Evaluation of *Pergularia daemia* and metformin in the treatment of PCOS in testosterone propionate induced albino wistar rats (*Rattus norvegicus*)

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Abstract- The study aimed to evaluate the effect of metformin and the combined activity of *P. daemia* and metformin in the treatment of polycystic ovary syndrome (PCOS) induced rats. Female albino wistar rats (150- 180 g) were injected with Testosterone propionate (TP) for the development PCOS. The PCOS induced rats were divided into two groups, one were treated with metformin and the other were treated with the combination of metformin and *Pergularia daemia* extract over a period of 15 days. The vaginal smear and histological studies proved the combined efficacy of metformin and the *P. daemia* extract by restoring the estrous cycle and re-establishment of the ovarian morphology. The biochemical and hormonal assays also confirms the same. Thus we conclude that the combined activity of metfromin and *P. daemia* extract is an effective medicine in treating PCOS than metformin alone.

Index Terms- Metformin, *Pergularia daemia*, polycystic ovary syndrome, Testosterone propionate, hyperandrogenism.

I. INTRODUCTION

Polycystic ovary syndrome is one of the most common endocrine disorders in women. The aetiology remains uncertain. PCOS is heterogeneous disorder affecting 6- 10% of women in their child bearing age. It is characterized by chronic anovulation, ovarian hyperandrogenism, amenorrhea, hirsutism, acne, and follicular cysts [1, 2]. The morbidity of PCOS includes hyperinsulinaemia, insulin resistance, type 2 diabetes, dyslipidaemia, cardiovascular disease, endometrial carcinoma and infertility [3, 4, 5, 6, 7]. PCOS was first described by Stein and Leventhal in 1935 and hence it is also known as Stein Leventhal syndrome [8, 9]. An association between insulin action and PCOS was first highlighted in 1980 [10]. Later studies have also shown that the insulin resistance is a vital feature of PCOS, mostly in obese women [11, 12].There is a direct effect on ovarian androgen secretion and abnormal follicular development leading to dysfunctional ovary and menstrual activity due to hyperinsulinemia [13].

At present the treatment for PCOS include insulin sensitizers, anti androgens and hormonal therapies. Metformin, the most commonly used agent for treating PCOS, is a biguanide insulin sensitizing drug. It inhibits hepatic glucose production and increase peripheral glucose uptake and moreover it does not encourage secretion of insulin or cause hypoglycemia but in due course of time, these therapies have slight to adverse side effects like hot flushes, arthiritis, joint pain, irritability, mood swings, depression and bloating [14, 15]. Allopathic treatments have severe to mild side effects but the usage of herbs has no or very mild side effect. So it is always better to go for a natural cure. *Pergularia daemia,* belonging to a milky weed family Asclepiadaceae is widely distributed in the tropical and subtropical areas growing on the road sides of Tamil Nadu state of India. It is commonly found at an altitude of 1000m in Himalayas and 900 m in Southern India.

The whole plant has an array of application in folk medicine as well as in Ayurveda. The fresh leaf juice is used for amenorrhea, dysmenorrhea, infantile diarrhoea, catarrhal infection and reduces body pain. The aerial parts of the plant have various pharmacological significance like hepatoprotective [16], anti-diabetic [17], anti-pyretic and anti inflammatory [18]. Therefore the present study compares the efficacy of leaf extract of the *P. daemia* plant with the combined therapy of metformin and metformin alone treatment in PCOS induced rats with Testosterone propionate.

II. MATERIALS AND METHODS

ANIMALS

Twenty four virgin female albino wistar rats (*Rattus norvegicus*) weighing 150- 180 g selected for the study. The animals were maintained at a temperature of about 22± 3^{o}C, photoperiod of 12h/ 12h light/ dark cycle and humidity of 45-50%. Animals were given free access to rat feed (Sai Durga Enterprises, Chennai, India) and water. The approval for carrying out the experiments was obtained from Institutional Animal Ethical Committee (437/C/CPCSEA) (Ref. 03/2013).

EXPERIMENTAL DESIGN

In the present experiment the animals were categorized into four groups including a control group that received vehicle only (olive oil). The three treatment groups' rats were injected intra peritoneally with TP (Himedia, Mumbai, India) at a concentration of 400mg (5g dissolved in 100ml of olive oil) once daily. The treatment period was 7 days. The first PCOS induced groups were left for natural recovery and its serves as a PCOS model. The second PCOS group was treated with metformin of

2mg/ 100g body weight/ day (Sigma Chemical Co; USA) for 15 days. The dosage of metformin was chosen so that it is similar to the dosages that were clinically used in human PCOS patients (200- 300 mg/kg/ day). The last group is given a combined treatment of metformin and fresh plant extract of *P. daemia*. The fresh leaves were washed thoroughly and grinded well in a mortar and pestle resulting in a fresh leaf extract. About 1ml of the fresh extract is given orally through a canula and followed by the metformin administration for every single day for 15 days.

After the experimental period, the animals were anesthetized and the blood were collected from the jugular vein to separate serum for hormonal and biochemical analysis. Vaginal smears were obtained to study the estrous cycle. Ovaries were also excised and fixed in Bouins fixative. Histological studies were carried out using standardized methods. 5µ thickness of sections were taken and stained with haematoxylin eosin and viewed under the microscope.

III. RESULTS

VAGINAL SMEAR

Testosterone propionate induced PCOS animals did not show any estrous stage and resulted in the irregularity in the reproductive cycle. The metformin and the plant extract treated rats regained estrous stage with regular phases. The metformin alone treated groups also showed some improvement but not to the level of the combination therapy of the metfromin and the plant extract which is represented in the Table 1. This shows that the combined therapy is much more effective than metformin alone.

HISTOLOGY

In Fig. 1- 4. the histological sections of the PCOS rats exhibited many small cysts whereas there are no histological abnormalities in the control group. There is a change in the metfromin and *P. daemia* combined therapy, where there is development of follicles and there were no cysts. Metfromin alone treated group also showed normalcy but it was not significant when compared it with the combination therapy.

Hormonal and biochemical parameters are important in knowing the main cause of PCOS. The LDL and HDL levels are measured and represented in the Fig. 5. The levels of HDL are normal in the control group and it increased in the metformin and *P. daemia* treated group. The metformin alone treated group had only slight increase but the PCOS induced group had lesser level of HDL than any other groups. In the case of LDL it is vice versa. There was a decrease in the level of LDL in the metformin and *P. daemia* treated group and also the metformin alone treated group had only small decrease but the PCOS induced groups had higher level of LDL than any other groups signifying that it causes obesity which is a major cause for PCOS. It also shows that the combination therapy of metfromin and *P. daemia* plant had a better efficiency in treating the syndrome than metfromin alone. There was an increased level of cholesterol in the PCOS induced groups and the level starts to decrease in the *P. daemia* and metfromin treated group. Only there is a minor decrease in the metfromin alone treated group. Fig. 6 shows the graphical representation of the levels of triglycerides and glucose. The lower levels of triglycerides are present in the control group and it is similar in the metfromin and *P. daemia* treated group. But the levels increased in the PCOS induced groups. Type 2 diabetes is commonly seen in PCOS patients so to study the link; the glucose level is also estimated. The glucose level was increased in PCOS induced groups but the levels are decreased in the metfromin treated group and the levels were further decreased in the metfromin and *P. daemia* treated group.

Hormonal imbalance is an important criterion in the PCOS patients, so the study was conducted to find out the fluctuations in the level of hormones like FSH, LH, estradiol, progesterone and testosterone. The hormones like FSH and LH regained their levels when the PCOS induced rat groups were treated with *P. daemia* and metformin (Fig 7). The other hormones like estradiol, progesterone and testosterone where brought to normalcy through the combined treatment rather than metformin alone therapy which is represented in the Fig 8.

IV. DISCUSSION

PCOS is a heterogeneous disorder characterized by insulin resistance, hyperandrogenism and obesity [19]. The underlying mechanism for the insulin resistance is indistinct. PCOS is chiefly manifested by anovulation and hyperandrogenism [20]. According to the biochemical analysis, there was an increase in the LDL, triglycerides and cholesterol levels. The results were in accordance with previous studies by Desai, et al., 2012; Sasi kala and Shamila, 2009. The reasons for the obesity in PCOS women are due to the high lipid and cholesterol content. In contrast the levels were decreased when the metformin and *P. daemia* was treated when compared to metformin. The HDL level decreased in the PCOS induced rats but slowly it regained normalcy. There is also a great change in the hormonal profile. There is an increased level of testosterone in the PCOS induced rats where it is a hallmark symptom of PCOS. Clinically metfromin therapy resulted in a significant decrease in the total serum testosterone. It was also corrected by the combined treatment of metfromin and *P. daemia*. Metfromin, an anti hyperglycemic drug has been shown to improve hyperandrogenism and hyperinsulinaemia, due to the effects on glucose utilization in insulin sensitive tissues. Hyperandrogenism was efficiently treated by reducing hyperinsulinaemia using metformin. Recent evidence suggested that one of the modes of action of metformin may be through phosphorylation of the insulin receptor and insulin receptor substrates. In addition, metformin appears to induce cardio-protective effects on plasminogen activator inhibitor (PAI)-1 as well as serum lipids by decrease in the release of FFAs (Free Fatty Acids) from adipose tissue. Since the plant has also anti diabetic property, the combined treatment resulted in the decrease in the glucose level than to metfromin treatment. The irregularity of the reproductive cycle was well studied by using the vaginal smear of the rats. There was no estrous phase in the reproductive cycle but other stages where prominent. It is exciting that combined treatment of the *P. daemia* and metfromin restored estrous cycle in the PCOS induced rats. Ota et al., (1983) concluded that TP administered ovarian histology are very much similar to the human PCOS. The histological results prove that the *P.daemia* with metformin re-established the ovarian architecture where the cysts got reduced and the follicle began to develop. Many follicles were present in the ovarian

cortex with various stages of development showing normal oogenesis.

V. CONCLUSION

According to the present study the metformin has a good healing effect on PCOS condition. Thus it is a potential merit as the first line of treatment for ovulation induction. But to increase the recovering time and minimize side effects, phytotherapeutic approach can be added to the treatment. Herbal drugs have promising role in the treatment of PCOS and have minimal effects. So metformin is used as a co- treatment with the plant *P. daemia.* From the results it is evident that combined therapy of metformin and *P. daemia* is better than metfromin treatment alone.

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Table 1: Comparison of Estrous Cycle of Various Treatment Groups of Albino Wistar Rats

DAYS	CONTROL	INJECTED	METFORMIN	METFORMIN+ PLANT
1	Estrous	Diestrous	Metaestrous	Diestrous
2	Estrous	Diestrous	Metaestrous	Diestrous
3	Estrous	Diestrous	Metaestrous	Diestrous
4	Metaestrous	Early proestrous	Metaestrous	Proestrous
5	Metaestrous	Proestrous	Diestrous	Proestrous
6	Diestrous	Proestrous	Diestrous	Estrous
7	Diestrous	Proestrous	Diestrous	Estrous
8	Diestrous	Proestrous	Diestrous	Metaestrous
9	Diestrous	Metaestrous	Proestrous	Metaestrous
10	Proestrous	Metaestrous	Proestrous	Diestrous
11	Proestrous	Metaestrous	Proestrous	Diestrous
12	Estrous	Metaestrous	Estrous	Diestrous
13	Estrous	Diestrous	Metaestrous	Proestrous
14	Estrous	Diestrous	Metaestrous	Estrous
15	Metaestrous	Diestrous	Metaestrous	Estrous

FIGURE LEGENDS

EFFECT OF *PERGULARIA DAEMIA* AND METFORMIN ON OVARIAN HISTOLOGY IN TESTOSTERONE INDUCED RAT MODEL

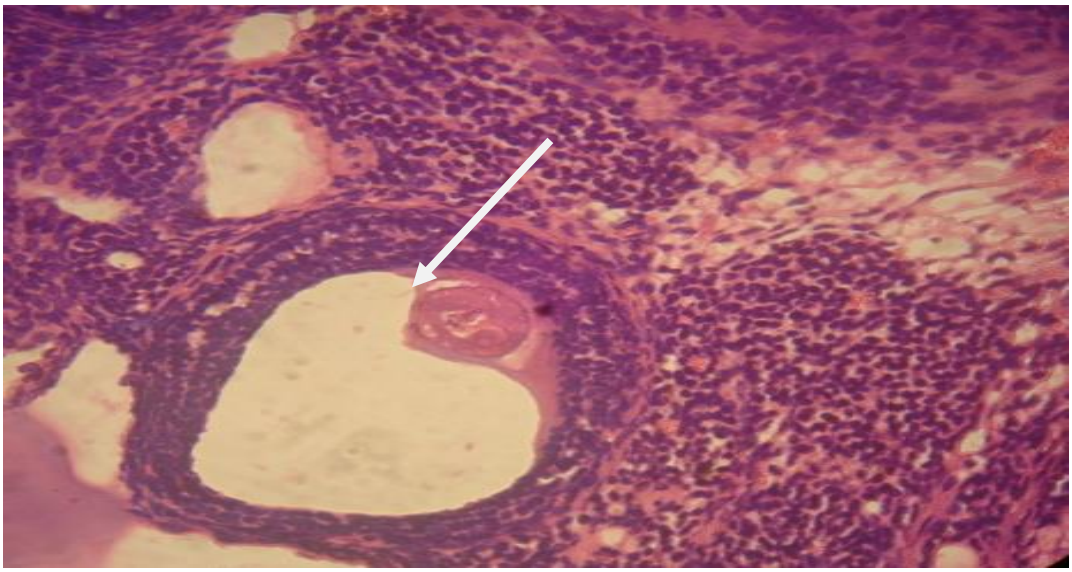

FIG 1: CONTROL 10X

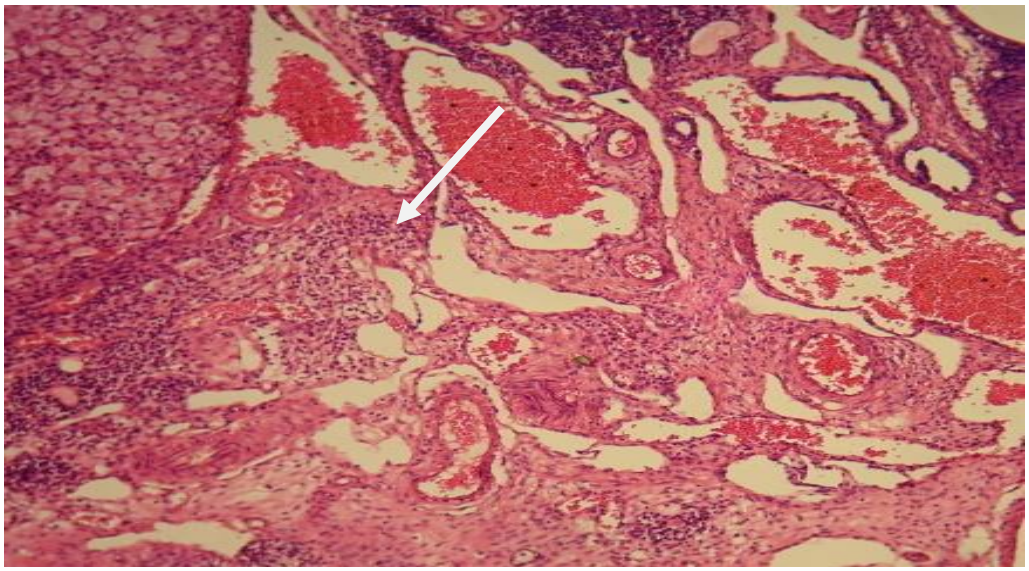

FIG 2: INJECTED 10X

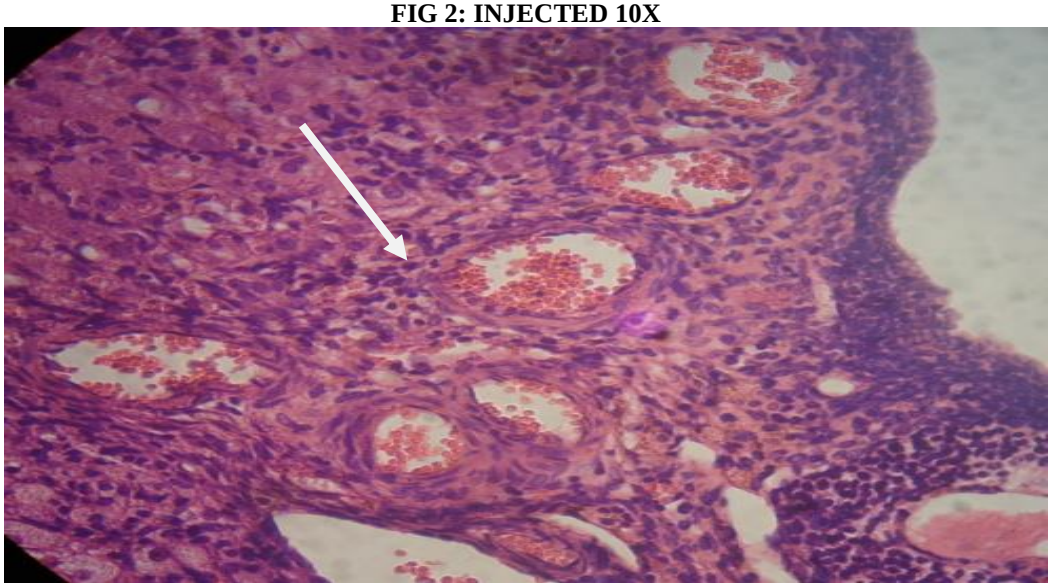

FIG 3: PERGULARIA DAEMIA+ METFORMIN 10X

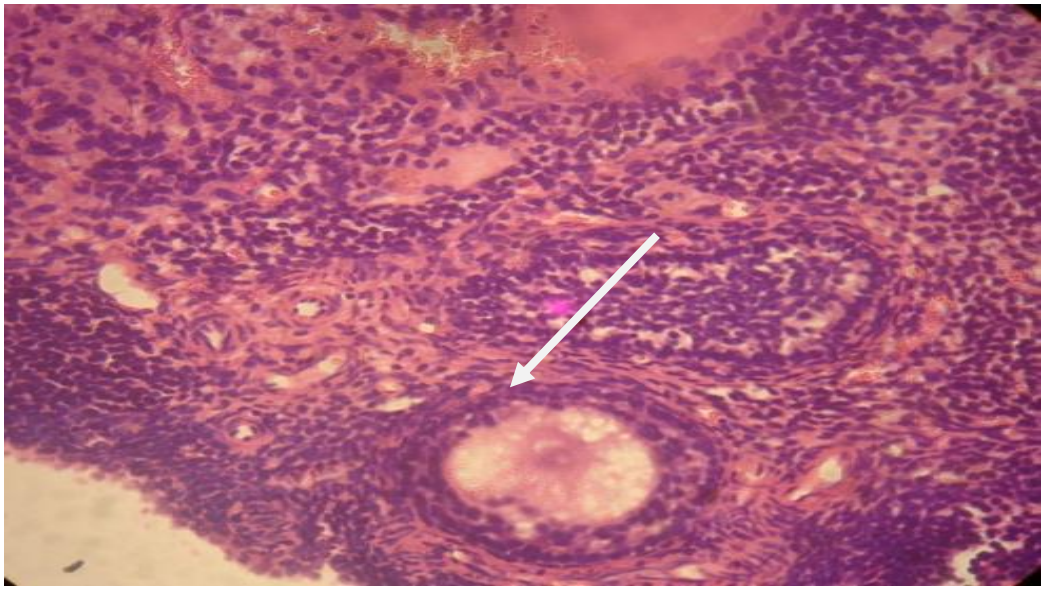

FIG 4: METFORMIN

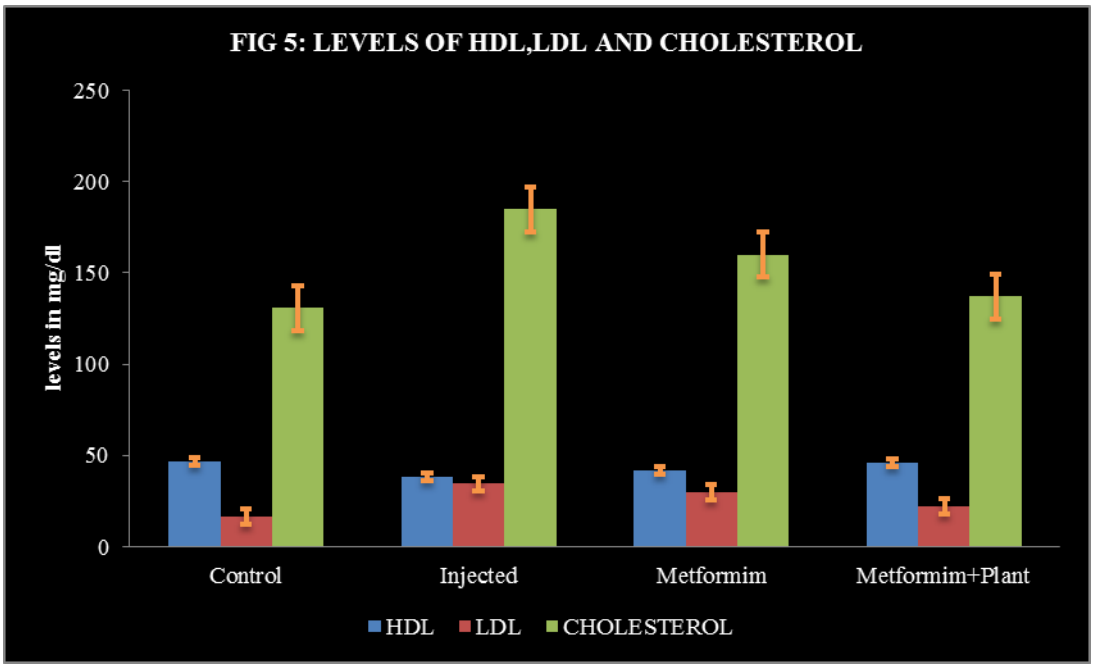
FIG 5: LEVELS OF HDL,LDL AND CHOLESTEROL
250
200
150
100
50
0
levels in mg/dl
Control
Injected
Metformim
Metformim+Plant
HDL
LDL
CHOLESTEROL

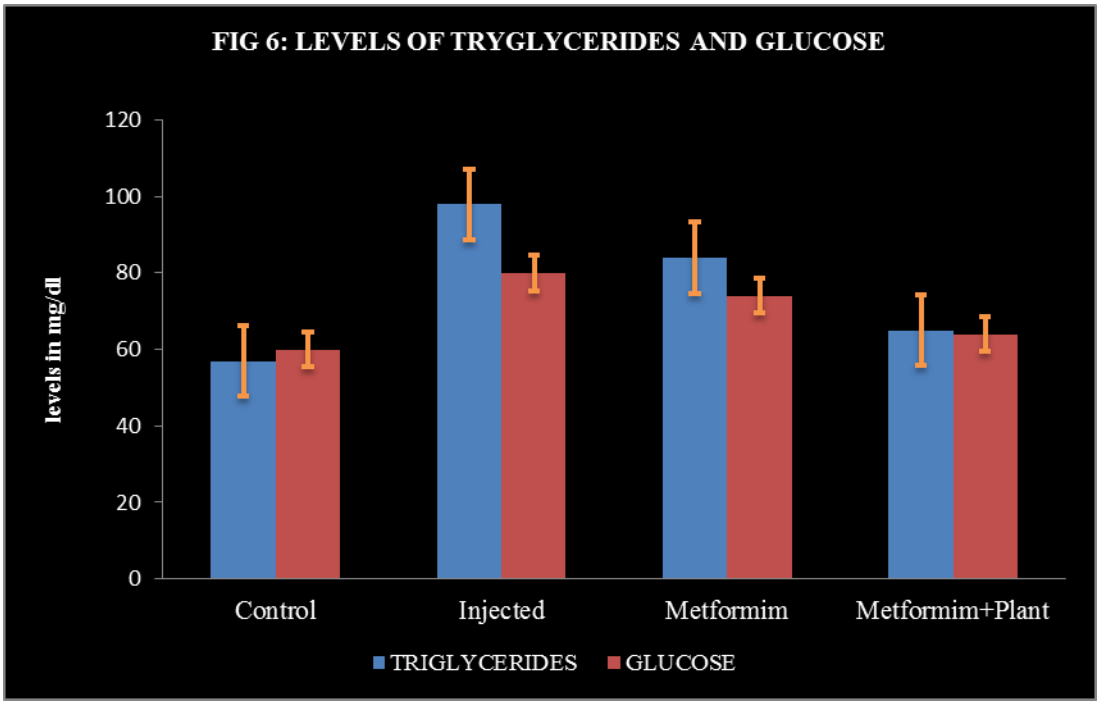
FIG 6: LEVELS OF TRYGLYCERIDES AND GLUCOSE
120
100
80
60
40
20
0
levels in mg/dl
Control
Injected
Metformim
Metformim+Plant
TRIGLYCERIDES
GLUCOSE

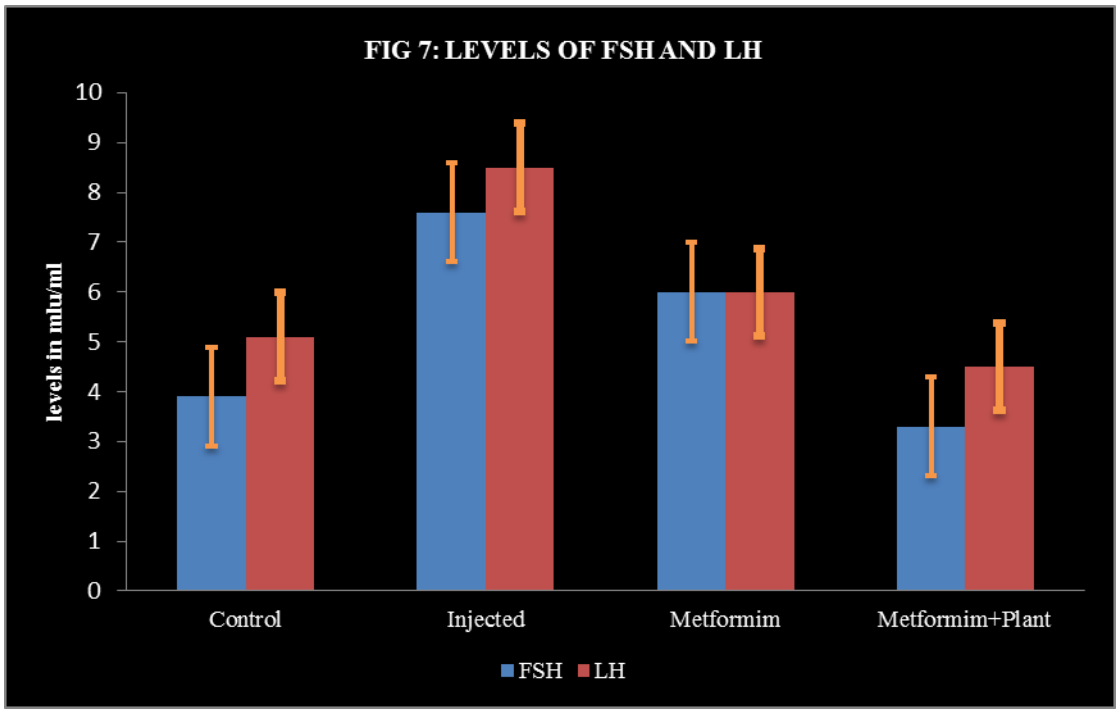
FIG 7: LEVELS OF FSH AND LH
levels in mlu/ml
10
9
8
7
6
5
4
3
2
1
0
Control
Injected
Metformim
Metformim+Plant
FSH
LH

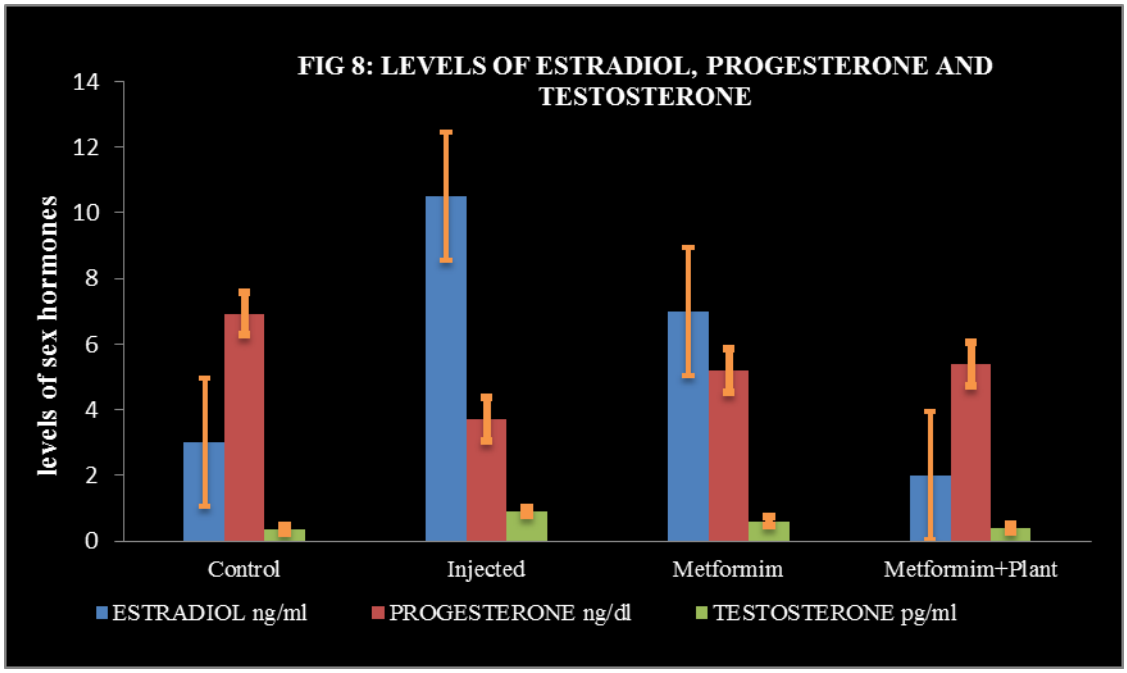
FIG 8: LEVELS OF ESTRADIOL, PROGESTERONE AND TESTOSTERONE
levels of sex hormones
14
12
10
8
6
4
2
0
Control
Injected
Metformim
Metformim+Plant
ESTRADIOL ng/ml
PROGESTERONE ng/dl
TESTOSTERONE pg/ml