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IN VIVO ANTIDIABETIC AND ANTILIPIDEMIC ACTIVITIES OF *PERGULARIA DAEMIA* ON PCOS INDUCED RATS

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ABSTRACT: This study focussed about the combined effect of methanolic extracts of *Pergularia daemia* with Metformin drug in the recovery of PCOS condition in female albino Wistar rats. The methanolic leaf extracts of *P. daemia* showed the presence of active biocompounds by column and Thin layer chromatography. The compounds were characterized by GCMS. Letrozole used to induce PCOS in albino Wistar female rats. *In vivo* antidiabetic studies showed increased levels of glucose in Group II - PCOS condition whereas the glucose levels reduced in Group III and V. While in Group IV - Metformin + Plant extract showed significant ($P<0.01$) levels with control group (Group I). *In vivo* lipid studies showed elevated levels of Total cholesterol were found in Group II and Group III than compared to Group I. Triglycerides levels also found significantly high in all four the treated groups than control. Group IV- Metformin + plant extract showed significant ($P<0.01$) results in Total cholesterol, HDL, LDL and Triglycerides with control. High antihyperlipidemic levels found in Group IV than compared to Group III and Group V. These results concluded that Metformin+plant extract group results showed significant results with the control which is an effective way to treat PCOS.

KEYWORDS: PCOS, *P. daemia,* antidiabetic, antilipidemic.

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1.INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the causes of female infertility which is affecting about 5–10% of women in age of fertility [1] due to ovulatory problems with absence of menstrual periods [2]. Stein and Leventhal [3] described the association between PCOS and infertility which is caused by anovulation in women with PCOS from 35% to 94% [4]. Polycystic condition may develop in

the ovaries due to the excessive production of male hormones (androgens), testosterone and excessive luteinizing hormone (LH) released from the pituitary or high levels of insulin in the blood (hyperinsulinaemia) in women who showed sensitive to this stimulus. Cysts are immature follicles developed from primordial follicles at an early antral stage due to ovarian dysfunction [5].PCOS is a multi-factorial disorder characterized by the co-existence of hyperandrogenism, dysfunction of ovaries, polycystic ovaries, infrequent menstrual periods and/or irregular bleeding, infertility or inability to get pregnant, increased growth of hair on the face, chest, stomach, back, thumbs, or toes, acne, oily skin or dandruff, pelvic pain, weight gain or obesity (30-75% of women with PCOS) usually carrying extra weight around the waist, type 2 diabetes, high cholesterol, high blood pressure, male-pattern baldness or thinning hair. Obese women with PCOS are at increased risk of anovulation and consequent subfertility [6]. The drug Metformin either alone or combining with clomifene, significantly lowered serum androgen levels, restoring of menstrual cyclicity and effectively improved ovulation [7]. The use of metformin improved the infertility therapies and reduced the risk of ovarian hyper stimulation when it is used one month prior to ovulation induction with FSH [8] and also improves fertilization and pregnancy rates in PCOS women those who undergoing *in vitro* fertilization [9]. According to Khare [10] plant drugs have been used to treat various diseases of female reproductive system caused by hormonal imbalance. *Pergularia daemia* widely used by tribal communities in Western Ghats of India for the treatment of different varieties of ailments and the whole plant is used as an antihelmintic, antiseptic, antivenin and expectorant [11].This work aimed to study the combined therapeutic effect of *P. daemia* and metformin in the treatment of Polycystic ovarian syndrome in the Letrozole induced PCOS rats and also to identify and characterize the bioactive compounds of *P. daemia* by Column, Thin Layer Chromatography and Gas chromatography-Mass Spectrometry (GC-MS) techniques.

2. MATERIALS AND METHODS

2.1. Experimental animal and Treatment

Female Albino Wistar Rats are procured from King's Institute of Preventive Medicine and Research, Guindy, Chennai (India) acclimatized in Holy Cross College animal house in polypropylene cages (8 × 12× 8 inch) with a steel grid on the top for fifteen days prior to the experiment with water and feed at *ad libitum.* Metformin and Letrozole were purchased from the Sigma Aldrich Company. The methanolic leaf extracts of the *Pergularia daemia,* used for this study.

2.2. Isolation of active compounds by column chromatography and Thin layer chromatography

The crude extract was loaded into the solvent layer above the silica gel in the packed column and the elution of the column first with hexane, increasing amount of ethyl acetate in hexane and finally with methanol, yielded a number of fractions, which were collected under the column [12]. 5 µl of isolated compounds were applied to silica gel plates, Merck (Germany) 20×20 cm, 0.25 mm in thickness. Plates were developed using the solvent system n-Hexane: Ethyl acetate: methanol (2:3:5

v/v/v). The separated zones were visualized with freshly Dragendroff's, Ninhydrin, LibermannBurchard, concentrated sulphuric acid, aluminium chloride, ferric chloride and heated at 100°C for 10 minutes. Chromatograms were then examined under daylight within 10 minutes [13].

2.3. Gas Chromatography-Mass Spectrometry Analysis

The GCMS analysis of the fraction 7 and 8 was performed using a GC model (451- GC) and Autosampler 8410 equipped with a Bruker column (0.25mm) and FID detector was used [14]. Helium was the carrier gas at a flow rate of 1 ml /min. the injector used was PTV and the oven temperature was programmed as follows: 400°C for 3mins, then gradually increased to 2800°C and the total time taken was 30mins. Then, the identification of the components was based on comparison of their mass spectra with those of NIST library (Version 12)

2.4. Biochemical studies

Five groups (n=6) such as Control group (Group I), PCOS induced group (Group II), Metformin treated group (Group III), Metformin +Plant leaf extract treated group (Group IV) and Plant leaf extract treated group (Group V) were maintained. After 15 days, the Wistar rats blood collected by intravenous method used for biochemical analysis.The antidiabetic and antilipid activities were assayed by the method of Glucose oxidase [15], Lowry *et al*. [16] and Trinder [17] respectively. Triglycerides, HDL, LDL and VLDL levels also estimated by Young and Pestaner [18], Hatch and Lees [19], Rifai*et al*., [20] and Friedewald *et al.,* [21] methods respectively.

2.5. Statistical Analysis

Data were represented in Mean±Standard Deviation (SD). One way Analysis of Variance (ANOVA) were used to test the significance between the control and the treated groups by SPSS (17.0 version) tool.

3. RESULTS AND DISCUSSION

3.1. Column chromatography and Thin layer chromatography

Five different solvents – Pure Hexane, Hexane + Ethylacetate (7:3), Hexane + Ethylacetate (5:5), Pureethylacetate, Ethylacetate + Methanol (5:5) and Pure Methanol were used in the column chromatography. The separated fractions were collected in separate container. Totally 12 fractions were observed in the total five solvent system (Table 1). Pure Hexane and Hexane + Ethylacetate (7:3) solvents showed high fractions as three, but two (1 & 2) and one (4) fractions are colourless respectively. Fractions colour varied from light green to Dark green. Based on the results from column chromatography, seven fractions such as 5, 6, 7, 8, 9, 10, 11 and 12 were selected for further Thin layer chromatography. Seven fractions were run in TLC and their Rf values ranges from 0.873 to 0.719. Fraction 8 was further sub fractioned with Hexane:Ethylacetate:Formic acid (31:14:5) solvent system by TLC to analysis their composition. Sub fraction results showed 5 spots with Rf 0.22, 0.44, 0.50, 0.36 and 0.38. with orange colour for first three spots, dark green colour next two spots respectively. Figure 1 showed spot development in TLC paper in daylight and UV light.

Table 1: Different solvent system and their fractions from column chromatography

Solvent system	Fraction	Color
Pure Hexane	1	Colorless
	2	Colorless
	3	Light green
Hexane + ethylacetate (7:3)	4	Colorless
	5	Yellowish green
	6	Green
Hexane + ethylacetate (5:5)	7	Dark Green
	8	Yellowish green
Pure ethylacetate	9	Green
Ethylacetate +methanol (5:5)	10	Light green
	11	Green
Pure Methanol	12	Light green

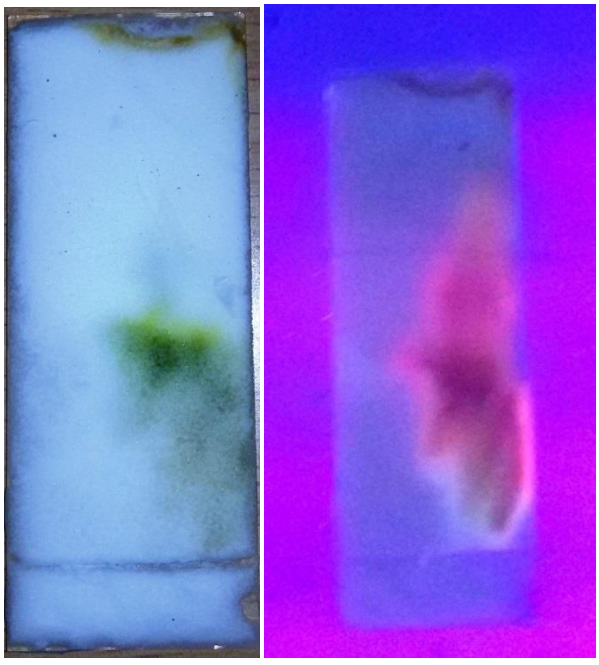

Figure 1: TLC Profile of crude extract – under daylight and UV light

3.2. GCMS

Gas Chromatography Mass Spectrometric analysis of extract (Figure 2) showed twelve different peak which indicated that 12 different compound. L- Glucose at Rt 7.490 with 2.998 peak area showed Antidiabetic activity. 2,2,4- Trimethyl-3-pentanol observed at Rt 17.533 with 3.752 peak area showed antidepressant activity. Cyclopentanol, 1-methyl- at 19.919 Rt and peak area was 41.189% showed Antiacne and Antidepressant activities. At Rt 23.215, Hexaethylene glycol monododecyl ether was identified with 0.322% peak area showed Antidiabetic and

Antihyperlipidemic activities. BIS(2- Ethylene)phthalate with 24.197Rt and their peak area is 9.002% showed sexual disorder inducer of ovulation and Antiacne activities.

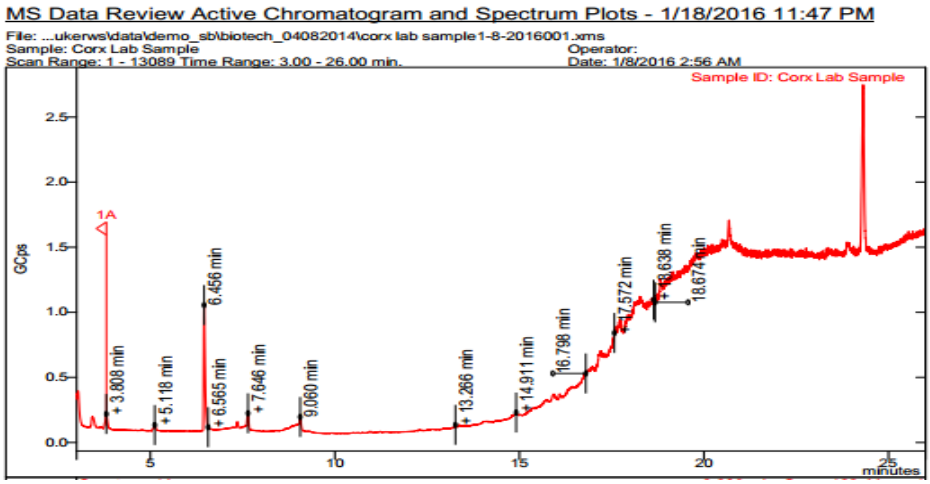

Figure 2: GCMS chromatogram of plant leaf extract

3.3. Biochemical analysis

3.3.1. *In vivo* antidiabetic studies

The total glucose levels in control sample (n=6) was found as 61.8±0.40mg/dL whereas in PCOS induced group the glucose levels found as twice as 183.88±0.26mg/dL. In Metformin (Group III) and Plant leaf extract (Group V) treated groups, the glucose levels found as 73.96±0.25mg/dL and 68.11±0.26 mg/dL respectively (Table 2). Among the treated groups, the Group V - Metformin + Plant leaf extract treated showed reduced glucose levels as 63.88±0.26mg/dL which was very close to the control group level (Figure 3). The ANOVA results showed significant ($P<0.01$) between the groups with F values as 189826.391. Rats treated with Poloxomer 407 compared with all rats treated with the *Tephrosia purpurea* extracts groups which showed that *Tephrosia purpurea* whole plant leaf extract at the dose of 600mg/kg significantly decreases the TC ($P<0.05$), TG ($P<0.05$), while increases the level of HDL ($P<0.001$) i.e., antihyper lipidemic activities were observed [22]. In liver tissues, peroxidation occurs in unsaturated lipids exposed to reactive oxygen species and recognized as the primary toxicological event due to the generation of free radicals.

Table 2: Total glucose levels (mg/dL) in control and experimental wistarrats blood

Experimental groups	Group I- Control	Group II - PCOS induced	Group III - Metformin treated	Group IV - Metformin + Plant leaf extract treated	Group V - Plant leaf extract treated
Glucose (mg/dL)	61.8±0.40	183.88±0.26	73.96±0.25	63.88±0.26	68.11±0.26

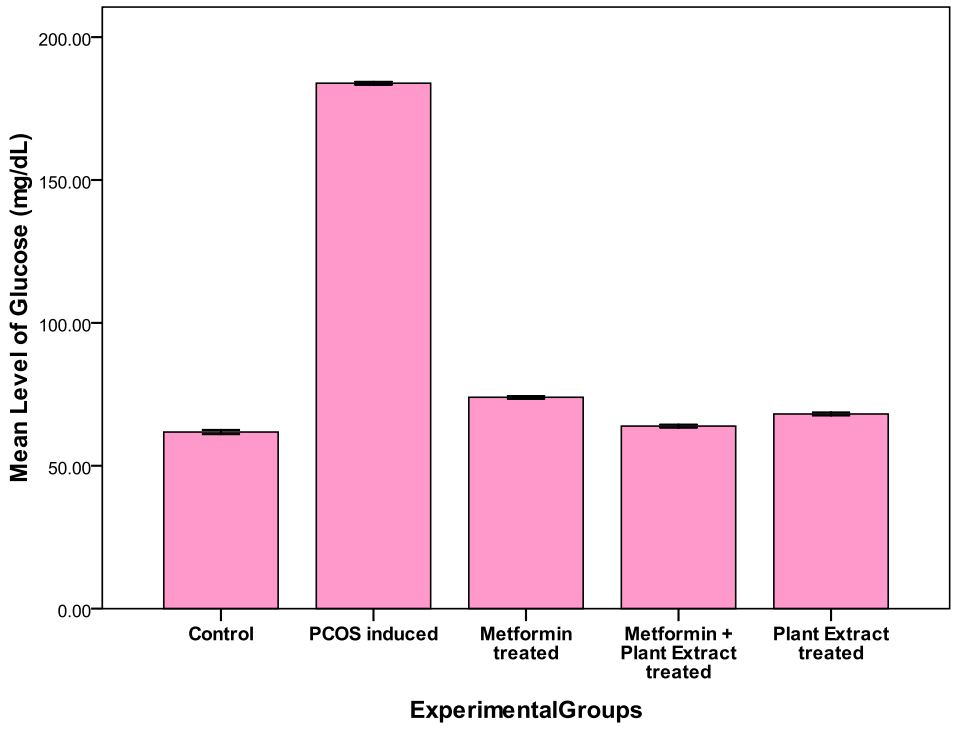

Figure 3: Mean±SD of Total glucose levels in blood of control and experimental groups (n=6)

3.3.2. *In vivo* - Lipid Studies

Elevated levels of Total cholesterol were found in Group II and Group III than compared to Group I (control). Triglycerides levels also found significantly high in all four the treated groups than control (Table 3). Among the four treated groups, Group IV- Metformin + plant extract showed significant ($P<0.01$) results in Total cholesterol, HDL, LDL and Triglycerides with control. Hyperlipidemic condition was observed in Group II as 189.76±0.51mg/dl and 97.95±0.28 mg.dl level of total cholesterol and triglycerides due to the PCOS induction whereas in Group III the levels were significantly reduced as 159.86±0.29 and 83.66±0.61 mg/dl respectively. Antihyperlipidemic levels found in Group IV than compared to Group III and Group V (Figures 4-7). Raised total cholesterol, low density lipoprotein (LDL), triglyceride and reduced HDL cholesterol levels were observed in women with PCOS [23, 24, 25]. Cibula*et al.* [26] observed increased prevalence of type 2 diabetes mellitus in PCOS women and 31% of them had impaired glucose tolerance and 7.5% had type 2 diabetes mellitus [27]. Increased serum total cholesterol, triglycerides and low density lipoprotein cholesterol levels are important risk factors for atherosclerosis development. Elevated levels of HDL exert an antiatherogenic effect by counteracting LDL oxidation and facilitating the translocation of cholesterol from peripheral tissue such as arterial walls to the liver for catabolism [22].

Table 3: *In vivo* blood plasma lipid profile of Wistar rats from various groups

Experimental groups	HDL (mg/dL)	LDL (mg/dL)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)
Group I-Control	47.03±0.42	16.66±0.21	130.85±0.22	56.96±0.25
Group II-PCOS induced	37.93±0.21	34.6±0.02	189.76±0.51	97.95±0.28
Group III- Metformin treated	41.96±0.21	29.95±0.18	159.86±0.29	83.66±0.61
Group IV- Metformin + Plant leaf extract treated	45.91±0.34	21.93±0.25	131.96±0.31	64.93±0.33
Group V-Plant leaf extract treated	39.93±0.28	23.95±0.18	139.86±0.28	69.58±0.55

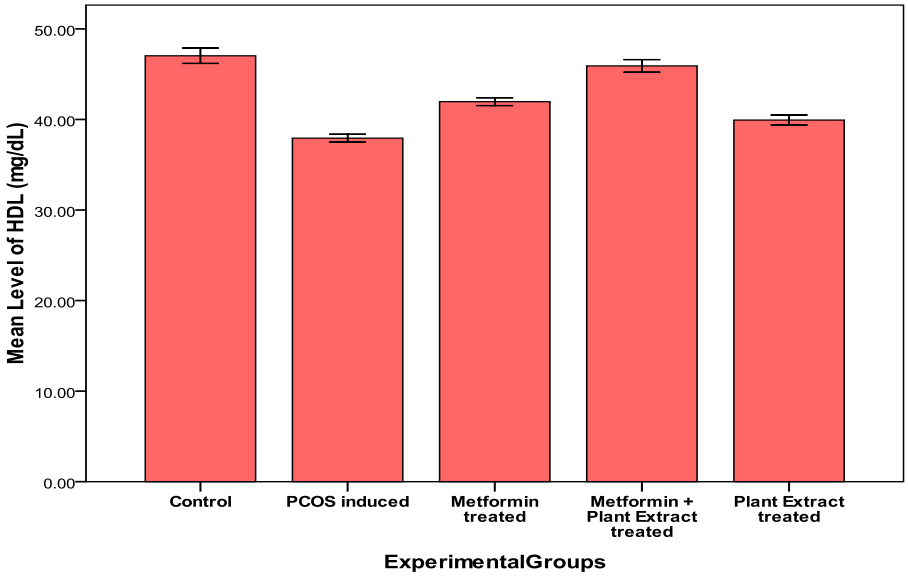

Figure 4: Blood Plasma HDL levels of Wistar rats from various Groups

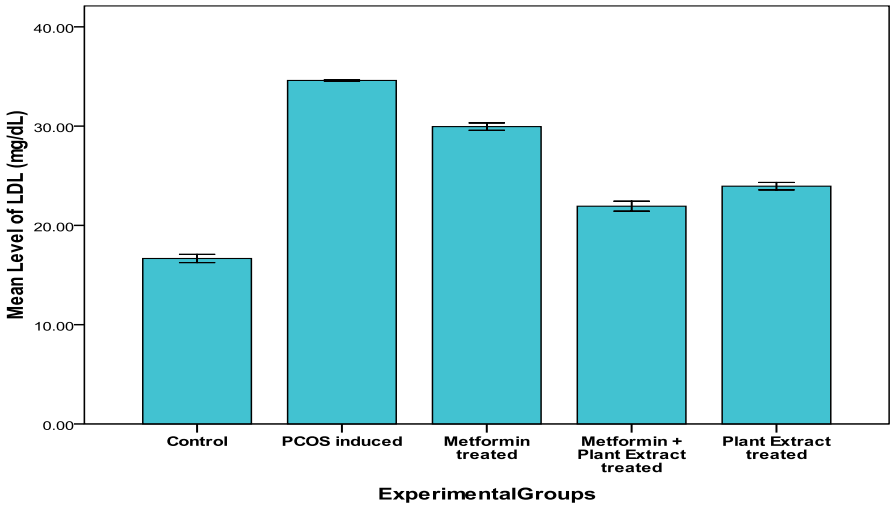

Figure 5: Blood Plasma LDL levels of Wistar rats from various groups

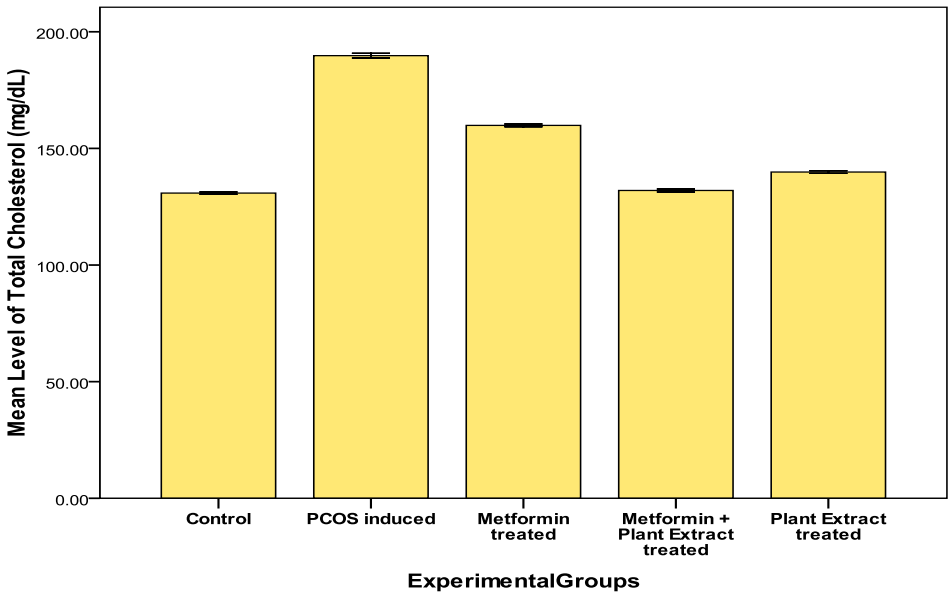

Figure 6: Blood Plasma Total cholesterol levels of Wistar rats from various groups

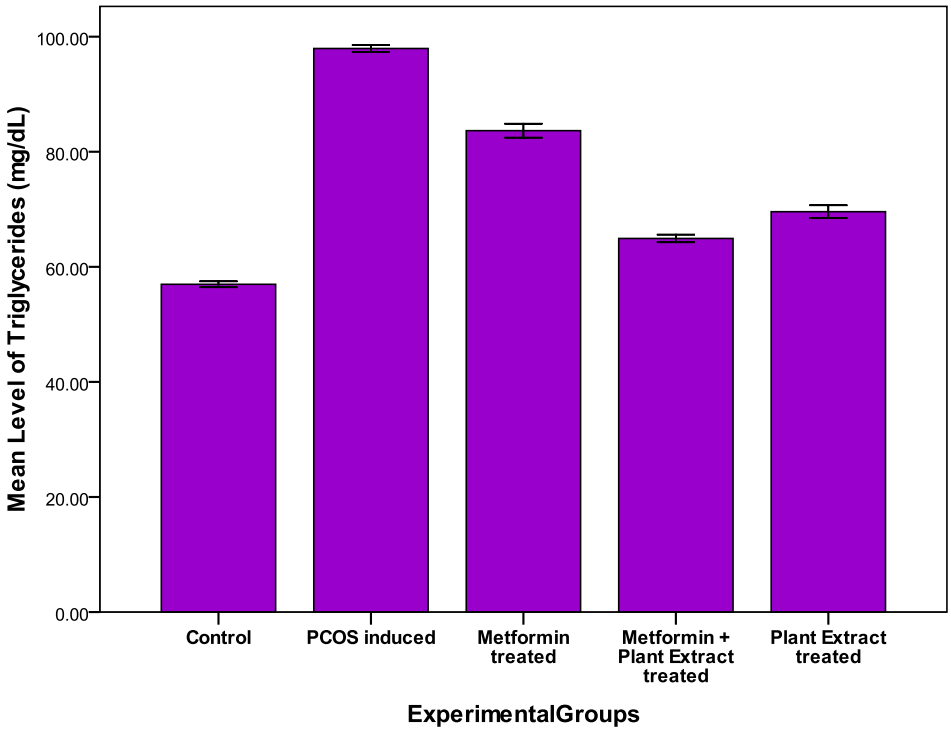

Figure 7: Blood Plasma Triglycerides levels of Wistar rats from various groups

According to Grundy [28] usage of metformin is associated with increased menstrual cyclicity, improved ovulation and reduced androgen levels. Also improves induction of ovulation in women with PCOS by reducing insulin levels and altered ovarian androgen biosynthesis, proliferation of theca cells and endometrial growth through reduced ovarian gluconeogenesis. Metformin is thought

to have primary effects on increasing peripheral glucose uptake in response to insulin, perhaps at the post receptor level, with some reduction in basal hepatic glucose production [29, 30]. Metformin used for the treatment of PCOS had improved the insulin metabolism and reduced the androgen concentration in the serum which was increased due to PCOS and normalization of menstrual cycle. Metformin can also be used as the first line of treatment for ovulation induction and decreases the infertility rate. Both Metformin alone or in combination with clomiphene citrate had been used in the treatment of PCOS and induction of ovulation thereby increases the pregnancy in women with PCOS [31].

4. CONCLUSION

This study focussed about the combined effect of methanolic extracts of *P. daemia* with Metformin in the recovery of PCOS condition in female albino wistar rats. Plant bioactive compounds were isolated and characterized by Column, Thin layer chromatography. Their active compounds were identified by GCMS. Among the four treated groups, Group IV showed significant antidiabetic and antilipidemic activities with the control group Wistarrats blood plasma. These results concluded that Metformin+plant extract group results showed significant results with the control which is an effective way to treat PCOS.

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CONFLICT OF INTEREST

No

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