enhance the health status in terms of improvement of biochemical and metabolic profile, mental health, ovulation, and menstrual regularity in PCOS subjects. Recent studies about PCOS focused on mineral supplementation in order to remove pathologic situations from PCOS. Micronutrients such as calcium, iron, selenium, zinc, magnesium, phosphorus chromium, fibre rich supplement was prepared using the seeds and grains available in the market.

Grains and seeds were powdered and supplement was formulated by mixing the powdered grains and seeds in the ratio 1: 5. Two top scored variations as per the sensory evaluation was selected for the intervention. The nutrition intervention group was asked to consume 30g of the supplement every day evening for a period of 90 days. Supplement I was asked to consume for the first 15 days followed by Supplement II for the remaining 15 days, they were also given a structured low calorie diet plan to be followed for 3 months. The nutrition education group was given detailed education along with structured diet plan,

they were asked to continue the plan for 3 months. The medication (control group) was asked to consume the medications alone. Microbial count of the nutritional supplement I and II was analysed. The shelf life evaluation was done after 1 month and the plate count was  $1.2 \times 10^4$  cfu/g for supplement I and  $1.8 \times 10^4$  cfu/g supplement II respectively. The supplement was prepared without preservatives and the subjects were asked to keep the supplement in the refrigerator throughout the supplementation period. The nutrient analysis of the supplement was also done. The ingredients used in the formulation of nutritional supplement is represented in figure 1

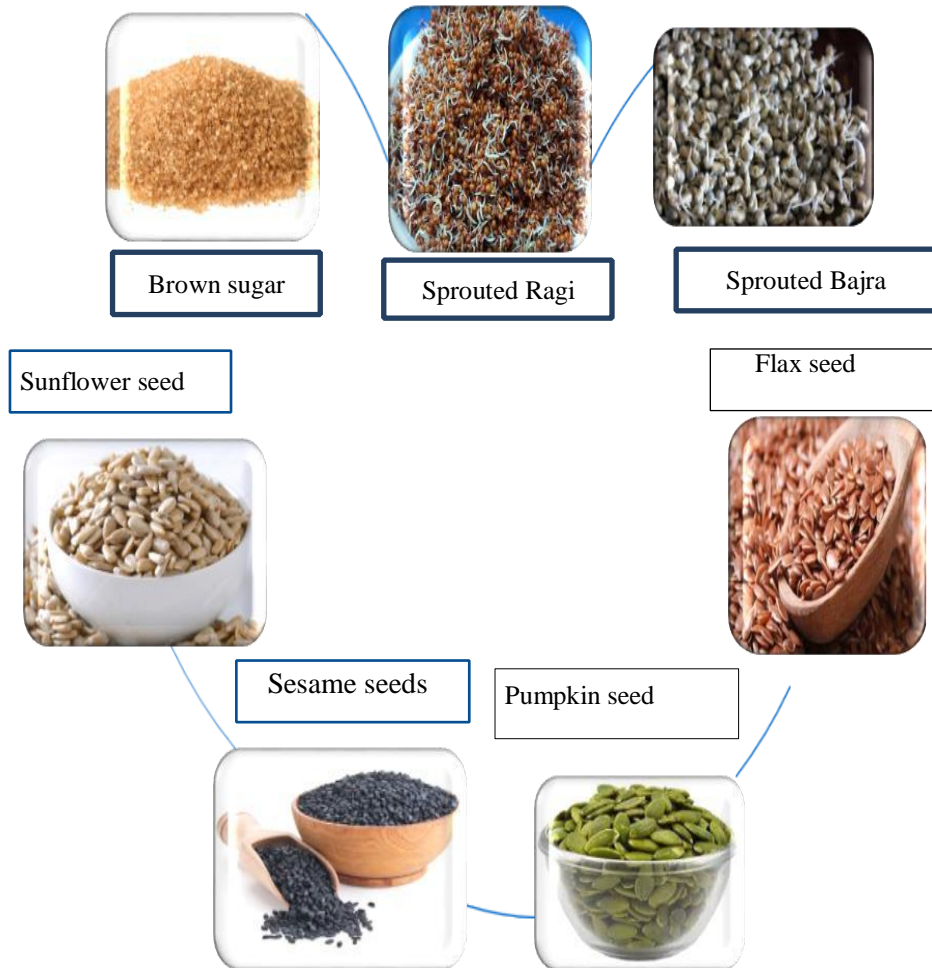


Figure 1. Ingredients for the Micronutrient rich supplement powder

### **Selection of subjects for the study**

Women in the age group of 20-45 years, diagnosed using Rotterdam criteria (2003) were considered for the Nutrition intervention study. The purposive sampling method was adopted for selection of participants for the intervention study. The sample size was calculated using the standard formula suggested for

clinical trials by considering type one error ( $\alpha$ ) of 0.05 and type two error ( $\beta$ ) of 0.20 (power = 80%). Based on a previous study (Garg et.al; 2015) 0.11 ng/mL as SD and 0.09 ng/mL as the difference in mean (d) of total testosterone concentrations as used primary variable. Based on this, 25 subjects were needed in each group .Considering a dropout of five subjects in each group, a total 30 subjects per group were selected. In the present study 93 subjects were selected and 32 subjects were systematically participated in the intervention for the period of three months.

### **Grouping of subjects based on anthropometric, biochemical and clinical parameters**

Selected subjects were further grouped based on the BMI criteria for Asians. Waist hip ratio was also checked and distributed as normal with  $\leq 0.8$  and abnormal as  $>0.8$ . Table II distributed the 93 subjects prior to intervention, 3.3 percent of subjects were in the BMI distribution 18.5-22.9 , 6.5 percent were between 23-24.9 ,43 percent were between 25-29.9 , 53.7 percent were between 30-39.9 and 4.3 percent were above 40years .Waist hip ratio was below 0.8 for sixteen percent of cases and above 0.8 for 83 percentage of cases. In the nutrition intervention group 75percent subjects were presented with PCOM morphology on USG scan and 25 percent without ovarian cyst. In the Nutrition Education group 93.5 percent and in the Medication group (Control) 90 percent of subjects were presented with PCO morphology and the remaining were not having any ovarian cyst .Forty three percent of subjects were having increased testosterone levels of  $>0.59$ ng/mmol . Menstrual cycles were irregular for 81 percent of subjects in the Intervention group and Education group and 87 percent of subjects in the control group

Table II Distribution of subjects prior to intervention based on Anthropometric and Biochemical Parameters

<b>BMI</b>					
	<b>18.5-22.9</b>	<b>23-24.9</b>	<b>25-29.9</b>	<b>30-39.9</b>	<b>&gt;40</b>
Nutrition intervention	1	2	13	13	1
Nutrition Education	1	2	12	14	2
Control	1	2	15	13	1
<b>WAIST HIP RATIO</b>					
	<b><math>\leq 0.8</math></b>		<b><math>&gt;0.81</math></b>		
Nutrition intervention	5		25		
Nutrition Education	8		23		
Control	2		30		
<b>OVARIAN CYST</b>					
	<b>PRESENT</b>		<b>ABSENT</b>		
Nutrition intervention	24		8		
Nutrition Education	29		2		
Control	27		3		
<b>TESTOSTERONE</b>					
	<b><math>\leq .59</math></b>		<b><math>&gt;0.59</math></b>		

Nutrition intervention	20	12
Nutrition Education	23	8
Control	17	13
<b>MENSTRUAL CYCLE</b>		
	<b>REGULAR</b>	<b>IRREGULAR</b>
<b>Intervention</b>	<b>6</b>	<b>26</b>
<b>Experimental II</b>	<b>6</b>	<b>25</b>
<b>Control</b>	<b>4</b>	<b>26</b>

## Result and Discussion

Totally 93 participants with a mean age of  $29.31 \pm 7$  were selected for the study. Selection of subjects for the Intervention study was done using purposive sampling method. Among the selected 93 subjects 32 subjects were systematically participated in the intervention, 31 subject participated in the Nutrition Education Programme and remaining 30 subjects were given medications for the period of three months. The mean age of the Nutrition intervention group was  $25.97 \pm 4.78$ , for Control  $25.00 \pm 5.45$  and for nutrition Education  $27.84 \pm 5.38$ . There were no significant differences between the three groups. Table III summarises the demographic profile of the participants

### Demographic profile of the selected subjects

Table III represented the systematic Grouping of PCOS subjects based on demographic and socio economic profile such as the age group, religion, marital status and socio economic class. The data revealed that the 93 subjects selected for the study were between 20-45 years age groups 73 percent cases were in the age group of 20-29, 24 percent belonged to age group 30-39 and 1 percent in the age group 40-45 years. The participants comprised of the study were 52.6 percentage Christians, 38.7 percent Hindus and 8.6 percent Muslims. Marital status of participants revealed that 54.8 percent were married and 44 percent were unmarried and 1 percent as separated. Socio Economic classification concluded that 43 percent subjects belonged to Lower middle class family, 36.5 percent in the upper middle class, 15 percent in upper lower class and 5.3 percent in the upper class

Table III Demographic profile of the selected subjects

AGE	Frequency	ExpI	Exp II	Control	Percent	Cumulative Frequency	Cumulative (%)
20-29	68	24	20	24	73.12	68	73.12
30-39	24	8	11	5	25.81	92	98.92
40-45	1	0	0	1	1.08	93	100
<b>RELIGION</b>							
Christian	49	17	17	15	52.69	49	52.69
Hindu	36	11	12	13	38.71	85	91.4
Muslim	8	4	2	2	8.6	93	100

MARITAL STATUS							
Married	51	16	12	23	54.84	41	44.09
Single	41	15	19	7	44.08	92	98.92
Separated	1	1	0	0	1.08	93	100
SOCIO ECONOMIC CLASS							
Upper	5	2	3	0	5.38	5	5.38
Upper middle	34	13	7	14	36.56	39	41.94
Lower middle	40	12	16	12	43.01	79	84.95
Upper lower	14	5	5	4	15.05	93	100

### **Impact of the Nutrition Intervention on Anthropometric parameters at baseline and following Interventions**

Table IV compares the the effect of administration of micronutrient rich nutritional supplement powder, Nutrition education and allopathic medication alone on the anthropometric measurements (weight, BMI, body fat, waist circumference, hip circumference, total Skin fold Thickness, body water and visceral fat )at baseline and following 12 weeks of the study. At the starting of the study, no significant differences were observed between the three groups in terms of any of the anthropometric variables. Findings of the present study showed that after 12 weeks of intervention there was a significant reduction in anthropometric measurements in the Nutrition intervention group and Nutrition Education group. All the anthropometric variables were comparable at baseline as there were no significant difference in age, weight, BMI, TSF, WC, HC, body fat, body water and visceral fat ( $P > 0.05$ ) among the subjects. Among the Nutrition Intervention group we could see larger drop in weight, BMI, waist circumference, hip circumference, body fat and visceral fat levels ( $p < 0.001$ ) with a mean weight reduction in the 3 month period was 4.7kg .The reduction in BMI, TSF, waist circumference, hip circumference, and body fat was 1.9kg/m<sup>2</sup>, 1.4cm, 1.5cm, 1.08cm and 1.5% respectively. However we could not see a significant difference in waist hip ratio ( $p = 0.324$ ) and body water ( $p = 0.968$ ). A significant difference between pre and post values among the Nutrition education group for Weight, BMI ,TSF and Body fat and has shown a drop of 1.9kg, 1kg/m<sup>2</sup> 0.54cm, 0.72cm respectively ( $p < 0.001$ ). Waist circumference ( $p = 0.640$ ), Hip circumference ( $p = 0.541$ ) and body water ( $p = 0.682$ ) was not significantly differed .In the Control we did not see a drop in any of the biometric variable in except for waist hip ratio ( $p = 0.004$ ), may be because the people were already taking medications before the study began. After the medication intervention there was no significant drop in weight , BMI Waist circumference , Hip circumference , body fat , body water and visceral fat (  $p > 0.05$ ). But at baseline all the biometric variables were comparable as represented in table IV.



Table IV Comparison of Anthropometric parameters between the groups at baseline and following intervention

Variable		Intervention ( N=32)	Nutrition Education(N=31)	Control (N=30)	P*
Weight	Baseline	74.77 ± 10.50	75.65 ± 16.27	74.73 ± 12.11	0.953
	After 3m	70.05 ± 10.43	73.73 ± 16.21	74.91 ± 11.32	0.304
	Changes	-4.7188± 2.6441	-1.9226± 2.1175	0.18±3.49	0.000
	P**	<.0001	<.0001	0.78	
BMI	Baseline	29.95 ± 4.35	30.82 ± 5.82	30.43 ± 4.18	0.776
	After 3m	28.05 ± 4.27	29.82 ± 5.73	30.53 ± 4.07	0.110
	Changes	-1.9031±1.0669	-1.0035±1.2706	0.10±1.30	0.000
	P**	<.0001	0.0001	0.68	
TSF	Baseline	27.98 ± 6.69	27.55 ± 7.62	27.77 ± 7.25	0.971
	After 3m	26.49 ± 5.82	27.00 ± 7.35	27.80 ± 6.99	0.745
	Changes	-1.4969± 2.2544	-0.5452±0.8477	0.03±1.17	0.01
	P**	0.0007	0.0012	0.89	
WC	Baseline	36.73 ± 3.12	37.97 ± 4.10	36.22 ± 4.90	0.231
	After 3m	35.18 ± 3.62	37.84 ± 4.75	36.21 ± 4.34	0.049
	Changes	-1.5469±1.2051	-0.129±1.5219	-0.01±1.28	0.000
	P**	<.0001	0.6403	0.97	
HC	Baseline	41.90 ± 3.47	43.39 ± 4.04	41.81 ± 4.58	0.231
	After 3m	40.82 ± 3.45	43.22 ± 4.19	41.99 ± 4.18	0.059
	Changes	-1.0844±1.089	-0.1677±1.5105	0.0180±1.16	0.000
	P**	<.0001	0.541	0.41	
BODY FAT	Baseline	35.85 ± 7.68	39.26 ± 6.86	35.77 ± 7.98	0.120
	After 3m	34.32 ± 7.93	38.54 ± 6.79	35.51 ± 7.84	0.079
	Changes	-1.5375±1.179	-0.7194±0.834	-0.26±1.12	
	P**	<.0001	<.0001	0.21	0.000
BODY WATER	Baseline	52.55 ± 2.53	51.55 ± 3.35	52.09 ± 2.48	0.372
	After 3m	52.56 ± 2.40	51.63 ± 3.05	52.29 ± 2.57	0.376
	Changes	0.0125±1.743	0.0871±1.1727	0.20±1.11	0.870
	P**	0.97	0.6822	0.34	
VISCERAL FAT	Baseline	9.30 ± 1.73	9.60 ± 2.66	9.39 ± 1.81	0.779
	After 3m	8.83 ± 1.96	9.44 ± 2.60	9.50 ± 1.86	0.357
	Changes	-0.4687±0.7213	-0.1645 ±0.243	0.11±0.64	.079
	P**	0.001	0.0007	0.33	

BMI body mass index, WC Waist circumference, HC Hip circumference, TSF Total skinfold thickness Data are presented as mean (SD) or geometric mean (SD). \* Calculated using one-way ANOVA. . \*\* Calculated using paired sample t-test

Impact of interventions in terms of anthropometric parameters between the groups showed that there were significant differences in weight loss, reduction in BMI, TSF, waist circumference, and hip circumference across the three intervention groups. The ANOVA results suggests that the weight difference (F<sub>2,90</sub>=24.215, P=0.000), BMI difference ( F<sub>2,90</sub>=20.502,p=0.000. Waist circumference difference ((F<sub>2,90</sub>=12.769,P=0.000) Hip-circumference (F=8.294,P=0.000), body fat percentage difference (F<sub>2,90</sub>=11.6,p=0.000)between

the groups differ significantly. There were no significant difference in the mean change values of body water ( $p=.870$ ) and visceral fat ( $p=0.079$ ) between the groups. The individual differences between groups indicated that the mean weight loss of nutrition intervention group ( $M=4.75$   $SD=2.652$ ) was significantly different from Education group ( $M=1.923$ ,  $SD=2.118$ ). Weight loss in the Control groups ( $M=1.767$ ,  $SD=3.49084$ ) also differed significantly from the Nutrition intervention group. A significant difference between the Nutrition education and Control was also observed. The Mean difference in BMI between Nutrition intervention ( $M=1.88$   $SD=1.07$ ) and Education ( $M=1.003$ ,  $SD=1.270$ ), Nutrition intervention and Control ( $M=-.100$ ,  $SD=1.304$ ) differed significantly. The mean difference in waist circumference in nutrition intervention ( $M=1.54$ ,  $SD=1.205$ ) differed significantly from Nutrition education ( $M=0.129$ ,  $SD=1.521$ ). No significant differences observed between Education group and Control ( $M=0.010$ ,  $SD=1.279$ ). Significant difference in hip circumference ( $M=1.084$ ,  $SD=1.088$ ) between nutrition intervention and Education group ( $M=0.167$ ,  $SD=1.510$ ) were observed, whereas the difference was not significant between Education and Control ( $M=-.176$ ,  $SD=1.558$ ). The mean difference in body fat percentage after Nutrition intervention ( $M=1.53$ ,  $SD=1.179$ ) differed significantly from Education group ( $M=0.719$ ,  $SD=0.834$ ) and Control group ( $M=0.264$ ,  $SD=1.117$ ). No significant difference seen between Education and Control group ( $p>0.05$ ). Previous studies have proved the beneficial effects of supplementation of multiple micronutrients vitamin, E, C Selenium, chromium, magnesium and calcium on PCOS-related symptoms such as immature oocytes, hyperinsulinemia, hyperandrogenism, increased BMI, cardiovascular disorders, and mental and psychological problems. (Günalan, 2018)

### **Impact of the Nutrition Intervention on Biochemical parameters at baseline and following Interventions**

Table V indicated that there were no differences between the interventions for haemoglobin and LDL levels. Significant difference between the treatment effects was seen in change in Total testosterone, cholesterol to HDL ratio, and change in total cholesterol levels ( $p<0.05$ ).

Table V Comparison of Biochemical parameters between the groups at baseline and following intervention

Variable	Nutrition Intervention (N=32)	Nutrition Education (N=31)	Medicine (Control) (N=30)	P*	
haemoglobin	Baseline	12.65±1.141	12.19±1.380	12.626±1.270	.278
	After 3m	12.80±.97 9	12.28 ± 1.00	12.54±.82	.090
	Changes	-.1531±.99709	-.0871±.69366	.0833±.90785	.554
Cholesterol	Baseline	206.87±37.47	195.16±39.72	197.86±40.86	.467
	After 3m	195.59±23.66	193.3871±38.51	207.1667±41.20	.264
	Changes	11.28±28.439	1.774±16.169	-9.30±24.833	.004
	P**	0.032	0.545	0.049	
Triglycerides	Baseline	126.06±65.10	139.22±92.13	133.60±77.64	.802

	After 3m	108.84±39.813	132.61±76.58	141.16±78.042	.146
	Changes	17.218±46.87	6.6129±23.408	-7.56±45.37	0.05
	P**	0.046	0.126	0.368	
Cholesterol to HDL ratio	Baseline	4.826±1.27	4.529±1.362	4.569±1.12	.599
	After 3m	4.27 ±.98	4.28 ±1.07	4.69 ±1.14	.221
	Changes	.5563±.905	.2484±.948	-.1223±.88	.017
	P**	0.0015	0.155	0.457	
LDL	Baseline	134.12±34.46	126.35±30.33	128.70±30.05	.609
	After 3m	128.71±25.50	121.35±30.5	132.06±28.76	.322
	Changes	5.4063±25.218	6.4516±11.70	-2.8333±15.24	.1040
	P**	0.234	0.062	0.242	
Testosterone	Baseline	.587±.39	.442±.286	.52±.27	.214
	After 3m	.442± .236	.440 ±.290	.538 ±.261	.263
	Changes	.1450±.238	.0023±.064	-.0147±.116	.000
	P**	0.0017	0.847	0.496	

The test indicated that the mean Cholesterol difference of Nutrition intervention group (M=11.28 SD=28.439) was significantly differed from Control groups (M=-9.30, SD=24.833), but no difference was observed between Nutrition intervention and Education group. Studies by Romualdi 2008 reported 36 mg/d soy isoflavone genistein treatment in women with PCOS for three months provided a significantly improved lipid profile. The difference in TG values showed significant difference between Nutrition intervention (M=17.218, SD=46.873) and Control (M=-7.56, SD=45.37), but was not significantly differed from Nutrition education group (M=6.612, SD=23.408). The difference in in cholesterol to HDL ratio indicated significant difference between the intervention (M=0.556,SD=0.905) and Control (M= -.1233, SD =0.948) but no significant difference between intervention and Education group (M= 0.248,SD=0.948).In terms of testosterone changes intervention group (M=0.145, SD 0.238) showed significant difference with education group (M=0.0023,SD=0.0646), difference was remarkable between Intervention and control group (M=-0.0147,SD=0.1167).Nutrition Intervention group had significant reduction in total cholesterol levels (M= 11.28, SD=28.439), Triglycerides (M=17.218, SD=46.87), Cholesterol ton HDL ratio and total testosterone levels. Haemoglobin levels had a significant increase (M=.1531, SD=.997) within the group as evidenced by paired sample t test.In the control group the only significant difference between the treatment effects was seen in cholesterol levels. In the nutrition education group any of the variable has shown significant difference within the group. Vitamin D and Myoinositol supplementation contribute to overcome complications of PCOS including hyperandrogenism (Jakimiuk 2014).Very long chain polyunsaturated fatty acids, including omega-3 and omega-6 fats have a hypotriglyceridaemia effect and may ameliorate inflammation in metabolic syndrome (Lopez 2012)

Table VI Comparison of Anthropometric and Biochemical parameters between the groups following intervention

Variables	Nutrition intervention (N=32)	Nutrition education (Experimental II (N=31))		Medicine Control (N=30)	
	Mean $\pm$ SD	Mean $\pm$ SD	Pr >  t	Mean $\pm$ SD	Pr >  t
Weight	70.05 $\pm$ 10.43	73.73 $\pm$ 16.21	0.2908	74.91 $\pm$ 11.32	0.0838
BMI	28.05 $\pm$ 4.27	29.82 $\pm$ 5.73	0.169	30.53 $\pm$ 4.07	0.0228
TSF	26.49 $\pm$ 5.82	27.00 $\pm$ 7.35	0.7582	27.80 $\pm$ 6.99	0.425
WC	35.18 $\pm$ 3.62	37.84 $\pm$ 4.75	0.015	36.21 $\pm$ 4.34	0.3137
HC	40.82 $\pm$ 3.45	43.22 $\pm$ 4.19	0.0154	41.99 $\pm$ 4.18	0.2324
Body fat	34.32 $\pm$ 7.93	38.54 $\pm$ 6.79	0.0268	35.51 $\pm$ 7.84	0.5538
Body water	52.56 $\pm$ 2.40	51.63 $\pm$ 3.05	0.1839	52.29 $\pm$ 2.57	0.671
Visceral fat	8.83 $\pm$ 1.96	9.44 $\pm$ 2.60	0.2985	9.50 $\pm$ 1.86	0.1707
HB	12.80 $\pm$ 0.97	12.28 $\pm$ 1.00	0.0388	12.54 $\pm$ 0.83	0.2634
Cholesterol	195.60 $\pm$ 23.67	193.40 $\pm$ 38.52	0.786	207.20 $\pm$ 41.21	0.1855
TG	108.80 $\pm$ 39.81	132.60 $\pm$ 76.58	0.131	141.20 $\pm$ 78.04	0.0482
HDL	48.86 $\pm$ 10.72	45.72 $\pm$ 6.07	0.1565	44.86 $\pm$ 7.42	0.0917
LDL	128.70 $\pm$ 25.51	121.40 $\pm$ 30.56	0.3026	132.10 $\pm$ 28.76	0.629
Cholesterol /HDL Ratio	4.27 $\pm$ 0.99	4.28 $\pm$ 1.07	0.9654	4.69 $\pm$ 1.15	0.1255
Testosterone	0.44 $\pm$ 0.24	0.44 $\pm$ 0.29	0.9742	0.54 $\pm$ 0.26	0.1378

### Effectiveness of Interventions between group following interventions

Table IV demonstrated comparison of Anthropometric and biochemical parameters between the groups following intervention. BMI ( $p=0.0228$ ) and TG ( $p=0.0482$ ) was significantly differed between the Nutrition intervention and Control group. There was a significant decrease in the waist circumference ( $P=0.015$ ), hip circumference ( $0.0154$ ), body fat percentage ( $p=0.0268$ ), haemoglobin levels ( $p=0.0388$ ) between the Nutrition Intervention and Nutrition education group

### Regularity of association of Intervention with Menstrual cycle

Level of regularisation of menstrual cycle post intervention is represented in figure 3. At base line there was no significant association of menstrual cycle regularity between groups. Those subjects who took Nutrition intervention had

9.1 times higher chance of getting regular periods than those on nutrition education alone (OR: 9.1, CI 2.84-29.146) and 4.3 times higher chance of getting regular periods compared to medication group ( Control ) (OR:4.33 CI 1.385-13.552). As per studies by Ramanand et al 2013 Irregular menstrual cycles is the consequence of the pathogenic feature anovulation in PCOS .Therefore, persistent menstrual irregularities due to anovulation can be considered as a better predictors of PCOS compared to biochemical parameters It is clear that nutrition-associated signalling pathways play a central role in the regulation of ovarian follicle growth and ovulation rates .Jakimiuk 2014 demonstrated the role of s Inositol and omega 3 supplementation in improving the metabolic and reproductive parameters associated with PCOS .Studies have demonstrated restoration of menstrual function with calorie controlled diets , even if there were mild or a moderate weight loss of < 5 percentage (Lefebvre, 1997)

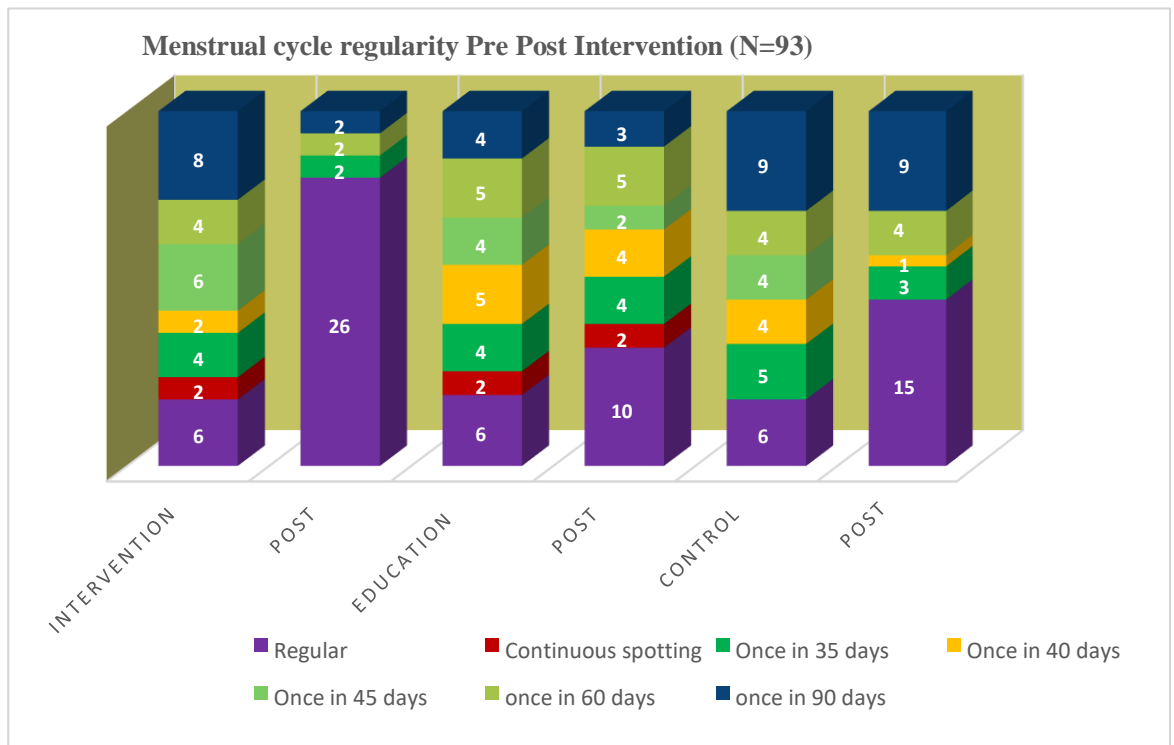


Figure 2 Menstrual cycle regularity pre post intervention

**Effectiveness of intervention on Clinical parameters**

Table VII depicted the significant difference in the Nutrition intervention group in terms of clinical symptoms of Acne and Stress level .Physical activity also showed a significant difference( p<0.001 ) with a mean increase of ( 5.03± 8.26 ) from the previous score. No significant difference seen in the hirsutism level between pre and post interventions .There was a significant considerable decrease in the mean value of Stress ( M=-4.22 ± 5.68) (p=0.000) .Acne score showed significant increase( M=5.56 ,SD =13.4) (p=0.026 ), Physical activity level also showed considerable increase ( M=5.03, SD=8.27 ) =0.0017).In the Education group and

control group a significant decrease in the mean values of stress ( $p < 0.05$ ) was observed. Hirsutism, acne and physical activity levels were not significantly differed among the subjects in the Education and Control group.

Table VII Effectiveness of intervention on Clinical parameters among group

Variable	Nutrition intervention (N=32)			Nutrition Education (N=31)			Control(N=30)		
	Mean $\pm$ SD	t	Pr >  t	Mean $\pm$ SD	t	Pr >  t	Mean $\pm$ SD	t	Pr >  t
Hirsutism	-0.47 $\pm$ 3.39	.783	0.439	0.16 $\pm$ 2.08	-.431	0.669	-0.63 $\pm$ 3.41	1.018	0.317
Acne	5.56 $\pm$ 13.45	-2.34	0.0259	0.65 $\pm$ 13.90	-.258	0.798	1.97 $\pm$ 16.18	-.666	0.511
Stress	-4.22 $\pm$ 5.68	4.20	0.0002	-6.35 $\pm$ 7.32	4.834	<.000	-3.10 $\pm$ 5.54	3.064	0.005
Physical activity Score	5.03 $\pm$ 8.27	-3.44	0.0017	2.39 $\pm$ 10.89	-1.221	0.232	2.83 $\pm$ 8.00	-1.941	0.062

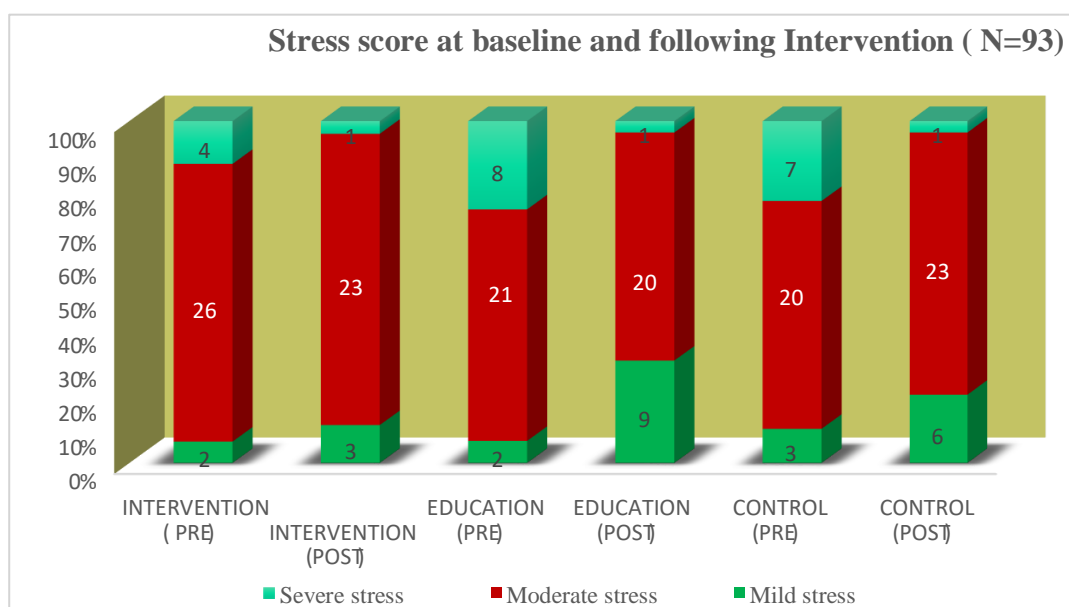


Figure 3 Stress level among the subjects at baseline and following intervention

### **Comparison of Stress at baseline and following interventions and the effectiveness between groups**

Figure 3 revealed that the stress score was significantly reduced with a mean decrease of (M±SD 4.2188±56), (M±SD 6.35±7.3191), (M±SD 3.1 ± 5.54) among the Nutrition Intervention group, education group, medication group respectively ( $p<0.001$ ). No significant difference in stress levels was seen between the groups following interventions. Recent studies by Sathyanarayana and Manjunatha 2019 confirmed that common mental disorders was as high as 33.5 percentage among the women of reproductive age group

### **Conclusion**

Nutrition intervention includes introduction of a micronutrient rich formula, which has shown significant reduction in Anthropometric parameters Weight, BMI, TSF, Waist circumference, Hip circumference, body fat and visceral fat levels. Among the Nutrition Intervention group we could see larger drop in weight, BMI, Waist circumference, Hip circumference, body fat and Visceral fat levels ( $p<0.001$ ) with a mean weight reduction in the 3 month period was 4.7kg. The reduction in BMI, TSF, Waist circumference, Hip circumference, and Body fat was 1.9kg/m<sup>2</sup>, 1.4cm, 1.5cm, 1.08cm and 1.5% respectively. Regarding the biochemical parameters, Haemoglobin, Total cholesterol, Triglycerides, Cholesterol to HDL ratio and total testosterone levels showed significant difference between the pre and post intervention. Acne and stress score has reduced significantly and physical activity score has increased considerably in the nutrition intervention group. Between the group comparison results showed significant weight loss ( $p=0.00$ ), reduction in BMI( $p=0.00$ ), reduction in TSF( $p=0.01$ ), Waist circumference( $p=0.00$ ), Hip circumference( $p=0.00$ ), but no difference in Body fat, body water and visceral fat. Cholesterol( $p=0.004$ ), Cholesterol to HDL ratio ( $p=0.0017$ ) and Testosterone( $p=0.00$ ) levels has shown significant difference between the groups. Comparison of effectiveness of the interventions was checked using t test following interventions showed a significant difference between Nutrition intervention and education in terms of waist circumference ( $p=0.015$ ) Hip circumference ( $p=0.0154$ ), body fat ( $p=0.026$ ) and Haemoglobin levels ( $p=0.038$ ). There was considerable difference in the BMI values between nutrition Intervention and education. Regularity of association of intervention with menstrual cycle revealed that the Nutrition intervention had 9.1 times higher chance of getting regular periods than those on nutrition education alone (OR : 9.1, CI 2.84-29.146) and 4.3 times higher chance of getting regular periods compared to medication group ( Control ) (OR:4.33 CI 1.385-13.552). There was a significant considerable decrease in the mean value of Stress in the Intervention, Education and control group ( $p<0.05$ ). Acne and Physical activity score considerably increased in the Nutrition intervention group. Hirsutism, acne and physical activity levels were not significantly differed among the subjects in the Education and Control group

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### Conflict Of Interest

The author(s) declare(s) that there is no conflict of interest

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