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COMPARATIVE STUDY ON EFFICACY AND SAFETY OF MYOINOSITOL VERSUS METFORMIN IN WOMEN WITH PCOS

Chinthana G.*

Assistant Professor, Department of Pharmacology, Thanjavur Medical College, Thanjavur, Tamilnadu, India.

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***Corresponding Author**
Prof. Chinthana G.
Assistant Professor, Department of Pharmacology, Thanjavur Medical College, Thanjavur, Tamilnadu, India.

ABSTRACT

Introduction: Polycystic ovarian syndrome (PCOS) or stein-leventhal syndrome is a most common endocrine disorder, affecting 20% of reproductive age women in terms of absence of ovulation and fertility, impaired glucose tolerance and a defect in the insulin signalling pathway which seems to be implicated in the pathogenesis of insulin resistance. For this reason, insulin lowering medications represent novel approach in women with PCOS. **Objective:** To evaluate the efficacy and safety of Myoinositol(MI) versus Metformin in women with PCOS. **Material and Methods:** Interventional prospective, open labelled, parallel arm, randomized control study conducted in OG department of Chennai Medical College Hospital and Research Centre, Irungalur, Tiruchirappalli. Total of 132 patients were recruited according to Rotterdams criteria, out of which 90 PCOS patients were randomly divided in to 3 groups of 30 each with Group I as placebo; Group II to receive Metformin 500mg; and Group III with Myoinositol 2mg. Hormonal parameters and ultrasonogram were carried out during the study period. Statistical analysis was carried out by Students's paired't' test with P value of < 0.05 was considered to be statistically significant. The end points noted were improvement in hormonal profile, ovulation and fertility rate in women with PCOS. **Results:** After treatment with Myoinositol our study showed significant reduction in F.Testo, DHEAS, LH, LH/FSH, Fasting insulin at 12^{th} wk, 12^{th} wk, 4^{th} wk, 8^{th} wk, 12^{th} wk (earlier weeks) compared to that of Metformin. This study showed improved ovulation rate and fertility rate in patients treated with MI compared to metformin. And moreover MI treated group did not showed any adverse events. **Conclusion:** Though metformin and MI can be considered as first line treatment in PCOS, MI seems to be more effective than metformin in improving the

hormonal profile, reproductive axis functioning with subsequent frequency of ovulation and pregnancy outcomes. It is a safe and effective drug in terms of very minimal or nil side effects compared to placebo and metformin. It could be included in the treatment protocol of infertility in the near future.

KEYWORDS: Polycystic ovarian syndrome, Myoinositol, Metformin, ovulation, fertility, hormonal profile.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) or stein - leventhal syndrome is one of the commonest endocrine disorders, affecting nearly 20% of women in reproductive age.[1] It is a multidimensional complex disorder with presence of polycystic ovaries in a women with cluster of symptoms that includes amenorrhoea, oligomenorrhoea, hirsutism, anovulation and other signs of androgen excess such as acne and crown pattern baldness.[2] The exact prevalence of PCOS is not known as the syndrome is not defined precisely. The estimated prevalence among the reproductive age women is 5-10%.[3] Global prevalence accounts for 2.2% to 26%, roughly 1 in 15 women worldwide, 36% of Indian women are suffering from PCOS.[4] PCOS receives more attention because of its high prevalence and reproductive, metabolic and cardiovascular consequences.

Environmental and genetic factors have a major role in PCOS.[2] Obesity with poor dietary choices and physical inactivity, worsens PCOS in susceptible individuals. PCOS may be common in our society owing to evolutionary advantages of the syndrome in ancient times, including smaller family sizes, reduced exposure to childbirth-related mortality, increased muscle mass and greater capacity to store energy.[5] Genetic sequence plays a strong role in the etiology of PCOS.[6] That is, it is inherited as a complex genetic trait. It is possible that a gene (CYP11A1, CYP17A1) may render the ovary susceptible to insulin stimulation of androgen secretion while blocking follicular maturation.[7]

In case of PCOS, the normal follicular growth seems to occur up to the mid-antral stage, after which the maturation ceases and follicle undergo atresia. The follicular fluid accumulates and follicle enlarges, the granulosa cell layer degenerates, appears like a thin-walled cyst.[8] The hyperandrogenism and anovulation in PCOS are caused by abnormalities in four endocrinologically active compartments such as ovaries, adrenal glands, periphery (fat) and hypothalamo-pituitary compartment. That is PCOS develops due to excessive luteinizing

hormones (LH) by the anterior pituitary gland with increase in LH/Follicle stimulating hormone (FSH) ratio and with high levels of insulin in blood and insulin resistance.[2] PCOS accounts for the main underlying cause of female infertility. The common immediate symptoms are anovulation, oligomenorrhea, amenorrhea and polycystic ovaries on ultrasound, excess androgenic hormones causing hirsutism, acne, alopecia, seborrhea and insulin resistance. Certain mood disorders such as depression, anxiety, bipolar disorder and binge eating disorder can also occur more frequently with PCOS.[9]

Though the diagnostic criteria for PCOS have been offered by three groups, the ultimate diagnostic criteria for PCOS was based on revised 2003 Rotterdam's Criteria, (2 out of 3 should be positive), a. Oligo ovulation and/or anovulation, b. Excess androgen activity (Clinical or biochemical signs of hyperandrogenism), c. Polycystic ovaries on ultrasonogram, after excluding other causes like congenital adrenal hyperplasia, cushings syndrome, hyperprolactinemia, thyroid disease, acromegaly and androgen secreting ovarian tumours.[10]

Types of PCOS include (a) Mild PCOS (16%) with irregular cycles, polycystic ovaries on USG, mildly elevated androgen levels, normal insulin. (b) Ovulatory PCOS (16%) with normal cycles, polycystic ovaries on USG, elevated androgen levels, increased insulin (c) Hyperandrogenism and chronic anovulation (7%) with irregular cycles, normal ovaries on ultrasound, elevated androgen levels, increased insulin and potential long-term risks (d) Severe PCOS (61%) with irregular cycles, polycystic ovaries on USG, elevated androgen levels, increased insulin and potential long-term risks.[11]

Complications of PCOS include reproductive complications such as increased incidence of miscarriage, endometrial cancer, infertility and cardiovascular complications such as coronary artery disease, hypertension and metabolic complications of obesity, insulin resistance, and type 2 diabetes.[2] Identification of patients worry is necessary when formulating a treatment plan. So the treatment depends on patients requirements of either hormonal contraception or ovulation induction. Lifestyle modification such as behavioral pattern, diet and exercise stays the first line of management. Other management for PCOS include oral contraceptive agents like ethinyl estradiol, medroxy progesterone to restore menstrual cyclicity, GnRH agonists; antiandrogens such as spironolactone, finasteride, flutamide, dexamethazone, selective estrogen receptor modulator, clomiphene citrate for ovulation induction; hypoglycemic agents such as metformin, thiazolidinediones, topical

eflornithine hydrochloride for hirsutism. Surgical management includes electrocautery, laser ovarian drilling and multiple biopsy.[12]

Though the insulin sensitizer-metformin in PCOS has improved metabolic disorders as a consequence of insulin resistance and the subsequent chronic sequalae, such as dyslipidemia, diabetes, hypertension and cardiovascular disease, it is associated with gastrointestinal side effects such as nausea, vomiting, diarrhea, lactic acidosis rarely.[13] Another drug, thiazolidinediones were associated with weight gain, cardiovascular events, fragile fractures and bladder cancer.[14]

Recently, Myoinositol (MI) - a novel insulin sensitizer has been developed for treating PCOS with infertility. MI could be used as an alternative to metformin treatment because the former can affect insulin target tissues and cells and potentiate insulin effects without the side effects of metformin. Certain studies have demonstrated that, treatment with MI is effective in reducing hormonal and oxidative abnormalities in PCOS patients by improving insulin resistance.[15] The need of doing this study in demonstrating the efficacy and safety of myoinositol was because of its limited studies available in India till now pertaining to supplementation of inositol for PCOS treatment.

AIMS AND OBJECTIVES

Based on the survey of literature, the effect of MI on PCOS, it was understood that limited works were carried out on the anovulatory PCOS and infertility cases. Keeping these in mind it was decided to carry out the comparative study of Metformin and MI on PCOS.

The objectives of the present study are

To compare the efficacy and safety of Metformin and MI, in terms of ovulation and fertility in PCOS patients.

To evaluate the hormonal effects of Metformin and MI on hyperandrogenism.

MATERIALS AND METHODS

Study centre

The study was conducted after obtaining institutional ethical clearance from institutional ethical committee (IEC), Research cell of Chennai Medical College Hospital and Research Centre Irungalur, Tiruchirappalli.

Methodology

The present study was a Interventional prospective, open labelled, parallel arm, randomized control study to compare the efficacy and safety of Metformin versus MI in women with PCOS during the study period of 24 weeks (from November 2015 to April 2016). Total of 132 patients were screened according to Rotterdams criteria, out of which 90 PCOS patients who fulfilled the inclusion and exclusion criterias were selected for this study after obtaining informed consent from the patients.

Inclusion Criteria

PCOS selected according to Rotterdam's criteria in the age group of 18-40 years were included in this study.

Exclusion Criteria

Patients on hormonal treatment for infertility in last 6 months, patient with increased risk of lactic acidosis, diabetic patients on insulin and insulin sensitizers, pregnancy and lactation, liver failure, renal failure, lung diseases, ischaemic heart disease and peripheral vascular disease were excluded from the study.

Withdrawal Criteria

Non compliance with protocol, protocol deviation, request for withdrawal by the patients and adverse effects (decision about withdrawal from the study made either by patients or investigator).

Treatment Protocol

Chemicals

Drugs used in this study were Metformin 500mg twice a day, Myoinositol 2gm twice a day.

The selected study subjects were randomly divided in to three groups of 30 each. Subjects in each group were treated as follows and continued without any changes in the treatment for the entire course of the study.

Group	No of subjects	Subject descriptions
1	30	Control -PCOS patient who delays specific treatment but follows Non-Pharmacological treatment
2	30	PCOS subjects + Tab. Metformin 500mg twice a day
3	30	PCOS subjects + Tab. Myoinositol 2gm twice a day

Clinical Assessment

All subjects were interviewed regarding the age, past history, personal history, family history, menstrual history, obstetric history, treatment history before starting the study. Height, weight, BMI, abdominal circumference, blood pressure and ultrasonogram(USG) were assessed for all the patient at baseline and periodically during the study period. Patients were advised to follow regular exercise and life style modifications during the study period.

Biochemical analysis

About 5ml of blood was collected in the morning hours after a 12-hours fast and a 30-minutes of rest in the supine position. Blood was added with an appropriate anticoagulant before estimation. Blood samples were obtained during the early proliferative phase (second through third day). Biochemical parameters such as LH, FSH, Fasting serum insulin, Serum-Total Testosterone & Free Testosterone, Dehydroepiandrosterone-sulfate(DHEA-S), Serum Androstenedione were evaluated at baseline, 4^{th} week, 8^{th} week and 12^{th} week, 16^{th} week, 20^{th} week, 24^{th} week of the study period.

SAFETY ASSESSMENTS

An adverse event reported by the subject or noted by the clinician during each visit was recorded. If continuation of the drug was considered harmful, the subject could be withdrawn from the study. Any adverse event was considered as serious if it was fatal, life threatening, disabling or if it prolonged hospitalization of the subject.

Statistical Analysis

Statistics were analyzed by SPSS statistical package before and after treatment. The results were tabulated and values were presented as mean (+ or -) and SD. Students's paired't' test P value of < 0.05 was considered to be statistically significant.

RESULTS

Age Distribution among the study population

The mean age of the study population are shown in Table 1 and Table 2. Among the three groups, 21 to 30 years of age subjects were involved maximum in the study. The mean age of subjects in group 1 was 25.63 ± 2.822. Group 2 was 24.20 ± 3.059 and Group 3 was 24.43 ± 3.685 respectively.

Table 1: Age distribution of the study population.

Age Group (in years)	No. of subjects (n=90)	Percentage (%)
11-15	01	1.11
16-20	09	10.00
21-25	40	44.44
26-30	39	43.33
31-35	01	1.11

Table 2: Distribution of Mean Age.

Group	Age (mean ± S.D)
Group 1	25.63±2.822
Group 2	24.20±3.059
Group 3	24.43±3.685
Total	24.75±3.189

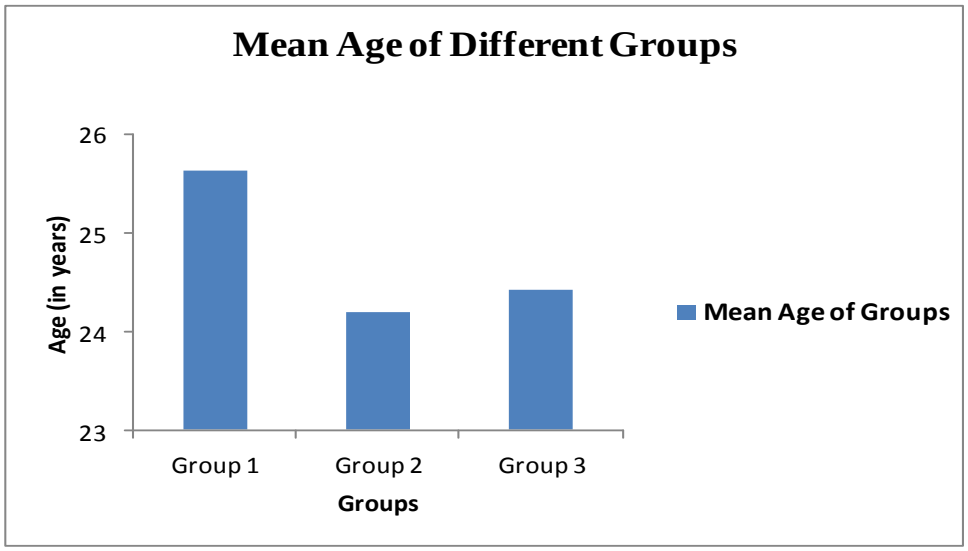

Figure 1: Distribution of mean age.

Marital Status Distribution of the Study population

Marital Status Distribution of the study population is shown in Table 3. The maximum number of married women were from group 2 and followed by group 1 and group 3. In the unmarried women distribution the maximum patients were from group 1 and group 3 followed by group 2. The distribution of mean age and Marital status is shown in Figure 1 and 2 respectively.

Table 3: Marital Status distribution.

Age Group	Married		Unmarried		Total
	No. of Patients	%	No. of Patients	%	
Group 1	17	56.66	13	43.33	30 (100)
Group 2	18	60	12	40	30 (100)
Group 3	17	56.66	13	43.33	30 (100)

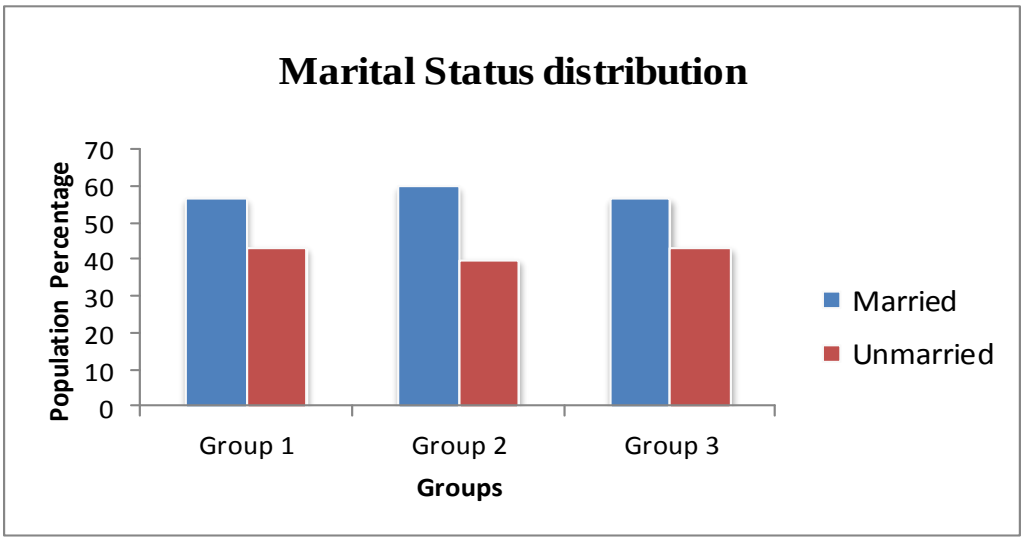

Figure 2: Marital status distribution.

Table 4: Effect of MI on weight in PCOS patient.

Groups	Weight (in Kgs.)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	61.68± 8.50	60.65± 8.11	59.16± 13.04	60.72± 8.48	60.63± 8.33	60.27± 8.49	60.60± 8.53
Group 2	62.90± 7.92	63.57± 9.75	61.80± 7.60	61.27± 7.11	63.86± 17.25	60.63± 6.45	61.50± 5.81
Group 3	63.12± 9.40	62.27± 9.23	61.07± 8.87	60.02± 8.58	59.50± 8.64	60.62± 7.05	61.25± 7.39

Table 5: Mean difference of Weight on treatment.

Groups	Mean difference of Weight (in Kgs.)					
	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	1.03	2.52	0.96	1.05	1.41	1.08
Group 2	-0.67	1.1	1.63	-0.96	2.27	1.4
Group 3	0.85	2.05	3.1	3.62	2.5	1.87

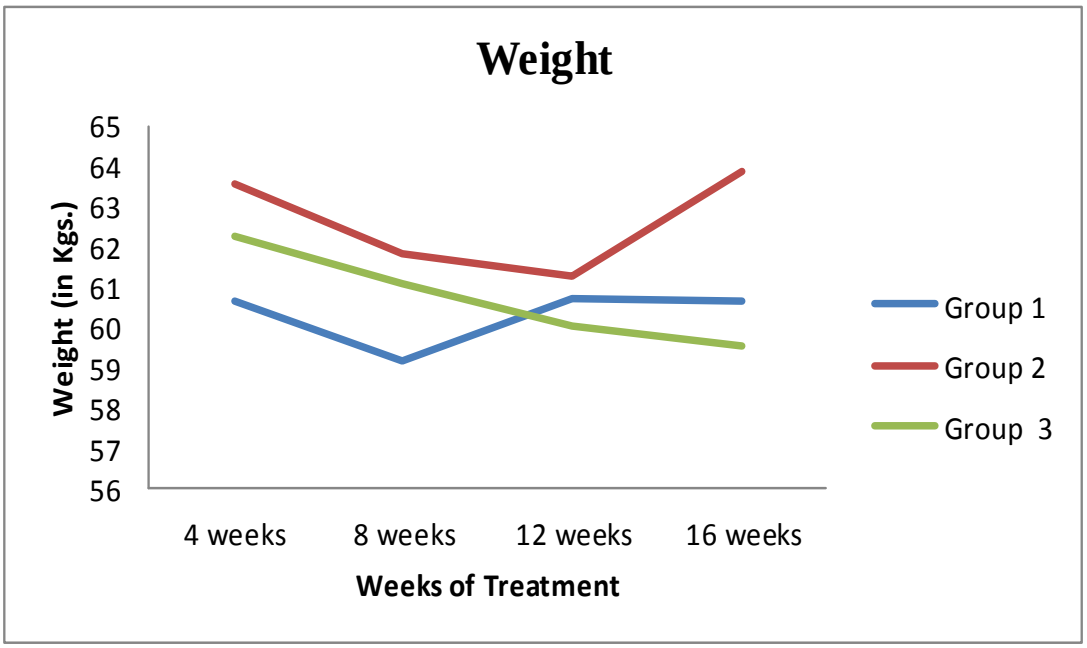

Figure 3: Effect of MI on Weight in PCOS patients.

Group 3 had maximum reduction of weight after 8,12,16,20 and 24 weeks and group 3 had a higher reduction of 3.1 kg at the end of 12 weeks and 3.62 kg at the end of the 16 weeks of treatment higher than all other groups. Group 1 had nominal reduction of 1.03 kg and 2.52 kg on weight at the end of 4 and 8 weeks which is higher than all other groups. It is observed that Group 3 achieved moderate control of weight than the Group 1 and 2.

From Figure 3, It is found that all the three groups were not statistically significant ($p>0.05$).

Effect of MI on BMI levels in PCOS patients

Table 6: Effect of MI on BMI levels in PCOS patients.

Groups	BMI (Kg/m^2)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	24.02± 3.17	23.60± 2.93	23.78± 2.93	23.62± 3.03	23.58± 2.89	23.44± 2.93	23.55± 2.90
Group 2	24.61± 3.17	23.48± 3.12	24.17± 2.99	23.96± 2.82	23.76± 2.47	23.78± 2.30	23.82± 2.05
Group 3	24.51± 3.83	24.16± 3.72	23.69± 3.45	23.26± 3.24	22.87± 3.27	23.31± 3.55	23.65± 4.14

Table 7: Mean difference of BMI levels on treatment.

Groups	Mean difference of BMI (Kg/m^2)					
	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	0.42	0.24	0.4	0.44	0.58	0.47
Group 2	1.13	0.44	0.65	0.85	0.88	0.79
Group 3	0.35	0.82	1.25	1.64	1.2	0.86

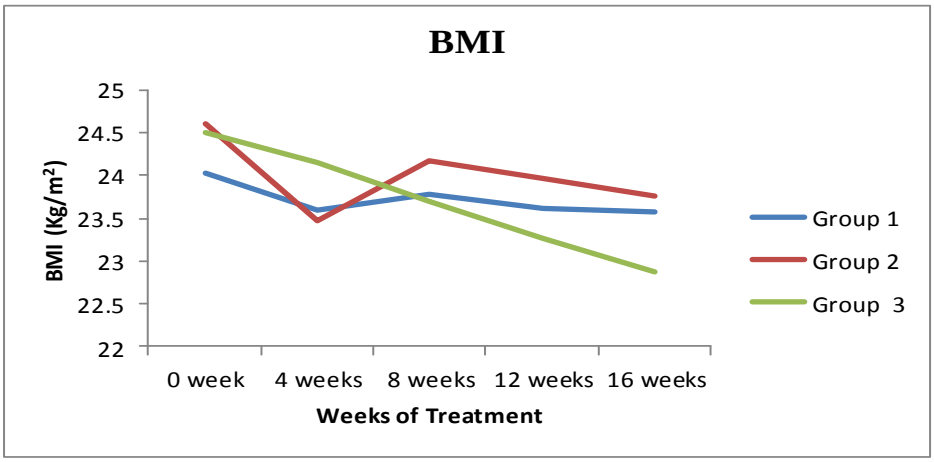

Figure 4: Effect of MI on BMI levels in PCOS patients.

Group 3 had maximum reduction of BMI after 8,12,16,20 and 24 weeks treatment and group 3 had a higher reduction of 1.64 kg/m^2 at the end of 16 weeks which is higher than all other groups. Group 2 had nominal reduction of 1.13 kg/m^2 on BMI at 4 weeks treatment which is higher than all other groups. It is observed that Group 3 achieved moderate control of BMI than the Group 1 and 2.

From Figure 4, It is found that all the three groups were not statistically significant ($p>0.05$).

Effect of MI on Fasting blood glucose level in PCOS patients.

Table 8: Effect of MI on fasting blood glucose in PCOS patients.

Groups	Fasting Blood Glucose (mg/dl)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	83.13± 8.47	83.10± 9.95	83.04± 8.96	83.00± 7.03	82.92± 7.71	82.86± 14.61	82.86± 8.17
Group 2	83.47± 9.71	83.42± 9.37	83.33± 8.66	83.27± 8.79	83.17± 9.85	83.17± 8.38	83.17± 6.02
Group 3	83.33± 8.84	83.27± 10.12	83.15± 8.34	83.02± 8.02	82.91± 8.70	82.71± 10.44	82.71± 8.60

Table 9: Mean difference of fasting blood glucose levels during treatment.

Groups	Mean difference of Fasting Blood Glucose (mg/dI)					
	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	0.03	0.09	0.13	0.21	0.27	0.27
Group 2	0.05	0.14	0.20	0.30	0.30	0.30
Group 3	0.06	0.18	0.31	0.42	0.62	0.62

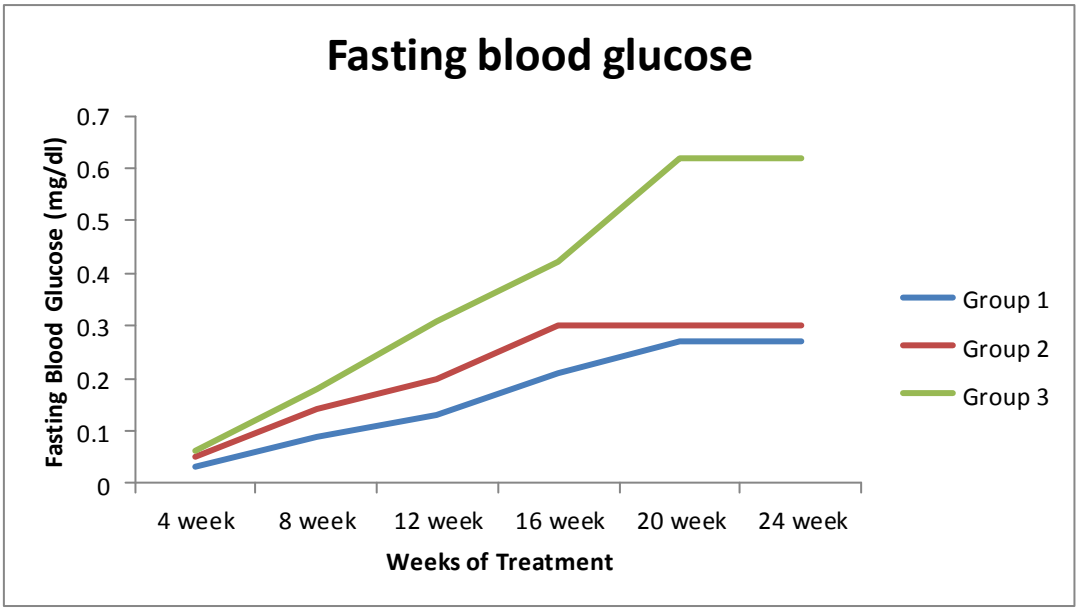

Figure 5: Effect of MI on fasting blood glucose levels in PCOS patients.

Mean reduction in FBS was shown in Table 6.Group 3 showed nominal reduction of FBS by 20 and 24 weeks (0.62 mg %) which is higher than group 1 & 2 but it was statistically not significant (P>0.05). The mean difference in reduction of fasting blood glucose after 4, 8, 12, 16, 20 and 24 weeks between the groups was not statistically significant which is shown in Figure 5.

Effect of MI on Post Prandial blood glucose level in PCOS patients

Table 10: Effect of MI on Postprandial blood glucose level in PCOS patients.

Groups	Postprandial Blood Glucose(mg/dl)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	102.13± 16.17	102.11± 14.03	102.03± 10.80	102.00± 11.52	101.97± 12.51	101.97± 11.75	101.97± 11.74
Group 2	94.43± 16.18	94.39± 14.25	94.35± 13.71	94.28± 25.68	94.15± 20.77	94.15± 13.78	94.02± 11.05
Group 3	103.93± 18.62	103.73± 12.34	103.63± 12.03	103.31± 12.04	103.01± 13.18	103.01.± 5.17	103.01± 4.17

Table 11: Mean difference of post-prandial blood glucose on treatment.

Groups	Mean difference of Postprandial Blood Glucose(mg/dl)					
	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	0.02	0.1	0.13	0.16	0.16	0.16
Group 2	0.04	0.08	0.15	0.28	0.28	0.41
Group 3	0.21	0.30	0.62	0.92	0.92	0.92

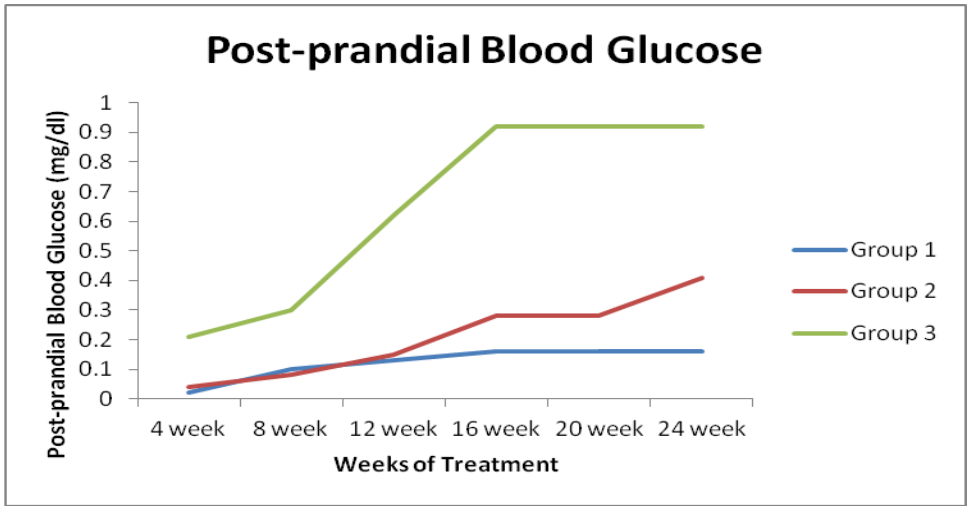

Figure 6: Effect of MI on postprandial blood glucose levels in PCOS patients.

Effect of MI on postprandial blood glucose levels in PCOS patients is shown in Figure 6 in which Group 3 subjects had nominal reduction of 0.92 mg % after 16 weeks but it as statistically not significant(P>0.05) followed by Group 2 which had a minimal reduction of 0.28 mg % after 16 week(P>0.05). It is found that all the three groups were not statistically significant.

Effect of MI on Fasting Insulin level in PCOS patients

Table 12: Effect of MI on Fasting Insulin level in PCOS patients.

Groups	Fasting Insulin (mg)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	10.55± 3.77	10.55± 3.77	10.50± 3.71	9.96± 3.39	9.987± 3.41	9.75± 3.28	10.22± 3.45
Group 2	10.04± 4.60	10.04± 4.20	9.78± 4.18	9.31± 3.64	9.16± 3.47	8.96± 3.26	9.06± 3.01
Group 3	10.31± 3.64	10.06± 3.38	9.69± 2.85	8.59± 2.33	8.27± 2.43	9.47± 2.87	10.15± 3.39

Table 13: Mean difference of Fasting Insulin on treatment.

Groups	Mean difference of Fasting Insulin					
	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	0	0.05	0.59	0.56	0.8	0.33
Group 2	0.01	0.26	0.73	0.88	1.08	0.98
Group 3	0.25	0.62	1.72	2.04	0.84	0.16

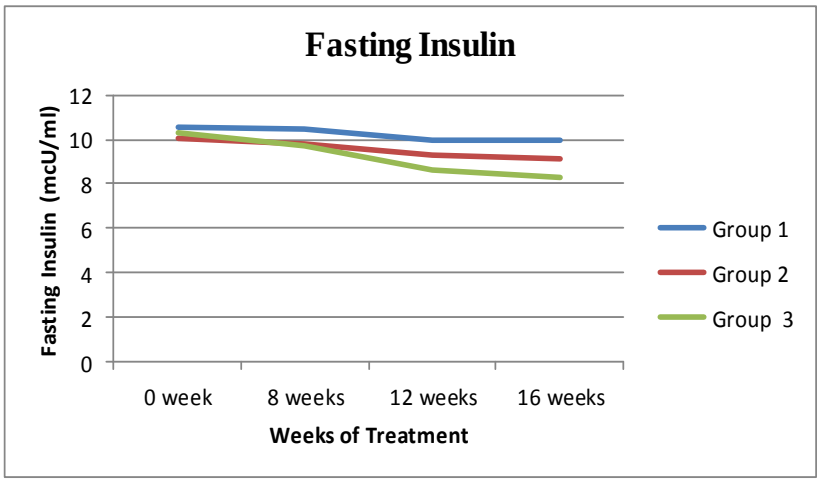

Figure 7: Effect of MI on Fasting Insulin levels in PCOS patients.

Group 3 had maximum reduction of fasting insulin after 4,8,12 and 16 weeks and group 3 had a mean reduction of 1.72 mg% at the end of 12 weeks and 2.04 mg % after the 16 weeks of treatment higher than the other two groups. Group 2 had nominal reduction of 1.08 mg% on fasting insulin after 20 weeks of treatment higher than all other groups. The mean difference in reduction of fasting insulin after 4 weeks between the groups was not statistically significant except for Group 3. It is found that Group 3 achieved average glycaemic control of fasting insulin than the Group 1 &2 which is shown in Figure 7.

The mean difference in reduction of fasting blood glucose after 8 weeks between the groups was not statistically significant except for Group 2 and Group 3. Myoinositol achieved moderate glycaemic control of fasting glucose than the Placebo and Metformin. The mean difference in reduction of fasting blood glucose for group 3 after 12 weeks and 16 weeks were statistically significant($P<0.05$) than the other groups. Group 3 achieved higher glycaemic control of fasting insulin than Group 1 & 2.

Effect of MI on LH levels in PCOS patients

Table 14: Effect of MI on LH levels in PCOS patients.

Groups	LH (mIU/mL)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	11.66± 1.64	11.37± 1.58	11.16± 1.74	11.13± 1.84	10.97± 1.99	10.79± 2.19	11.03± 2.11
Group 2	12.33± 1.58	11.87± 1.55	11.11± 1.46	10.47± 1.38	9.40± 1.90	9.21± 1.26	8.75± 1.30
Group 3	12.03± 1.42	11.03± 1.68	9.43± 1.57	7.99± 1.66	7.56± 1.61	8.68± 1.73	10.00± 0.25

Table 15: Mean difference of LH levels on treatment.

Groups	Mean difference of LH (mIU/mL)					
	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	0.29	0.5	0.53	0.69	0.87	0.63
Group 2	0.46	1.22	1.86	2.93	3.12	3.58
Group 3	1	2.6	4.04	4.47	3.35	2.03

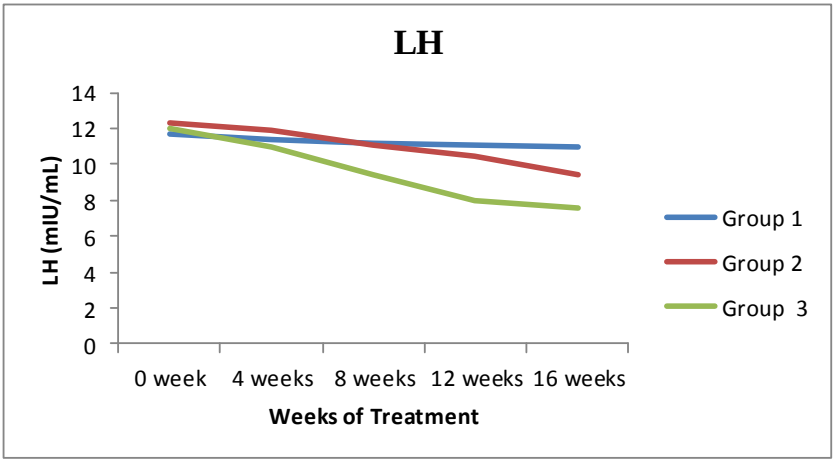

Figure 8: Effect of MI on LH levels in PCOS patients.

Group 2 subjects had moderate reduction of LH after 8,12,16,20 and 24 weeks with the corresponding p values of (p=0.003728000<0.01), (p=0.000015000<0.0001), (p=0.000000035<0.0001), P=0.000000000<0.0001) and (p=0.000000001 <0.0001) respectively. Group 2 was statistically most significant during 12, 16, 20 and 24 weeks and Group 2 achieved moderate control of LH than the Group 1.

Group 3 subjects had significant reduction of LH after 4,8,12,16,20 and 24 weeks with the corresponding p values of (p=0.01787<0.05), (p=0.0000000156<0.0001), (p=0.0000000000000465<0.0001), (p=0.0000000 0000252<0.0001), (p=0.001057<0.001) and (p=0.000000293<0.0001) respectively. It is found that Group 3 achieved significant

control of LH than the Group 1 and Group 2. Group 3 was statistically most significant during 8, 12 and 16 weeks with p<0.0001 which is shown in Figure 8.

Effect of MI on FSH levels in PCOS patients

Table 16: Effect of MI on FSH levels in PCOS patients.

Groups	FSH (mIU/mL)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	4.94±0.94	4.83±0.97	4.95±1.04	5.18±1.28	5.08±1.36	5.87±2.13	5.72±1.95
Group 2	5.30±0.86	5.10±0.83	5.30±0.78	5.13±0.73	5.49±1.30	5.57±1.21	5.47±0.94
Group 3	4.89±0.69	4.64±0.65	4.66±0.55	4.53±0.71	4.83±0.82	4.77±0.45	5.05±0.85

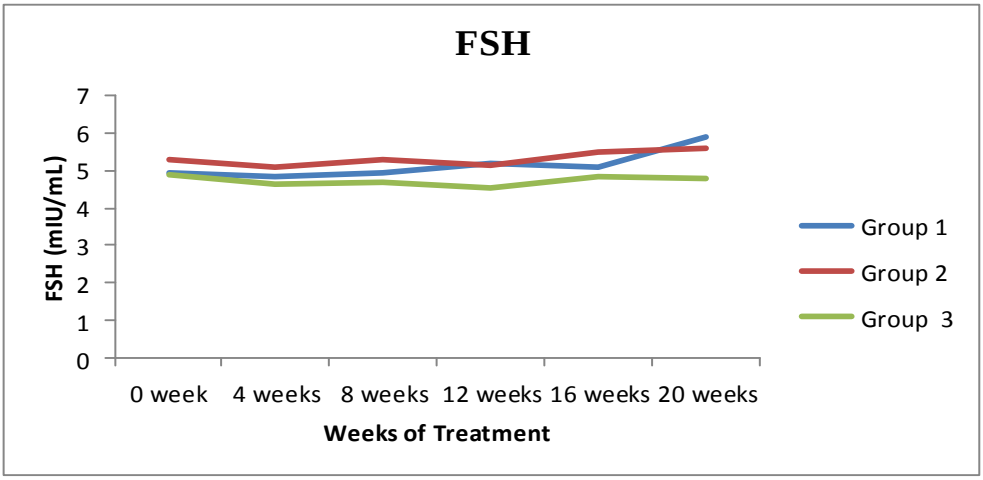

Figure 9: Effect of MI on FSH levels in PCOS patients.

Group 3 had maximum reduction of FSH after 4,8,12,16,20 and 24 weeks treatment and group 3 had a higher reduction of 0.36 at the end of 12 weeks than all other groups. The mean difference in reduction of FSH after 4 weeks for the group 1 and group 2 was not statistically significant except for group 3. It is found that Group 3 achieved average control of FSH than the Group 1 and 2 which is shown in Figure 9. Group 1 was statistically significant at the end of 20 week (p<0.05) compare to other groups.

Effect of MI on LH /FSH Ratio in PCOS patients

Table 17: Effect of MI on LH /FSH Ratio in PCOS patients.

Groups	LH/ FSH Ratio						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	2.42±0.45	2.41±0.42	2.31±0.41	2.23±0.43	2.26±0.54	2.05±0.66	2.13±0.68
Group 2	2.37±0.42	2.37±0.42	2.04±0.51	1.99±0.48	1.81±0.50	1.74±0.46	1.64±0.36
Group 3	2.50±0.40	2.43±0.50	2.05±0.39	1.81±0.47	1.61±0.41	1.84±0.3	2.03±0.33

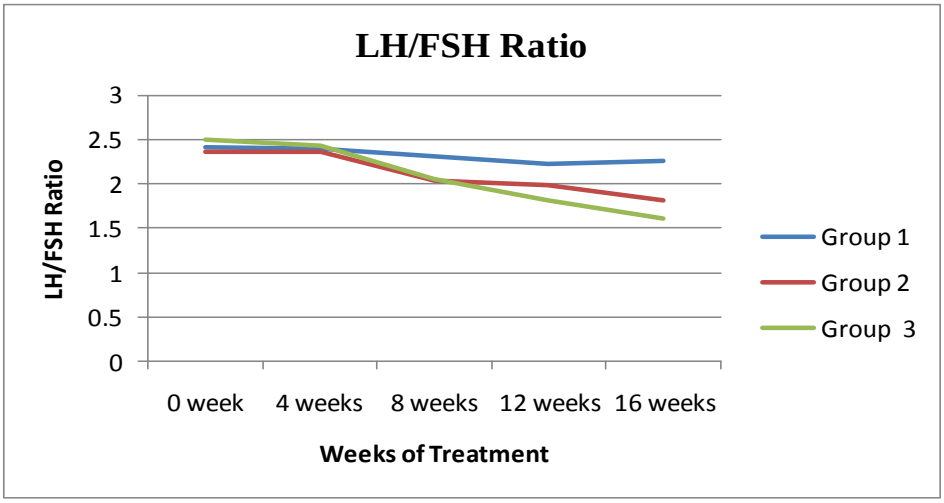

Figure 10: Effect of MI on LH/FSH ratio in PCOS patients.

The Effect of MI on LH/FSH ratio in PCOS patients is shown in Figure 10. The mean LH/FSH level were lowered by the drugs Group 3 and Group 2 and this reduction of LH/FSH level by both drugs after 8,12,16,20 weeks was statistically significant ($p<0.05$). Maximum reduction of LH/FSH level after 16 weeks was observed in group 3. It is observed that group 3 had significant reduction of LH/FSH ratio after 4,8,12,16 and 20 weeks than Group 2 and Group 1. Group 3 achieved significant control of LH/FSH than the Group 1 and 2.

Over all Group 2 was statistically significant at the end of 8 and 12 weeks ($p<0.01$) and statistically most significant during 16, 20, 24 weeks ($p<0.0001$) and Group 3 shows statistically most significance from 8 weeks onwards ($p<0.0001$). It is observed that Group 3 had higher control of LH/FSH compared with other groups.

Table 18: Effect of MI on T. Testosterone levels in PCOS patients.

Groups	T.Testo (ng/dL)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	0.89±0.38	0.95±0.53	0.88±0.39	0.78±0.36	0.78±0.36	0.78±0.36	0.85±0.37
Group 2	0.97±0.24	1.24±1.38	0.96±0.24	0.88±0.28	0.88±0.27	0.93±0.22	0.93±0.16
Group 3	1.09±0.99	1.08±0.99	1.08±0.99	1.08±0.99	1.23±1.12	1.40±1.34	1.77±1.75

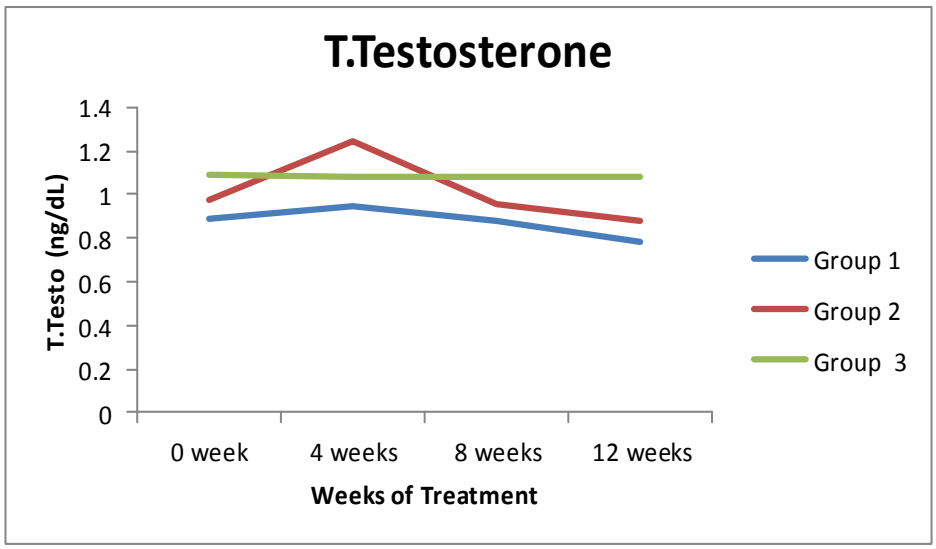

Figure 11: Effect of MI on T.Testosterone levels in PCOS patients.

The mean difference in reduction of T.Testo after 4 weeks for group 3 was comparatively better than group 1 and 2. The mean difference in reduction of T.Testo at 8 weeks for all the groups were significant. All the three groups are equally contributed towards the reduction of T.Testo over the periods and not statistically significant which is shown in Figure 11.

Effect of MI on Free Testosterone levels in PCOS patients

Table 19: Effect of MI on Free Testosterone levels in PCOS patients.

Groups	Free Testosterone (pg/ML)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	10.78±5.19	10.76±5.18	10.70±5.18	10.29±4.93	10.31±4.92	10.21±4.99	10.50±5.29
Group 2	10.91±4.11	10.46±4.41	10.59±4.04	9.27±3.18	9.08±3.24	8.36±3.17	8.71±2.56
Group 3	11.85±6.33	10.87±5.53	10.66±5.42	7.61±3.29	7.26±3.10	8.43±3.67	8.52±2.93

Table 20: Mean difference of Free Testosterone levels on treatment.

Groups	Mean difference of Free Testosterone					
	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	0.02	0.08	0.49	0.47	0.57	0.28
Group 2	0.45	0.32	1.64	1.83	2.55	2.20
Group 3	0.98	1.19	4.24	4.59	3.42	3.33

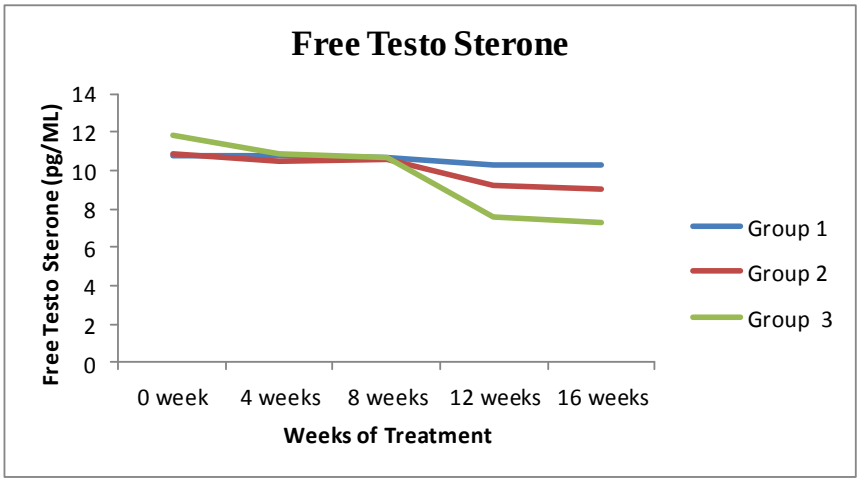

Figure 12: Effect of MI on Free Testosterone levels in PCOS patients.

Reduction of Free Testosterone after 4,8,12,16,20 and 24 weeks among the Group 3 and Group 2 was statistically significant and was maximum in Group 3 at the end of 16 weeks (4.59%) and group 2 at the end of 20 weeks (2.55%). The significant improvement in the reduction of Free Testosterone was shown in the group 3 at the earlier stage and also at end of 4,8,12,16,20 and 24 weeks. The mean difference in reduction of Free Testosterone after 4 weeks between the group 3 and group 2 was statistically significant except for Group 1. It is found that Group 3 achieved higher control of Free Testosterone than the Group 1 & 2.

The mean difference in reduction of Free Testosterone after 8 weeks for the group 3 was statistically significant than Group 1 and Group 2. Group 3 achieved higher control of Free Testosterone than the Group 1 & 2.

The mean difference in reduction of Free Testosterone for group 2 after 20 week was statistically most significant ($P<0.01$) and 24 weeks was statistically significant ($P<0.05$).

The mean difference in reduction of Free Testosterone for group 3 after 12 week was statistically significant ($P<0.01$) and 16 weeks was statistically most significant ($P<0.001$). Group 3 achieved higher control of Free Testosterone and treatment with Group 3 was better than the Group 1 and Group2 which is shown in Figure 12.

Effect of MI on S. Androstenedione levels in PCOS patients

Table 21: Effect of MI on S.Androstenedione levels in PCOS patients.

Groups	Serum Andro						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	2.41±0.70	2.41±0.70	2.41±0.70	2.32±0.69	2.32±0.69	2.32±0.69	2.41±0.70
Group 2	2.57±0.89	2.57±0.88	2.54±0.88	2.41±0.83	2.43±0.84	2.32±0.77	2.20±0.86
Group 3	2.41±0.83	2.40±0.88	2.40±0.83	2.40±0.82	2.46±0.95	2.29±0.65	1.94±0.66

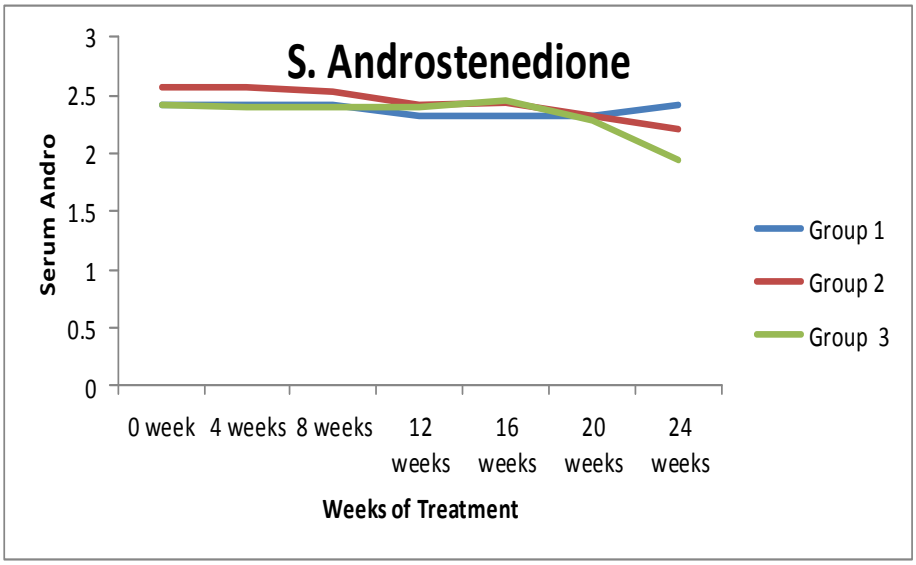

Figure 13: Effect of MI on S. Androstenedione levels in PCOS patients.

The Effect of MI on S. Androstenedione levels in PCOS patients is shown in Figure 13.

The mean difference in reduction of Serum Andro after 8,12,16,20 weeks for group 2 was better than group 1 and 3. The mean difference in reduction of Serum Andro at 4 weeks for group 3 was significant. Group 2 followed by Group 3 achieved moderate reduction of Serum Andro over the periods.

Effect of MI on DHEA-S levels on PCOS patients

Table 22: Effect of MI on DHEA-S levels on PCOS patients.

Groups	DHEAS (mg/DL)						
	0 week	4 week	8 week	12 week	16 week	20 week	24 week
Group 1	259.95± 45.02	259.77± 45.22	252.47± 54.95	245.94± 63.03	255.90± 44.87	255.33± 44.97	258.37± 44.10
Group 2	293.21± 62.04	292.46± 59.63	289± 61.14	278.90± 55.85	276.90± 56.97	273.81± 65.95	284.11± 61.59
Group 3	287.78± 85.28	278.40± 86.46	277.60± 80.09	257.51± 73.40	264.61± 77.20	260.75± 15.29	267.25± 11.12

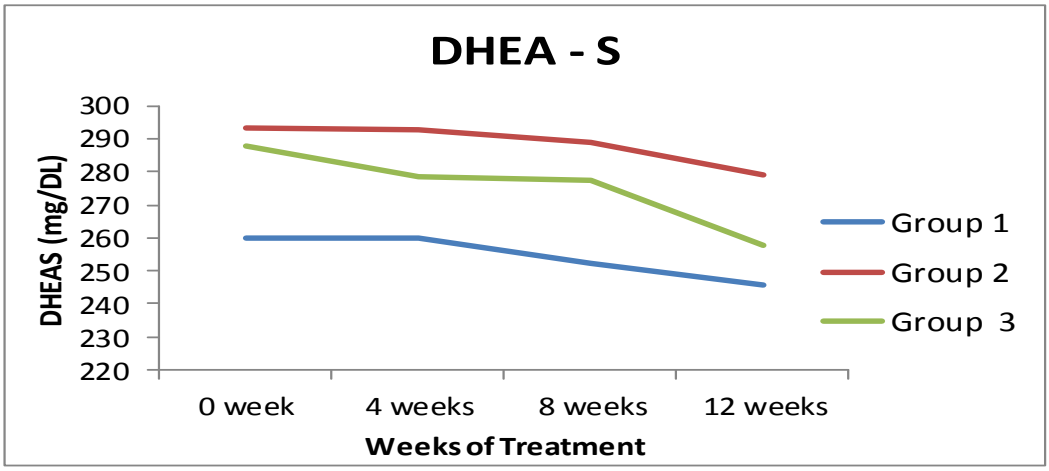

Figure 14: Effect of MI on DHEA-S levels in PCOS patients.

The mean difference in reduction of DHEA-S for group 2 after 16 and 20 weeks was statistically significant with the p value of 0.030895 and 0.016644 which is less than the chosen significance level ($p<0.05$). Group 2 achieved moderate control of DHEA-S.

The mean difference in reduction of DHEA-S for group 3 after 12, 16,20 and 24 weeks was statistically significant with the values ($p= 0.03446<0.05$), ($p= 0.045497<0.05$), ($p= 0.011193<0.01$), ($p= 0.02332<0.05$). It is found that Group 3 was most significant by 20 week ($p<0.01$)**.** Group 3 achieved higher control of DHEA-S and treatment with Group 3 was better than the Group 1 and 2 which is shown in Figure 14.

Table 23: Response of Group 1 with their p values for various clinical parameters.

Parameters / Weeks	4th Week	8th Week	12th Week	16th Week	20th Week	24th Week
F.Testo	0.992221	0.953387	0.715826	0.726729	0.671659	0.841435
DHEAS	0.987707	0.945042	0.732743	0.732743	0.697443	0.893894
LH	0.499624	0.266666	0.256650	0.153671	0.091896	0.219810
LH/FSH	0.989457	0.372393	0.118064	0.240141	0.017413	0.070779
WT	0.639503	0.387901	0.668356	0.638442	0.529727	0.631920
BMI	0.607807	0.765766	0.633776	0.588604	0.474442	0.563788
BP	0.869128	0.847933	0.823880	0.872273	0.838781	0.773700
FBS	0.530037	0.226233	0.186795	0.214962	0.882341	0.350785
PPBS	0.808728	0.699787	0.985639	0.474514	0.538052	0.632111
FSH	0.662285	0.969422	0.431206	0.665763	0.038340	0.063492
T.Testo	0.641182	0.927652	0.263128	0.246134	0.258703	0.696024
Sevum Andro	0.998552	0.994218	0.624289	0.607775	0.625778	0.997110

The result of p value after two tailed t-tests for the clinical parameters LH/FSH ratio and FSH are found to be significant with p values(=0.01743 and =0.03834) which is less than 0.05. So,

the response of Group 1 shows profound effect on reduction of LH/FSH ratio and FSH values.

Table 24: Response of Group 2 with their p values for various parameters.

Parameters / Weeks	4th Week	8th Week	12th Week	16th Week	20th Week	24th Week
F.Testo	0.691412	0.76685	0.095086	0.064771	0.012309	0.033106
DHEAS	0.963057	0.79558	0.122366	0.030895	0.016644	0.183391
LH	0.264354000	0.003728000	0.000015000	0.000000035	0.000000000	0.000000001
LH/FSH	0.962706	0.009073	0.002371	0.000021600	0.000001920	0.000000400
WT	0.776091	0.591542	0.591542	0.412027	0.246733	0.495219
BMI	0.869218	0.588835	0.412651	0.256057	0.271544	0.300999
BP	0.178396	0.519626	0.097286	0.205549	0.231268	0.225565
FBS	0.700975	0.484494	0.144141	0.321593	0.074449	0.115505
PPBS	0.403964	0.373511	0.638242	0.361414	0.109978	0.065439
F.I	0.993132	0.822581	0.503286	0.416134	0.313996	0.393144
FSH	0.353747	1	0.402621	0.521104	0.362678	0.559258
T.Testo	0.303379	0.87407	0.194921	0.209327	0.576482	0.513282
Serum Andro	0.994318	0.91705	0.497322	0.545187	0.273956	0.182143

The result of p value after two tailed t-tests for the clinical parameters F.Testo, DHEAS, LH, LH/FSH are found to be significant with p values (=0.012309,=0.016644,=0,=0.0000192) which are all less than 0.05. So, the response of Group 2 shows pronouncing effect on F.Testo, DHEAS, LH, and LH/FSH ratio.

Table 25: Response of Drug 3 with their p values for various clinical parameters.

Parameters/ Weeks	4th Week	8th Week	12th Week	16th Week	20th Week	24th Week
F.Testo	0.535254	0.449837	0.002546	0.001583	0.077263	0.158255
DHEAS	0.67899	0.356797	0.03446	0.045497	0.011193	0.02332
LH	0.01787	0.0000000156	0.0000000000000465	0.00000000000252	0.001057	0.000000293
LH/FSH	0.556073	0.0000573	0.000000131	0.00000000282	0.00094	0.083772
WT	0.728857	0.39649	1.671553	0.171306	0.446686	0.705938
BMI	0.730426	0.395014	0.18609	0.11654	0.448112	0.748683
BP	0.258199	0.920065	0.453158	0.475638	0.711113	0.826872
FBS	0.320624	0.3192	0.158457	0.151677	0.704724	0.059300
PPBS	0.58164	0.699123	0.541109	0.767224	0.060207	0.39047
F.I	0.785509	0.473717	0.036675	0.022235	0.523877	0.941334
FSH	0.984572	0.721108	0.729234	0.837296	0.678972	0.347046
T.Testo	0.979574	0.97549	0.973461	0.639755	0.580906	0.552175
Serum Andro	0.308918	0.982672	0.996268	0.843839	0.696086	0.320649

The result of p value after two tailed t-tests for the clinical parameters F.Testo, DHEAS, LH, LH/FSH, FI are found to be statistically significant with p values(=0.00255, =0.03446, =0.01787, =0.0000573, =0.03668) which are all less than 0.05. So, the response of Group 3 shows most positive response in almost all the clinical parameters such as F.Testo, DHEAS, LH, LH/FSH, FI. Hence the Alternate Hypothesis is proved.

Table 26: Response of Drugs for various clinical parameters over a period of 24 weeks.

Parameters / Drugs	Drug 1		Drug 2		Drug 3	
	NH	AH	NH	AH	NH	AH
F. Testo	✓			✓ 20th week		✓ 12th week
DHEAS	✓			✓ 16th week		✓ 12th week
LH	✓			✓ 8th week		✓ 4th week
LH/FSH		✓ 20th week		✓ 8th week		✓ 8th week
WT	✓		✓		✓	
BMI	✓		✓		✓	
BP	✓		✓		✓	
FBS	✓		✓		✓	
PPBS	✓		✓		✓	
F.I	✓		✓			✓ 12th week
FSH		✓ 20th week	✓		✓	
T.Testo	✓		✓		✓	
Sevum Andro	✓		✓		✓	

From the Table 26, it is being inferred that, For Group 3 more number of clinical parameters shows significance response for different age group of populations. The p values for the clinical parameters F.Testo, DHEAS, LH, LH/FSH, FBS, PPBS, F.I, are statistically significant ($p<0.05$) for Group 3 than Group 1 and Group 2.

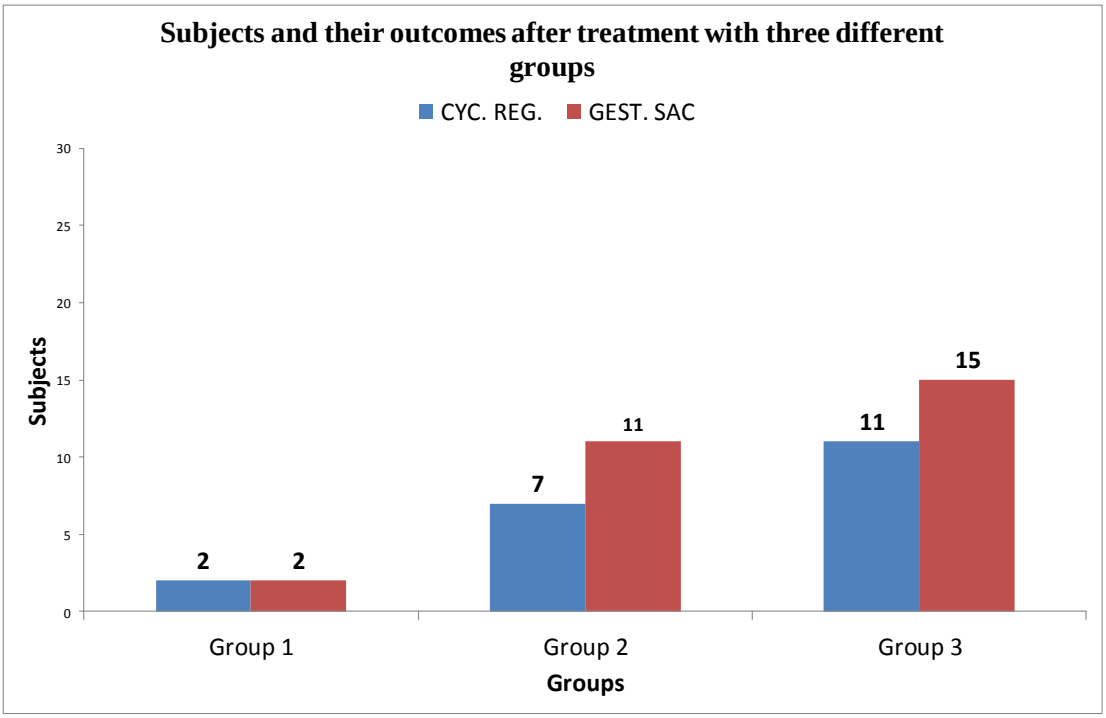

Figure 15: Subjects and their outcomes after treatment with three different groups.

Positive results in treatment were seen for 26 out of 30 patients in Group 3 and maximum positive outcome of 87% was achieved only by Group 3 and Group 2 achieved next level of positive outcome for 18 out of 30 patients with 60% and Group 1 provided positive outcome only for 4 out of 30 patients which is 13.3% only which is shown in Figure 15.

DISCUSSION

Polycystic ovarian syndrome is a complex heterogeneous clinical condition characterized by hyperandrogenism with chronic oligo/anovulation. The factors influencing PCOS include genetic predisposition, high levels of insulin in blood, obesity, excessive production of masculine hormones, abnormality in hypothalamic-pituitary-gonadal axis, environmental pollutants, food adulterants and chronic inflammation.[16]

In our study to evaluate the efficacy of MI on 30 PCOS patients compared to placebo (30 patients) and metformin (30 patients) for a period of 24 weeks, MI treated group showed significant reduction in Fasting insulin at the end of the study period . This was similar to the previous studies done by Genazzani *et.al* and Unfer V *et.al*, Martino M zacche *et.al*.[17,18]

Our study showed significant reduction in LH from 4th week onwards and reduction in LH/FSH ratio from 8th week onwards in MI treated group. This was similar to the previous studies done by Genazzani *et.al*, Martino M zacchi.et.al, Antonio simone lagana *et al.*[18]

Our study showed significant reduction in free testosterone from 12th week onwards in MI treated group and the same reduction was from 20th week only in metformin treated group. This was similar to the previous studies done by costantino.et.al, Nestler.J E *et al*., Martino M zacchi *et al.*[7,10]

Our study showed significant reduction in DHEA-S from 12th week onwards in MI treated group and the same reduction was from 16th week only in metformin treated group. This was similar to the previous studies done by Costantino *et al.*[10]

Our study does not show any significance with respect to the following parameters such as FBS, PPBS, FSH, hormonal parameters such as total testosterone, androstenedione.

Regularization of menstrual cycle

In our study 11 out of 13 unmarried women got their cycles regularized after treatment with MI followed by 7 out of 12 women in metformin group and 2 out of 13 in placebo group. These were similar to the previous studies done by Genazzani *et al.*[18] who stated that normal menstrual cycles restored with MI were maximum compared to placebo.

Pregnancy Rate

In our study MI group, 15 out of 17 married infertile PCOS women got conceived followed by 11 out of 18 in metformin group and 2 out of 17 in placebo. These were similar to the previous studies done by Gerli *et al*, who stated that MI has significantly increased the frequency of ovulation and pregnancy rate compared to placebo.[19]

Ovulation Rate

In the present study, in the MI group, total of 26 patients (87%) out of 30 ovulated showing positive results compared to metformin group showing 18 out of 30 patients(60%) showing positive results and placebo group with 4 out of 30 patients (13.3%) with positive results. These were similar to the previous studies done by costantino et.al, who stated that ovulation restored with MI was maximum compared to placebo.[10]

In a study done by Raffone *et al* comparing the effect of metformin and MI in 120 PCOS patients, out of 60 women 50% restored spontaneous ovulation and spontaneous pregnancy in 11 women and seven drop out because of side effects on treatment with metformin. In MI treated group, out of 60 women 65% restored spontaneous ovulation and spontaneous pregnancy in 18 women. He stated that though metformin and MI can be considered as first line treatment for restoring normal menstrual cycles in patients with PCOS, MI treatment seems to be more effective than metformin.[20]

In this study, the results revealed that MI was found to be better than metformin and placebo in improving the hormonal profile with subsequent frequency of ovulation and pregnancy outcomes.

In our study, 5 out of 90 PCOS women showed mild hair growth (hirsutism) on the upper lip which according to Ferriman -gallway visual scale (R) comes under the score of <8 which was regarded as normal.

Adverse Drug Reaction

In our study 4 out of 30 patients treated with metformin showed mild GIT distutbances but there were no drop out. MI treated group did not show any adverse events. These were similar to the previous studies done by Carlomagno *et al* who stated that dosage of 4gm/day commonly used in clinics is completely free of side effects.[21]

CONCLUSION

As PCOS is an emerging disorder during adolescence, early intervention is necessary to improve the reproductive health of adolescents and to prevent future complications. It is concluded from the present study that MI (non-hormonal drug) was effective in the treatment of PCOS in terms of regularization of menstrual cycle, induction of ovulation and improving the success rate of pregnancy in infertility cases. It was a safe and effective drug in terms of very minimal or absence of side effects compared to placebo and metformin. The observations made from this study justify the use of MI for the treatment of PCOD. Hence it could be included in the treatment protocol of infertility in the near future. Further research with the ideas of finding out the exact mechanisms including genetic expression for multiple illnesses of the PCOS is required.

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CONFLICT OF INTEREST: Nil.

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