

The Effect of *Bougainvillea spectabilis* Leaf Extract on Polycystic Ovary Syndrome (PCOS) in Rat Model

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Received September 17, 2022; Revised October 23, 2022; Accepted November 03, 2022

Abstract Polycystic ovary syndrome (PCOS) is the most common endocrine disorder that affects females of reproductive age. Clomiphene citrate (CC) is used as a first line treatment for induction of ovulation in PCOS patients. *Bougainvillea spectabilis* is a Phytochemicals containing plant used as herbal medicine in treating PCOS. The study was aimed to compare the efficacy of Clomiphene citrate and *Bougainvillea spectabilis* extract against PCOS in rats. Estradiol valerate was used for PCOS induction in all rats except control group. Normal estrous cycle was detected using vaginal smear method. Thirty young female rats were divided into five groups: 1. Control group. 2. Positive control group (0.2ml EV), 3. CC group (20mg CC). 4. BSL group (20mg of BSL extract in 0.5ml water). BSL+ CC group (20mg of CC and 20mg of BSL extract). Biochemical measurements of serum levels of LH, FSH, estrogen, progesterone and testosterone showed a significant change after treatment (p value < 0.05). Combined therapy group showed most promising results in restoring the normal hormonal balance (p value < 0.05). Histological investigation showed multiple cystic follicles in positive control group. Few cystic follicles were observed in BSL group while one cystic follicle was observed in CC group. The combined therapy group showed no cystic follicle in ovaries of the rats. Significant improvement in hormonal and histological observation was seen in combined therapy group suggesting this combination as an effective treatment against polycystic ovary syndrome.

Keywords: PCOS, Clomiphene citrate, Estradiol valerate, BSL extract

Cite This Article: Sania Riaz, Iqra Ejaz, Rizwan-ur-Rehman, Beenish Aftab, Maria Zahid, and Zahra Andleeb, "The Effect of *Bougainvillea spectabilis* Leaf Extract on Polycystic Ovary Syndrome (PCOS) in Rat Model." *Journal of Food and Nutrition Research*, vol. 10, no. 11 (2022): 762-771. doi: 10.12691/jfmr-10-11-3.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common reproductive disorder that affects 6–10% females. The infertility rate among PCOS patients is about 40% [1]. The main causes of sub-fertility or infertility are anovulation and hyperandrogenism [2]. It is also called as Stein-Laventhal syndrome. In PCOS, the ovaries of the affected female become puffy with many small cysts. These cysts are the undeveloped follicles [3].

Symptoms of PCOS may appear at any age period. They may range from premature puberty in childhood, hirsutism and menstrual disturbance in young age leading to infertility in middle age and finally diabetes mellitus and coronary diseases in older age. The other characteristic features include hyperandrogenism, insulin resistance, obesity, acne, multiple immature follicles, many atretic follicles and others indicating failure of folliculogenesis [4].

Insulin resistance is also a characteristic feature of PCOS. Insulin regulates the function of ovaries. Excess release of insulin leads to Anovulation. By the production of androgens, ovaries respond to this condition. Anovulation is the leading cause of infertility [5].

The pathogenesis of PCOS involves Luteinizing hormone (LH) and Follicle stimulating hormone (FSH). These hormones are secreted by the pituitary gland. In normal conditions, the level of FSH is higher than LH, which is essential for the normal menstrual cycle. While in PCOS, the increased level of LH will produce more androgens than normal, leading to increased production of testosterone and estrogen. This condition is associated with excess body and facial hairs and menstrual irregularity [6].

Estradiol valerate (EV) induced PCOS models were associated with morphological, endo-crinological and metabolic changes. Estradiol Valerate (EV) causes hormonal changes in the animal body, leading to PCOS (Walters et al., 2012). EV is an estrogen with a long term effect. It could activate the peripheral sympathetic neurons

innervating the ovary. This activation may increase the content of ovarian nor epinephrine, and decrease the number of ovarian beta-adrenergic receptors. This subsequently down regulates the ovary receiving catecholamine innervations [7].

Previous studies reported that hormonal changes due to EV can produce symptoms of PCOS [8]. For the induction of PCOS, Estradiol Valerate was used by Brawer in 1996. The concentration of Luteinizing hormone is changed and the ovulation process was arrested, leading to many cysts in the ovaries of rats [9]. The animals used in PCOS experiments mostly includes rodents [7], rhesus monkeys [10], and sheep [11]. Among these animals, rodents are most frequently used for PCOS studies because of lower cost, and short time period for the induction of PCOS.

To avoid PCOS development, proper diet and exercise are the modifications in lifestyle. Certain drugs are also used to counter disease symptoms. These drugs include Spironolactone and Flutamide (Androgens), Metformin as an insulin lowering agent, and oral Contraceptives (Estrogen and Progestin). Such pharmacological treatments certainly show different side effects, like gastrointestinal abnormalities, obesity and higher insulin resistance [12].

Ovulation induction is a way to treat infertility in PCOS which can be done by medication or surgery. Clomiphene citrate (CC) is the most commonly used first line treatment for induction of ovulation. It is a non-steroidal estrogen receptor modulator that has predominant anti-estrogenic action resulting in long lasting estrogen receptor depletion. CC has side effects like multi-follicular development, hyper stimulation syndrome and cyst formation [13]. Due to these side effects, the animal researchers are currently focusing on experimental trials involving plant based material.

Bougainvillea spectabilis (family Nyctaginaceae) is a thorny woody plant with flowers. The plant is also called as "Paper Flower" because of its papery textured flowers. Purple and pink are the most commonly found colors of Bougainvillea spectabilis; others include white and orange. The leaves are simple and dark green in color, having fine leathery texture. The length and width of a leaf ranges from 5-10cm and 2-6cm respectively [14]. It contains many components. One of these compounds is Phytochemicals. Phytochemicals include flavonoids, quinines, saponins, triterpenoids, phenols, sterols and sugars. Bougainvillea spectabilis also contains amylase inhibitor, oxidase and pinitol (hypoglycemic element). Flavonoids present in this plant are responsible for antioxidant activity [15]. The other properties may include Antidiabetic, anti-inflammatory, thrombolytic, and amylase inhibitory effects [16].

Due to these therapeutic properties of flavonoids present in Bougainvillea spectabilis, we sought to analyze the pre and post experimental levels of LH and FSH in the blood circulation of PCOS induced rats using Estradiol valerate administration.

2. Materials and Methods

2.1. Animals

Thirty healthy young female rats aged 10-12 weeks were obtained from the animal house of Institute of Molecular Biology and Biotechnology, The University of

Lahore. Prior to the experiment, weight of each rat was recorded between 160-180g. All the rats were kept in clean cages at room temperature and were exposed to normal light and dark cycle. All the rats had a free access food and water. Before experimental trail, the animals were acclimatized for 2 weeks in controlled conditions.

2.2. PCOS Induction

For the induction of polycystic ovary syndrome in female albino rats, hormonal induction method was performed using Estradiol Valerate (EV). Estradiol valerate was purchased from Bayer Pharma (Pvt) Ltd as brand name of Gravibinan 2ml injections. Except control group; all experimental rats received 0.2ml of EV via oral gavage method for. At 10th day of the treatment, persistent vaginal cornification was observed in all rats using vaginal smear method. Daily observation of vaginal smears through microscopy of stained slides, confirmed the PCOS condition in rats.

2.3. Standard Drug

Clomiphene citrate (CC) was used as a standard drug against PCOS [17].

2.4. Bougainvillea Spectabilis Leaves (BSL) Extract Preparation

Bougainvillea spectabilis leaves were collected from Jinnah Garden, Lahore Pakistan in June' 2021. The flowering and fruiting time of the plant is March-July. The identification of the plant species was verified from Flora and phytotaxonomy research department of the Botany, UOL.

The leaves were dried in air and grinded into powdered form. About 500ml of absolute ethanol was used for the percolation of 200g of leaves powder for approximately 2 weeks [18]. The percolated mixture was filtered and vaporized at room temperature [19]. 0.5ml of 0.25% Carboxy methylcellulose was used to make aqueous extract just before use.

2.5. Vaginal Smear

Normal estrous cycle was detected using vaginal smear method. Smear sample was collected between 8:00AM and 12:00AM. For the collection of smear sample, about 0.2-0.3ml of normal saline was flushed into vaginal orifice of each experimental rat using a small dropper. A drop of the obtained fluid was placed on a glass microscope slide, dried in air. Giemsa stain was used for staining purpose [20]. The rats with three 4-5 days' regular estrous cycles will be used for the experiment [15].

2.6. Experimental Design

Group I. Control group included six rats that received normal saline solution 1mg/kg/day orally.

Group II. Positive Control group/PCOS group included six rats that were administered with a dose of 2ml/kg (0.4 ml) of estradiol valerate via single oral gavage method.

Group III. Clomiphene citrate group/PCOS group included six rats that were given 100mg/Kg/day (20mg) Clomiphene citrate after the development of PCOS.

Group IV. BSL group/PCOS group included six rats that were given BSL extract in a daily dose of about 100 mg/kg (20mg) in 0.5ml of distilled water orally by gavage method for 10 days.

Group V. Combined therapy group (BSL + Clomiphene citrate group/PCOS group) included six rats that were given about 20mg of BSL extract and 20mg of Clomiphene citrate in 0.5ml of distilled water for 10 days.

2.7. Sample Collection

After the completion of trial, the treated rats were weighed and anesthetized using chloroform. The blood samples were directly taken from cardiac puncture and centrifuged at 3000 rpm for 15min. The collected serum was stored at -70°C . Serum was analyzed for hormonal assay [15].

2.8. Histopathological Analysis

The abdomen was rapidly dissected and ovaries were removed, cleansed gently with normal saline and weighed. Then, they were immediately fixed in 10% formalin and processed for paraffin blocks and stained with hematoxylin and eosin stain [21].

2.9. Hormonal Assay

Biochemical measurements of serum levels of testosterone, LH, FSH, estrogen and progesterone were done using ELISA method [15]. The FSH and LH plasma levels were measured by using Cusabio Kit (China) which were specific for rats with 0.15Mlu/ml using ELISA method [22].

2.10. Statistical Analysis

The data were expressed as mean $\pm$ SD. ANOVA test was used for comparison of the data between different groups [22].

Table 1. Mean values of LH, FSH, Progesterone, Testosterone and Estrogen in control group, Positive control group and treatment groups

Hormone	C	PC	CC	BSL	BSL+CC
LH	1.21	1.58	0.99	1.08	1.14
FSH	1.21	0.79	0.85	1.01	0.98
Progesterone	16.27	38.51	18.97	9.56	8.02
Testosterone	1.23	1.78	1.19	0.88	1.54
Estrogen	23.05	57.08	30.7	24.58	22.98

3. Results

3.1. Estrous Cycle Detection

Estrous cycle was monitored of all rats before induction of polycystic ovary syndrome. Vaginal smear method was used for this purpose. Stained slides were observed under microscope at 40X. The results showed pro-estrous phase

of estrous cycle consisting of round nucleated epithelial cells (Figure 1 a). Estrous phase was monitored by anucleated cornified cells (Figure 1 b). Metestrus phase was detected by the presence of leucocytes and cornified cells (Figure 1 c). Abundance of leucocytes marked the diestrus phase of estrous cycle (Figure 1 c). Presence of all four phases of estrous cycle showed that the estrous cycle is normal.

Biochemical results of serum concentration of different reproductive hormones in control group, PCOS group and treatment groups (CC, BSL and BSL+CC) showed a significant variation among all groups. According to the results, Luteinizing hormone, Progesterone, Testosterone and Estrogen significantly increased in positive control group compared with the control group after administration of estradiol valerate. While the serum level of Follicle stimulating hormone is significantly decreased in positive control group compared with the control group. This change in concentration of LH, FSH, Progesterone, Testosterone and Estrogen hormone confirmed the development of polycystic ovaries in all estradiol valerate treated rats (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6).

Treatment groups include treatment with Clomiphene citrate, *Bougainvillea spectabilis* leaf extract and combined therapy group (CC, BSL and BSL+CC). Clomiphene citrate group showed a minimum change in hormonal levels of LH, FSH and estrogen compared with the PCOS group, while Progesterone and Testosterone levels are significantly increased compared with positive control group.

Bougainvillea spectabilis leaf extract group showed significant change in concentration of LH, FSH and Estrogen compared with positive control group. While change in Progesterone and Testosterone level is less significant in BSL extract treated group compared with the positive control group.

In combined therapy group, BSL extract and Clomiphene citrate was given to evaluate the effect of this novel combination. This combined treatment proved to be most effective against polycystic ovary syndrome in estradiol valerate induced rats. Biochemical analysis of this group revealed the most significant change in serum concentration of LH, Testosterone and Estrogen. While the change in FSH and Progesterone hormone was also significant in combined therapy group compared with the positive control group.

3.2. Histological Observations

3.2.1. Control Group

Histological observation of the control group ovary with fallopian tube reveals normal ovarian stroma and vasculature (Figure 7). There were four follicular cysts present in the section. No evidence of any inflammatory disease, granuloma or malignancy seen.

3.2.2. Positive Control Group

Histological examination of the ovarian tissue revealed normal ovarian stroma and vasculature (Figure 8). The ovary includes enlarged, sclerotic multiple cystic follicles that were six in number. No evidence of any inflammatory disease, granuloma or malignancy seen. These features were suggestive of Polycystic Ovary.

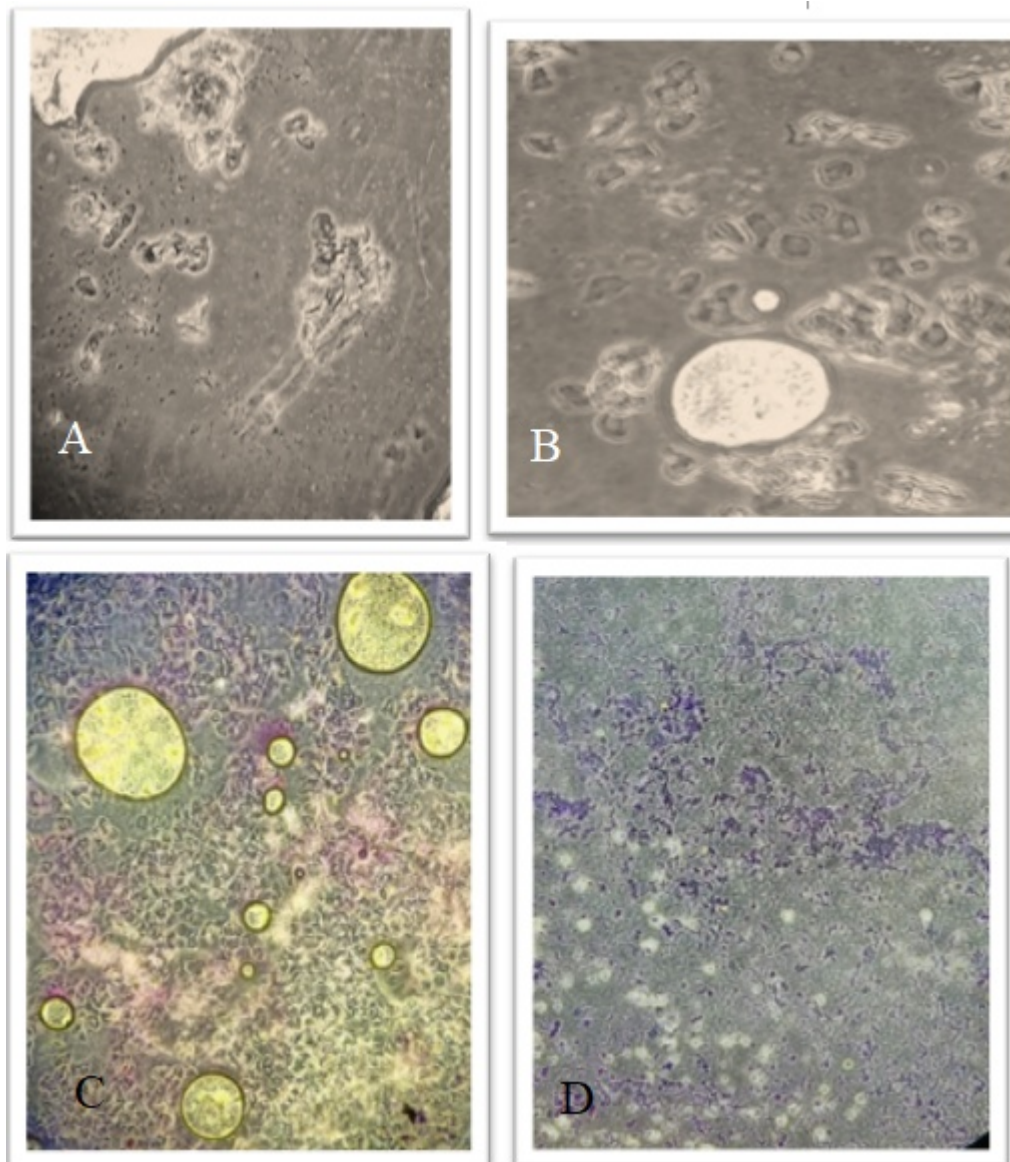


Figure 1. Stages of estrous cycle, A) Pro-estrous phase B) Estrous phase C) Metestrus phase and D) Diestrus phase

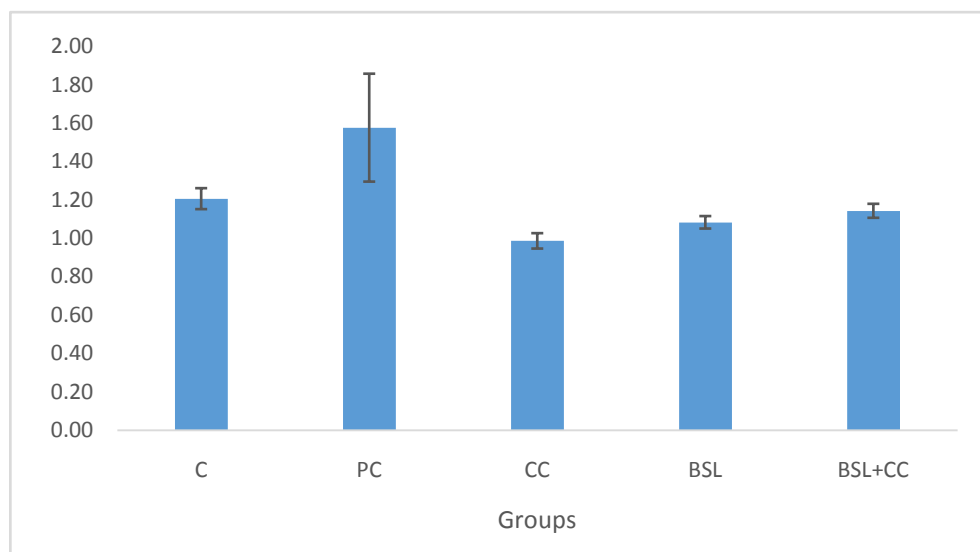


Figure 2. Mean serum levels of Luteinizing hormone (mIU/ml) in control (group I), positive control (PCOS induced group, group II), Clomiphene citrate treatment group (group III), *Bougainvillea spectabilis* leaf extract treated group (group IV), combined therapy group (BSL+CC treated rats, group V)

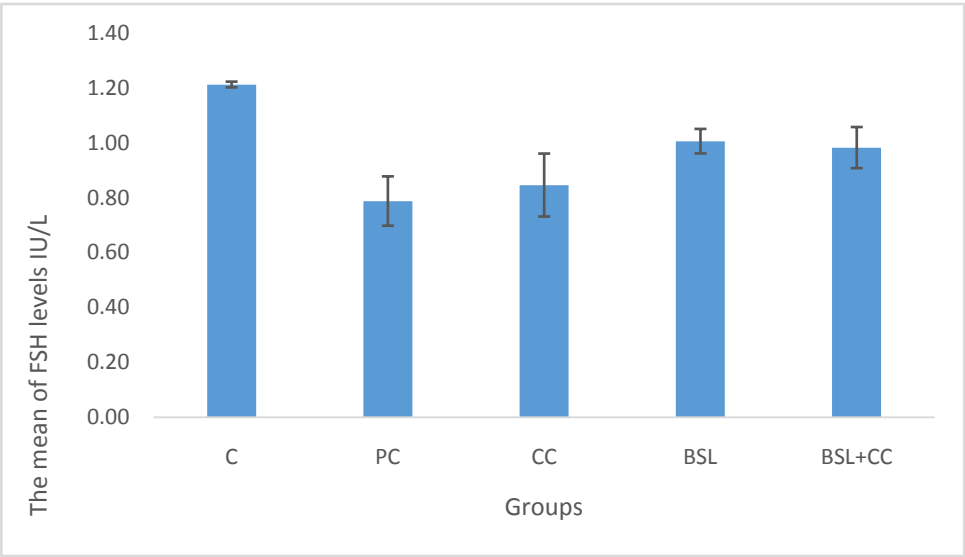


Figure 3. Mean serum levels of Follicle stimulating hormone (IU/L) in control (group I), positive control (PCOS induced group, group II), Clomiphene citrate treatment group (group III), *Bougainvillea spectabilis* leaf extract treated group (group IV), combined therapy group (BSL+CC treated rats, group V)

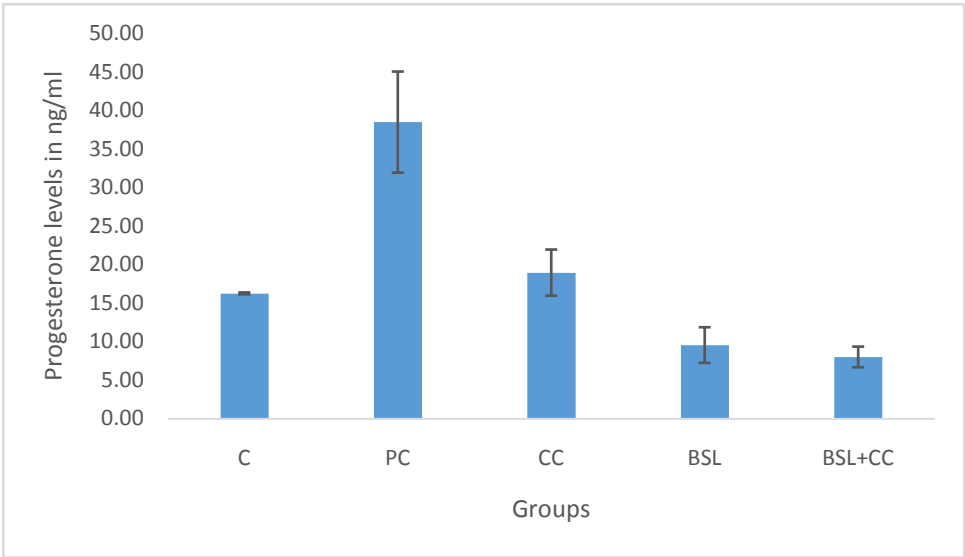


Figure 4. Mean serum levels of Progesterone hormone (ng/ml) in control (group I), positive control (PCOS induced group, group II), Clomiphene citrate treatment group (group III), *Bougainvillea spectabilis* leaf extract treated group (group IV), combined therapy group (BSL+CC treated rats, group V)

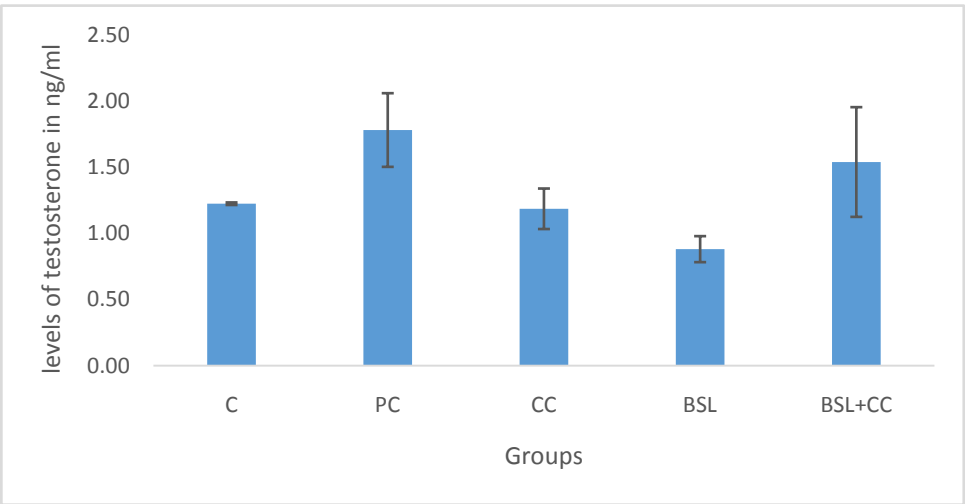


Figure 5. Mean serum levels of Testosterone hormone (ng/ml) in control (group I), positive control (PCOS induced group, group II), Clomiphene citrate treatment group (group III), *Bougainvillea spectabilis* leaf extract treated group (group IV), combined therapy group (BSL+CC treated rats, group V)

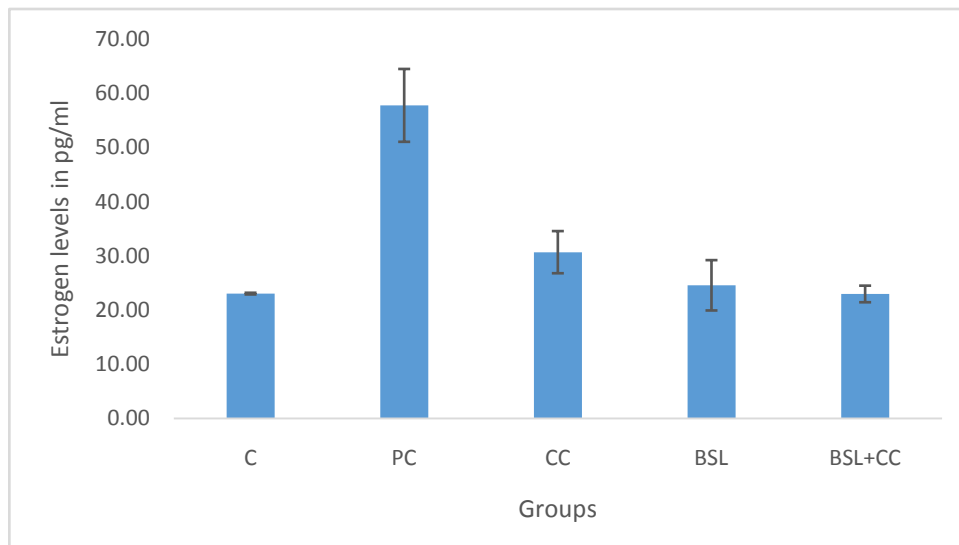


Figure 6. Mean serum levels of Estrogen hormone (pg/ml) in control (group I), positive control (PCOS induced group, group II), Clomiphene citrate treatment group (group III), *Bougainvillea spectabilis* leaf extract treated group (group IV), combined therapy group (BSL+CC treated rats, group V)

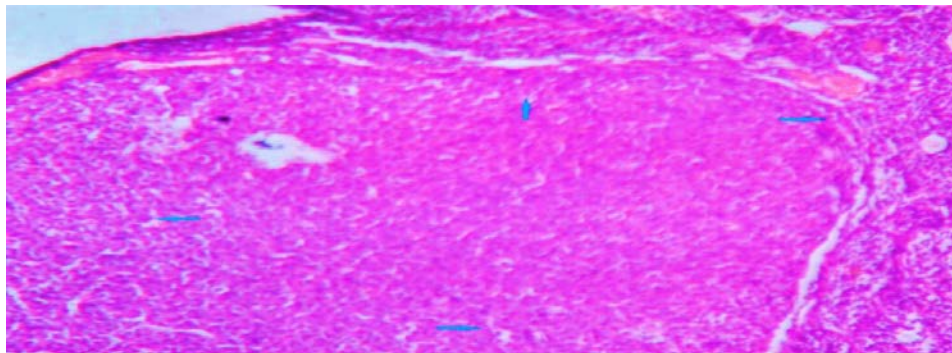


Figure 7. Histological analysis of ovarian tissue of Control group (staining with H and E stain). Follicular cysts (Black arrows), Graafian follicle (Blue arrow), early ovarian follicle and corpus luteum

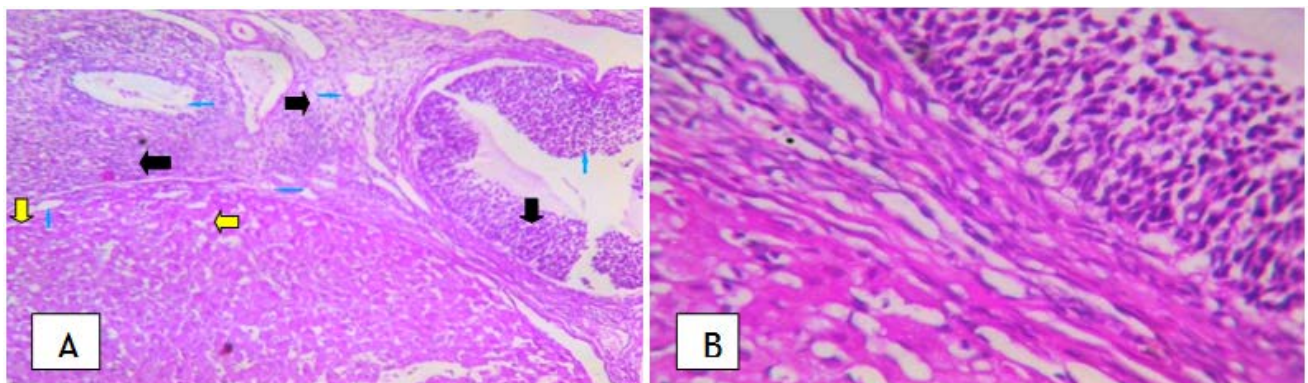


Figure 8. Histological analysis of ovarian tissue of Positive Control group (staining with H and E stain). A: Low magnification (40X). B: High magnification (100X). Cystic follicles (blue arrows)

3.3.3. Clomiphene Citrate Group

Histological examination of the submitted ovary reveals normal ovarian stroma and vasculature (Figure 9). The ovary includes three mature griffin follicles. One cystic follicle is also present. No evidence of any inflammatory disease, granuloma or malignancy seen. These features were suggestive of maturation of graafian follicles.

3.3.4. BSL Group

Histological examination of the submitted ovary revealed normal ovarian stroma and vasculature (Figure 10). The

ovary includes one griffin follicle. Few cystic follicles were also present. No evidence of any inflammatory disease, granuloma or malignancy seen. These features were suggestive of maturation of griffin follicles.

3.3.5. BSL + CC Group

Histological examination of the submitted ovary revealed normal ovarian stroma and vasculature (Figure 11). The ovarian sections contain two mature Graafian follicles. No cystic follicle was seen. These features were suggestive of maturation of Graafian follicles.

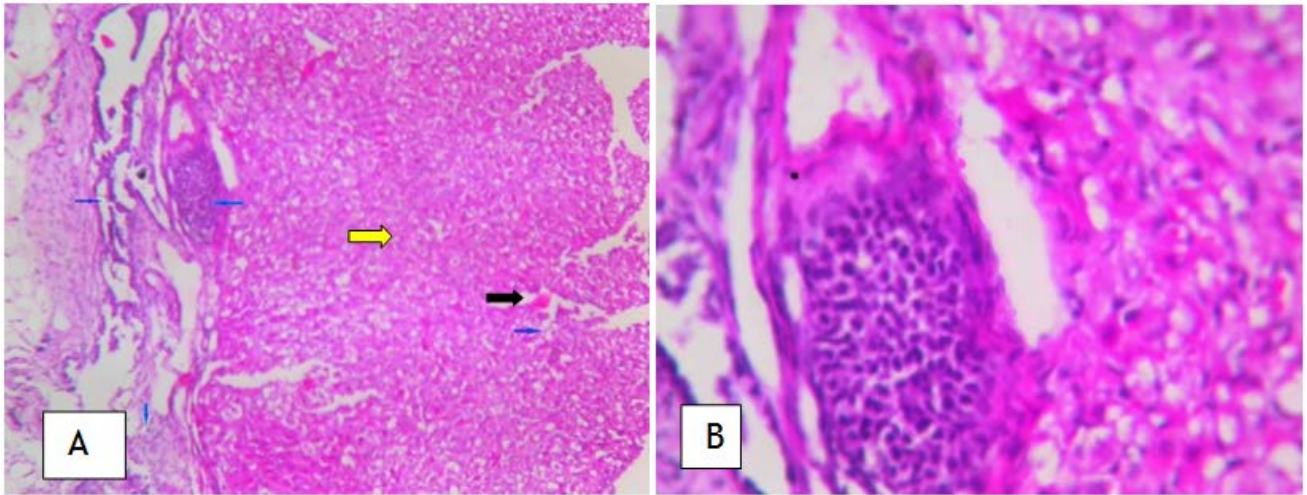


Figure 9. Histological analysis of ovarian tissue of Clomiphene citrate group (staining with H and E stain). A: Low magnification (40X), B: High magnification (100X). Cystic follicle (Black arrow), Corpus luteum (yellow arrow) and mature Graafian follicles (Blue arrow)

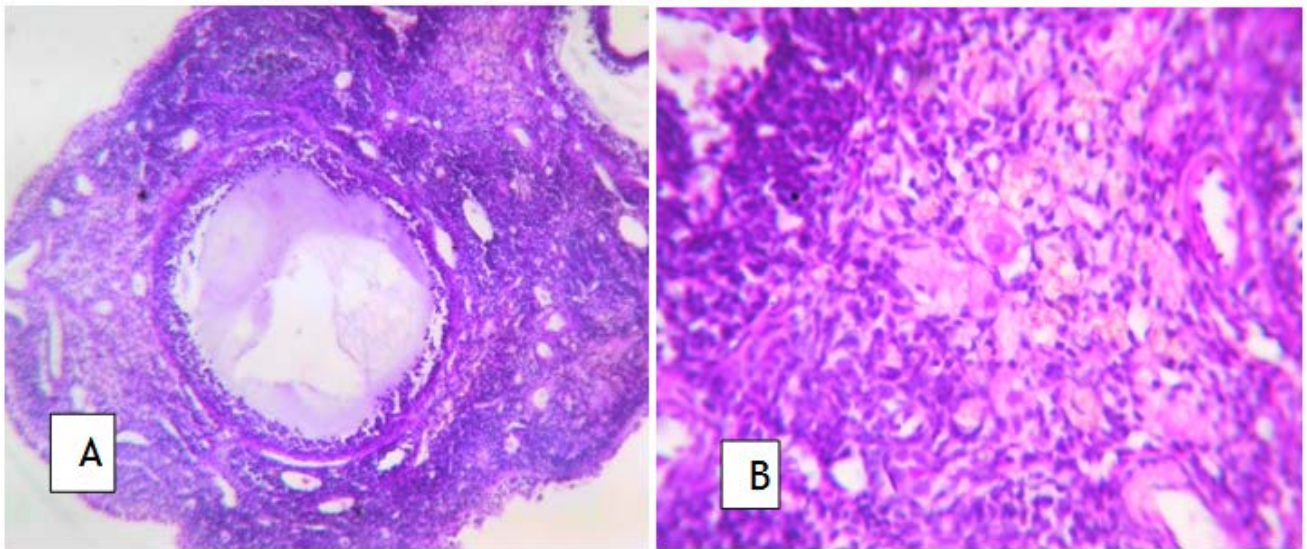


Figure 10. Histological analysis of ovarian tissue of BSL extract treatment group (staining with H and E stain). A: Low magnification (40X), B: High magnification (100X)

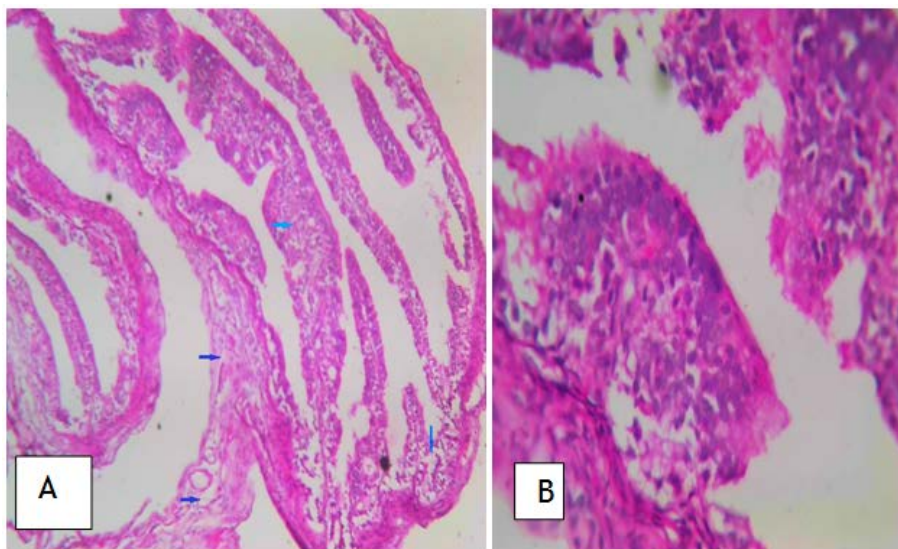


Figure 11. Histological analysis of ovarian tissue of combined therapy group (staining with H and E stain). A: Low magnification (40X), B: High magnification (100X)

4. Discussions

Polycystic ovary syndrome is a hormonal disorder in adolescent females characterized by a large number of cysts in the ovaries of the patient leading to menstrual disturbances, hirsutism, acne and obesity [23]. Polycystic ovary syndrome affects 10% of female population of reproductive age [24]. Endocrine and metabolic mechanisms are involved in the pathogenesis of polycystic ovary syndrome that causes infertility due to anovulation [25]. The other symptoms of PCOS are polycystic ovaries, hyperandrogenism, increased luteinizing hormone and oligomenorrhea [26].

Currently the standard care treatments for the PCOS include changes in modes of living such as exercise and changing food habits and pharmaceutical therapy [3].

Clomiphene Citrate (CC) is the most frequently used first line treatment, is an estrogen-receptor modulator that will promote ovulation, regulates menstrual cycle increases the production of gonadotropins from pituitary. However, it may have a lot of side effects such as decrease in the thickness of endometrium, vomiting, ovarian hyper stimulation syndrome, nausea and breast discomfort [27].

As the side effects of these pharmaceutical treatments have significant importance, so many research studies including case studies, animal experimental studies and randomized controlled trials are investigated for herbal treatments [28].

Many beneficial herbs showing antioxidant and hypoglycemic activity may have protective effects against polycystic ovary syndrome. *Bougainvillea spectabilis* is a thorny woody plant, containing different types of active compounds such as flavonoids, saponins, sterols, glycosides, phenols, tannins and small amounts of sugars. The other properties may include Antidiabetic, anti-inflammatory, thrombolytic, and amylase inhibitory effects [16].

In this study, PCOS rats were developed using Estradiol valerate that has been reported to cause polycystic ovary syndrome in animal models by through physiological and metabolic processes [15]. Thirty female rats were divided into five groups including Control, Positive control, Clomiphene citrate group, BSL treated group and combined therapy group (BSL+CC).

Estrous cycle was monitored for each group before induction of PCOS. Four phases of estrous cycle was observed under microscope using vaginal smear method. Proestrous stage was detected by the presence of round, nucleated epithelial cells. Estrous phase was confirmed by anucleated cornified cells. Some leucocytes and cornified cells marked the Metestrus phase. The abundance of leucocytes confirmed the diestrus phase of estrous cycle. These results were similar to a recent study by [29]. After induction of PCOS by estradiol valerate, estrous cycle was disturbed, however in treatment groups the estrous cycle was restored to normal.

In this experiment, a significant change in the levels of reproductive hormone was observed in all groups, except control after administration of Estradiol valerate which indicated the development of polycystic ovary syndrome. The results showed a significant increase in the levels of Luteinizing hormone in PCOS group compared with the control group, 1.58 ± 0.28 and 1.21 ± 0.05 respectively.

Elevated levels of LH produced excess amount of androgen substrate leading to hyperandrogenism and causing the development of PCOS symptoms. A study conducted by Eyvaznejad *et al.*, [30] also reported an increase in the level of luteinizing hormone in PCOS group compared with the control group. The results of this study were also similar to the work of Ghafurniyan *et al.*, [20] in which the level of LH was raised in PCOS rats.

Follicle stimulating hormone levels, as shown by the biochemical analysis of serum, were significantly decreased in positive control group compared with the control, 0.79 ± 0.09 and 1.21 ± 0.01 respectively. Due to this decrease in the level of FSH, ovaries were not stimulated for menstruation and then ovulation. This is the reason that PCOS patients experience difficulty in conceiving and have menstrual disturbances. Similar results have been reported by Zeba *et al.*, [31]. Eyvaznejad *et al.*, [30] also reported that the level of follicle stimulating hormone decreased after administration of estradiol valerate in rats. While in contrast, Barzegar *et al.*, [32] reported an increase in serum concentration of follicle stimulating hormone after induction of PCOS using estradiol valerate.

Increased levels of LH resulted in an increase in the production of progesterone hormone elevating its levels as observed by hormonal assay of this experiment. The levels of progesterone hormone have been increased in PCOS group as compared with the control group, 38.51 ± 6.56 and 16.27 ± 0.12 respectively. Similar results have been observed by Mehraban *et al.*, [25]. While in contrast, Khazaei *et al.*, [33] reported a study in which the level of progesterone hormone decreased in PCOS group after administration of estradiol valerate. According to Atashpour *et al.*, [17], progesterone hormone level was decreased in PCOS group after PCOS induction by estradiol valerate.

Testosterone is a male hormone but it is also produced in the females. In PCOS females, excess production of testosterone causes acne, facial and body hairs. In this experiment, increased serum concentration of testosterone was observed in PCOS group compared with the control group, 1.78 ± 0.28 and 1.23 ± 0.01 respectively. These results are similar to the work carried out by Abtahi-Eivari *et al.*, [26]. Ghafurniyan *et al.*, [20] used estradiol valerate for PCOS induction, reported that serum level of testosterone hormone increased in PCOS group compared with the control group. The results of Barzegar *et al.*, [32] also support the finding of this study.

Estrogen serum concentration as shown by the results of biochemical analysis, have been significantly increased in PCOS group compared to control group, 57.80 ± 6.72 and 23.05 ± 0.15 respectively. These results found similarity with those reported by Mehraban *et al.*, [25]. A study performed by Khazaei *et al.*, [33] reported that the level of estrogen hormone increased in PCOS group after administration of estradiol valerate.

Restoring the normal levels of reproductive hormones is the first option in the treatment of polycystic ovary syndrome. Clomiphene citrate has been reported to treat PCOS conditions by the induction of ovulation and regulating the hormonal levels. The results of this study showed that CC administration reduced the levels of LH 0.99 ± 0.04 , testosterone 1.19 ± 0.15 , progesterone 18.97 ± 3.00 and estrogen levels 30.70 ± 3.89 in Clomiphene

citrate treated group compared to PCOS group. The level of FSH increased in the CC treated group compared with the PCOS group 0.85 ± 0.11 and 0.79 ± 0.09 respectively. These results are similar to the findings of Ainehchi *et al.*, [27]. A similar study was conducted by Atashpour *et al.*, [17] in which the serum concentrations of LH, FSH and Estrogen were restored to normal values after treatment with Clomiphene citrate.

Bougainvillea spectabilis leaves extract have been reported to restore the normal levels of hormonal parameters. The biochemical analysis of this experiment showed that BSL extract significantly reduced the levels of LH 1.08 ± 0.03 , testosterone 0.88 ± 0.10 , progesterone 9.56 ± 2.31 and estrogen 24.58 ± 4.6 . While the levels of FSH were increased as compared to PCOS group 24.58 ± 4.65 and 0.79 ± 0.09 respectively. These results are similar to the finding of Badawi *et al.* [15].

Combined therapy group (BSL+CC) showed that LH, testosterone, progesterone and estrogen levels were reduced compared with the PCOS group. The results showed that FSH serum concentration increased significantly compared with PCOS group as reported by Atashpour *et al.*, [17], Ainehchi *et al.*, [27] and Badawi *et al.* [15].

Histological examination of the ovarian tissue of rats showed that the number of normal follicles was significantly reduced after induction of PCOS while the number of cystic follicles was significantly increased in positive control group. Hyperandrogenism is the cause of reduction in number of normal follicles resulting in the formation of cystic follicles. Abtahi-Eivari *et al.*, [26] reported that the number of cystic follicles is increased in PCOS group compared to control group. This was also in agreement with Badawi *et al.* [15] that reported a massive loss of primordial follicles in ovarian tissue estradiol valerate treated group. After treatment with *Bougainvillea spectabilis* leaf extract and Clomiphene citrate, cystic follicles were significantly reduced in number restoring the normal follicular growth.

5. Summary

Polycystic ovary syndrome (PCOS) is the most common reproductive disorder that affects 6-10% females. In PCOS, the ovaries of the affected female become puffy with many small cysts. These cysts are the undeveloped follicles. Symptoms of PCOS may appear at any age period, ranging from premature puberty in childhood, hirsutism and menstrual disturbance in young age leading to infertility in middle age and finally diabetes mellitus and coronary diseases in older age. The pathogenesis of PCOS involves Luteinizing hormone (LH) and Follicle stimulating hormone (FSH). In PCOS, the increased level of LH will produce more androgens than normal, leading to increased production of testosterone and estrogen. Certain drugs are also used to counter disease symptoms. Clomiphene citrate (CC) is the most commonly used first line treatment for induction of ovulation. CC has side effects like multi-follicular development, hyper stimulation syndrome and cyst formation. Due to these side effects, the animal researchers are currently focusing on experimental trials involving plant based material.

The study was aimed to investigate the effect of *Bougainvillea spectabilis* leaf extract on polycystic ovary syndrome in rats and to compare the efficacy of Clomiphene citrate and *Bougainvillea spectabilis* extract against PCOS.

Thirty healthy young female rats aged 10-12 weeks were divided into five groups (6 rats in each group). Estradiol valerate was used for PCOS induction in all groups except control group. Before induction, normal estrous cycle was detected using vaginal smear method. The experimental groups included: 1. Control group. 2. Positive control group received Estradiol valerate (0.2ml EV for 10 days) for PCOS induction. 3. CC treatment group received 20mg Clomiphene citrate. 4. BSL extract treatment group was given 20mg of *Bougainvillea spectabilis* leaf extract in 0.5ml water. BSL+ CC group received 20mg of Clomiphene citrate and 20mg of BSL extract for 10 days. Biochemical measurements of serum levels of LH, FSH, estrogen, progesterone and testosterone were estimated using ELISA method. Ovarian changes were observed through histological study.

Biochemical results showed a significant change in serum concentration of LH, FSH, estrogen, progesterone and testosterone after induction of PCOS by Estradiol valerate compared with the control group. This hormonal imbalance was significantly restored by treatment with Clomiphene citrate, BSL extract and using combined therapy of CC and BSL. Combined therapy group showed most promising results in restoring the normal hormonal balance. Histological investigation showed multiple cystic follicles in positive control group. Few cystic follicles were observed in BSL treatment group while one cystic follicle was observed in CC group. The combined therapy group showed no cystic follicle in ovaries of the rats.

Significant improvement in hormonal and histological observation was seen in combined therapy group suggesting this combination as an effective treatment against polycystic ovary syndrome.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

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