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Original Article

Combination of Phytochemicals in the Management of Polycystic Ovarian Syndrome Induced in Rats

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is a heterogeneous reproductive disorder and the most common endocrinopathy that leads to anovulatory infertility. PCOS is associated with wide spectrum of complications in various health aspects including anovulation, hyperandrogenism, hirsutism, infertility and menstrual disturbance. Metabolic dysfunctions like obesity, dyslipidemia, diabetes mellitus, cardiovascular risk and changes in psychological feature such as mood disorders and decreased quality of life are also associated with PCOS. Disruption in secretion rates and metabolism of androgens, estrogens and other reproductive hormones is the major characteristic of PCOS. Balancing the disrupted hormone levels is therefore an effective treatment strategy in managing PCOS. **Materials and Methods :** PCOS was induced in female Sprague Dawley rats with body weights between 180-200g by administrating oral doses of letrozole for 21 days. After PCOS induction, different combinations of mimosine, β-sitosterol and diosgenin along with clomiphene citrate were administered for 15 days. Body weight, food intake, water intake and cage side observations were recorded during the study. Serum testosterone, FSH, LH, estrogens and progesterone, oxidative enzymes and lipid profile were measured to determine the efficacy of treatments. Ovarian histopathological features were also examined by light microscopy. **Results:** Rats after letrozole induced PCOS showed significant aberrations in hormonal levels ($p \leq 0.001$, 0.01) compared with the normal control group indicating induction of PCOS. The treatment with combinations of phytochemicals was found to be effective in resolving and normalizing the disrupted levels of hormones and other biochemical parameters caused due to PCOS. Significant histological changes in ovarian histology after PCOS induction was found to have resolved among all treatment groups, indicating the ovarian recovery following therapy. **Conclusion:** Results indicate the effectiveness of treatment with a combination of bioactive phytochemicals for balancing reproductive hormone levels. The results suggest that abnormality in hormonal level and changes in ovarian histoarchitecture due to PCOS may be reversed with treatment regimen of administering bioactive phytochemicals as an adjunct with clomiphene citrate.

Keywords: PCOS, Bioactive phytochemicals, Clomiphene citrate, *Mimosa pudica* L., β-sitosterol, Mimosine, Diosgenin.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a heterogeneous reproductive disorder of uncertain etiology. Approximately 10% of women of child bearing age are affected by this disorder.[1] It is the most common endocrinopathy causing anovulatory infertility.[2] PCOS is associated with wide spectrum of complications affecting different health aspects, including anovulation, hyperandrogenism, hirsutism, infertility, menstrual disturbance, obesity, dyslipidemia, diabetes mellitus, cardiovascular risk, mood disorders and decreased quality of life.[3]

Although the definite cause of PCOS has not been clearly understood, disruption in secretion rates of hormones and changes in metabolism of androgens and estrogens are major characteristics of PCOS. The serum concentrations of testosterone, androstenedione and dehydroepiandrosterone are generally high in PCOS women.[4] The resulting increase in insulin levels causes gonadotrophin-releasing hormone (GnRH) levels to escalate, which results in increased luteinizing hormone (LH) secretion. Insulin resistance caused by increased insulin level and increased LH causes abnormal secretion of androgens and decreases the synthesis of oestradiol.[4] Studies have shown that conversion of progesterone into testosterone by ovarian theca cells is increased in women with PCOS compared with healthy women.[5] In response to increased LH levels, theca cells produce androgens; therefore, androgen levels also in blood increase in PCOS women. These increased levels of androgens (especially testosterone) disrupt synthesis of follicle stimulating hormone (FSH), causing dysfunction of ovulation and other reproductive organs.[6,4] Many studies have suggested that oxidative stress also contributes to PCOS, along with hormonal imbalance.[7] Indeed, treatment with antioxidant supplements improves insulin sensitivity.[8]

Clomiphene citrate, Metformin and Tamoxifen are currently used in the management of PCOS. However, treatment with these drugs results in many side effects including nausea, vomiting and loss of appetite. Clomiphene citrate along with Metformin is the most widely used treatment regimen used in the management of PCOS. Clomiphene citrate has a structure similar to estrogens; it binds to estrogen receptors and mimics a situation of low estrogens levels.[9,10] Reduced level of estrogen stimulates increased secretion of FSH, which triggers ovulation.[11] On the other hand, many scientific studies have proven that various phytoconstituents are effective against female reproductive disorders. Consumption of phytochemicals in sufficient amounts is also effective in management of several metabolic disorders.[12] Thus, use of phytochemicals could potentially be an alternate strategy to manage PCOS.

MATERIALS AND METHODS

Bioactive Phytochemicals: Mimosine, β-sitosterol, Diosgenin

Mimosa pudica L. ('Touch me not' plant) is an annual, perennial herb commonly used in management of various female reproductive disorders. Mimosine is the major compound in *Mimosa pudica* and has strong estrogenic and tumor suppressing properties.[13-15] In the current study mimosine (M) ($C_8H_{10}N_2O_4$), purchased from Sigma Aldrich with 99% purity was used in Sprague Dawley rats induced with PCOS. A 6mg/kg dose of mimosine was used for evaluating the efficacy.[16,17]

β-sitosterol (B) is an important phytosterol found in many medicinal plants and useful in management of urinary problems. It has potent glucose lowering effect, lowers cholesterol level and improves insulin receptor sensitivity.[18,19] β-sitosterol ($C_{29}H_{50}O$) was purchased from Sigma Aldrich (99% purity) and was used at a dose of 400mg/kg for evaluating the efficacy.

Diosgenin (D) is a steroid saponin which is used in lowering glycemic index and as an anti-inflammatory, antiproliferative and antioxidant agent.[20] Diosgenin is also used in the treatment of type II diabetes mellitus due to its inhibitory activity against α-amylase and α-glucosidase.[21,22] Diosgenin ($C_{27}H_{42}O_3$) was purchased from Sigma Aldrich (99% purity) and was used at a dose of 40mg/kg dose in the current study.

The correlation between PCOS and oxidative stress was evaluated in the current study by recording levels of the antioxidant enzymes like catalase, superoxide dismutase (SOD), glutathione peroxidase, lipid peroxidase (LPO) and glutathione-s-reductase. An attempt has been made to combine clomiphene citrate (CC) with bioactive phytochemicals to lower the side effects of clomiphene citrate treatment.[23] In the current study, therefore, a combination of phytochemicals is used as an adjunct with clomiphene citrate in the management of PCOS.

In current study Super oxide dismutase (SOD) was assayed by the method of Kakkar *et al.* (1984); Glutathione peroxidase activity was measured by the method of Rotruck (1973), and Catalase activity was measured by the method of Patterson *et al.* (1984). Lipid peroxidation assay was carried out by estimating Thiobarbituric acid reactive substances (TBARS) by the method suggested by Okhawa *et al.* (1989).[24-27]

All hormonal assays were conducted using specific kits manufactured by ABNOVA, Taoyuan city, Taiwan. (FSH-Catalog Number KA2797; Estradiol - Catalog Number KA3641; LH- Catalog Number- KA2796; Progesterone - Catalog Number KA2803).

PCOS Model

Healthy adult (7–10 weeks old) nonparturient female albino Sprague Dawley rats, weighing between 180-200 gm were procured from Bharat Serum and Vaccines Pvt. Ltd., Thane, Mumbai, India. Animals were housed in the clean and dry polypropylene cages (B. I. K Industries, Mumbai, India) at the animal house facility (CPCSEA No.: 315/PO/Re/S/2000/CPCSEA) of Ramnarain Ruia Autonomous College, Matunga, Mumbai, India. They were provided with sterilized, clean and dry corn cob bedding and acclimatized at 22 ± 3°C, relative humidity of 50-55% with 12 hr light-dark cycle (artificial light, 6.00 a.m. to 6.00 p.m.) for seven days prior to the experiment. Before experimentation the stool samples were collected and sent for examination for parasitic infection to the department of veterinary parasitology, Bombay Veterinary College, Mumbai, India. The stool samples were found to be clinically normal and devoid of parasites. Letrozole being an aromatase enzyme inhibitor is known to induce PCOS in rats and the model has been used in earlier studies. A 1mg/kg dose of letrozole was administered for 21 days to induce formation of cysts in the rat ovary.[22] PCOS induced rats were randomly grouped in six animals per group and were given different treatment regimen for 15 days post PCOS induction (Table 1). The protocol used in the study was approved by the Institutional Animal Ethics Committee of Ramnarain Ruia Autonomous College, Matunga, Mumbai, India (Protocol No. RRC/IAEC/03/2018).

Various hormonal and biochemical parameters were evaluated to determine the therapeutic potential of the treatment regimen used. Each rat was examined every day for fur color, hair loss, eyes, general activity, genitals, tail and mouth. Individual body weights of animals were recorded, along with the water and food intake in each cage for the entire duration of the study. Animals in the letrozole control group were sacrificed by CO_2 intoxication after 21 days of letrozole treatment. Normal control and treatment groups were sacrificed on 37th day of treatment. Blood was collected from retro-orbital plexus. All blood parameters were evaluated in the serum. Ovaries and uterus were excised and used for biochemical and histopathological evaluation after recording their weights. For histological evaluations, ovarian tissue was excised from all rats during autopsy at the end of the study period. Small pieces of ovarian tissue were fixed in neutral formalin for 24 hr. After fixation, the tissue was washed in water to remove the excess fixative. Washed tissues were then dehydrated through a graded series of ethyl alcohol, cleared with xylene and embedded in paraffin. Sections of 5 to 7 µm thicknesses were cut, mounted on glass slides, stained with hematoxylin and eosin and observed under a light microscope. A collage of multiple images was prepared using the automatic panorama stitching software; Autostitch™ (Copyright University of British Columbia, 2005).

Table 1: Treatment groups of the study.

Test groups	Treatment	Dose administered
Normal control	D/W	1mL/kg body wt
Letrozole control	Letrozole	1mg/kg body wt in CMC
CC	Clomiphene citrate	1mg/kg body wt in CMC
CC+M+B	Clomiphene citrate (1mg/kg) + Mimosine (6mg/kg) + β-sitosterol (400mg/kg)	407mg/kg body wt in CMC
CC+M+B+D	Clomiphene citrate (1mg/kg) + Mimosine (6mg/kg) + β-sitosterol (400mg/kg) + Diosgenin (40mg/kg)	447mg/kg body wt in CMC
M+B+D	Mimosine (6mg/kg) + β-sitosterol (400mg/kg) + Diosgenin (40mg/kg)	446mg/kg body wt in CMC

Statistical Analysis

The raw data obtained was systematically categorized and statistically evaluated for significance. All values are expressed in tables as mean± SEM (standard error of the mean). One way ANOVA followed by Tukey test was used for multiple comparisons between various treatment groups. Statistical analysis was performed using Microsoft Excel®, and GraphPad Prism®. The significance of the difference was fixed at $p < 0.05$.

RESULTS

During the letrozole induction period, slimy stools were observed in 50% of the treated rats, although other cage-side observations remained normal during the study period. The food intake of letrozole control rats was significantly increased compared to the normal control rats (Figure 1). No significant difference was found in water intake of all treated rats (Figure 2). Body weight changes of rats in all treatment groups showed no significant difference as compared with normal control. Weight of ovaries was significantly increased in letrozole control group as compared with the normal control group. Other treatment groups showed no significant difference in ovary and uterus weights when compared with those of normal control group (Figure 3).

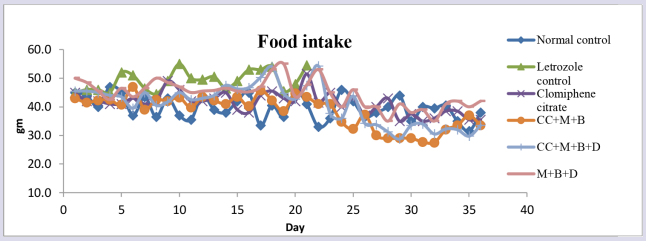


Figure 1: Effect of treatments on food intake of rat.

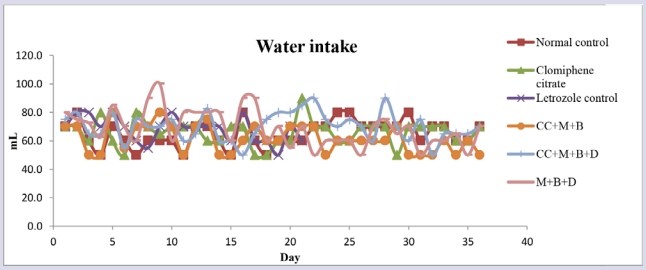


Figure 2: Effect of treatments on water intake of rat.

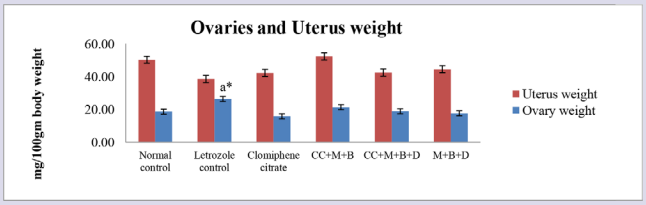


Figure 3: Effect of different treatments on weight of ovary and uterus of rats (a* - compared with normal control group, $p \leq 0.05$).

Effect of Treatment on Hormonal Levels, Blood Biochemistry and Antioxidants

Measurement of hormonal levels (testosterone, LH, estradiol, progesterone and FSH) is one of the effective diagnosing strategies in PCOS. The consistent feature in PCOS is the elevated levels of serum testosterone, LH, FSH, and decreased levels of serum estradiol and progesterone. The testosterone level in Letrozole control group was significantly elevated ($p \leq 0.001$) as compared to all other treatment groups and normal control group (Table 2). The testosterone levels in all treatment groups were significantly decreased ($p > 0.05$). In the present study, 15 days of treatment with all combinations significantly decreased the testosterone level, which correlates with the trends reported in earlier studies.[28] The level of LH was highest in the letrozole control group and showed significant differences as compared to the normal control group, as well as the other treatment groups. LH level in letrozole control group was 2.5 times more than normal control group ($p < 0.001$). The levels of LH in CC+M+B, CC+M+B+D, M+B+D and clomiphene citrate treated groups indicated almost complete recovery after treatment (Table 2). The levels of FSH were also significantly elevated in the letrozole-induced PCOS group as compared to clomiphene citrate, CC+M+B, and CC+M+B+D treated groups. Treatment given with pure phytochemicals M+B+D showed FSH levels closest to normal control group as compared to other treatment groups. The results correlate with the observations of Kafali *et al*. 2004, who reported decrease in estrogen concentrations corresponding with increased FSH in PCOS. In comparison to the Letrozole control group, all treatment groups showed significant recovery in FSH levels. The progesterone level in Letrozole control group was significantly low and was about 78% less than that in normal control group. Progesterone level in other treatment groups recovered after treatment and were comparable to those of the normal control group.

Hormonal imbalance in PCOS leads to the insulin resistance, which in turn increases blood sugar levels and further elevates the levels of cholesterol and triglycerides. In the current study, the levels of blood sugar was significantly elevated in letrozole control group ($p \leq 0.001$) (Table 3). Rats of all treatment groups showed recovery in blood sugar level as compared with normal control group. The recovery was best in the groups treated with combinations, CC+M+B+D (Table 3). Similarly, the recovery in levels of cholesterol was significant in rats treated with clomiphene citrate, CC+M+B, CC+M+B+D (Table 3). Triglycerides levels in all treatment groups were found to be similar to the normal control group, although they were significantly decreased in the clomiphene citrate treated groups.

With respect to the antioxidants, all treatment groups showed catalase levels similar to those of normal control group. There were also no significant differences found in SOD levels in rats of all treatment groups when compared to normal control group. The rats of the treatment group, M+B+D, however, showed significant reduction in SOD activity as compared to the Clomiphene citrate group (Table 4). Lipid peroxidation activity was significantly elevated in the rats of Letrozole control group while there were no significant changes in lipid peroxidation activity in rats of all other treatment groups as compared to normal control group (Table 4). There was no significant change found in activity of glutathione peroxidase in all treatment groups as compared to normal control rats. The rats treated with CC+M+B and CC+M+B+D, however, exhibited significant increase in glutathione s-transferase levels as compared to clomiphene citrated treated group (Table 4). It is interesting to note that the rats treated with pure phytochemicals, M+B+D showed the levels of antioxidants similar to other treatment groups indicating the efficacy of pure phytochemicals in the management of oxidative stress associated with PCOS.

Histology of the Ovary

Ovaries of rats from the control group exhibited normal histological features (Figure 4a). In the rats of letrozole control group, the ovary exhibited many cystic follicles and damaged thecal layer (Figure 4b). In rats treated with CC after induction of PCOS, the ovary showed graafian follicles, secondary follicles, corpus luteum and cystic follicles (Figure 4c). In rats treated with CC+M+B, graafian follicles were observed more in number as compared to other treatment groups (Figure 4d). Ovaries of rats treated with CC+M+B+D showed increase in corpus luteum, primordial and secondary follicles, as well as few well-developed graafian follicles. There were many secondary follicles in the corpus luteum (Figure 4e). In rats treated with pure phytochemicals combination, M+B+D, the ovary showed normal cytoarchitecture with thecal and granulosa cells along with well-developed follicles. The follicular development is indicated with the presence of developing secondary and graafian follicles without any presence of cystic follicle which clearly indicates the efficacy of pure phytochemicals in the management of PCOS.

DISCUSSION

In rats with letrozole induced PCOS, the serum levels of testosterone, FSH, and LH were increased, while progesterone level decreased with

Table 2: Effect of treatments on testosterone, LH, estradiol, progesterone and FSH levels.

Test Groups	Testosterone ng /mL	Luteinizing Hormone (LH) mIU/mL	Estradiol pg/mL	Progesterone ng /mL	Follicle stimulating Hormone (FSH) mIU/mL
Normal control	19.14±1.56	0.12±0.02	24.29±8.81	8.39±3.61	0.86±0.39
Letrozole control	245.76±31.61 (a***)	0.31±0.08 (a***)	26.18±1.11	1.77±0.31(a*)	1.65±0.44
Clomiphene citrate	19.64±9.78 (b***)	0.15±0.06 (b**)	23.93±1.36	7.55±1.31	0.36±0.08 (b***)
CC+M+B	10.99±7.28 (b***)	0.15±0.08 (b**)	21.32±6.49	12.41±11.65	0.73±0.35 (b**)
CC+M+B+D	19.14±3.11 (b***)	0.17±0.05 (b**)	24.6±6.16	8.99±0.42	0.49±0.13 (b***)
M+B+D	6.06±0.71 (b***)	0.05±0.01 (b***)	23.29±4.49	6.38 ± 0.39	0.88±0.39 (b*)

All values listed as Mean± SE, (*) – $p \leq 0.05$, (**) – $p \leq 0.01$, (***) – $p \leq 0.001$.
a – compared with normal control group, b – compared with the letrozole control group

Table 3: Effect of treatments on blood sugar, cholesterol and triglycerides levels.

Test Groups	Random Blood Sugar mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
Normal control	82.88±8.16	41.48±2.12	163.87±26.77
Letrozole control	110.67±13.41 (a***)	39.33±3.61	157.33±3.44
Clomiphene citrate	94.48±6.92	33.69±5.03	111.04±40.17 (a*)
CC+M+B	86.01±11.8 (b**)	40.12±5.59 (b*)	135.33±43.82
CC+M+B+D	79.83±9.28 (b***)	42.17±8.35	142.67±40.58
M+B+D	87.83±10.44 (b**)	43.83±5.78	124.33±36.62

All values listed as Mean± SE, (*) – $p \leq 0.05$, (**) – $p \leq 0.01$, (***) – $p \leq 0.001$.
a – compared with normal control group, b – compared with the letrozole control group

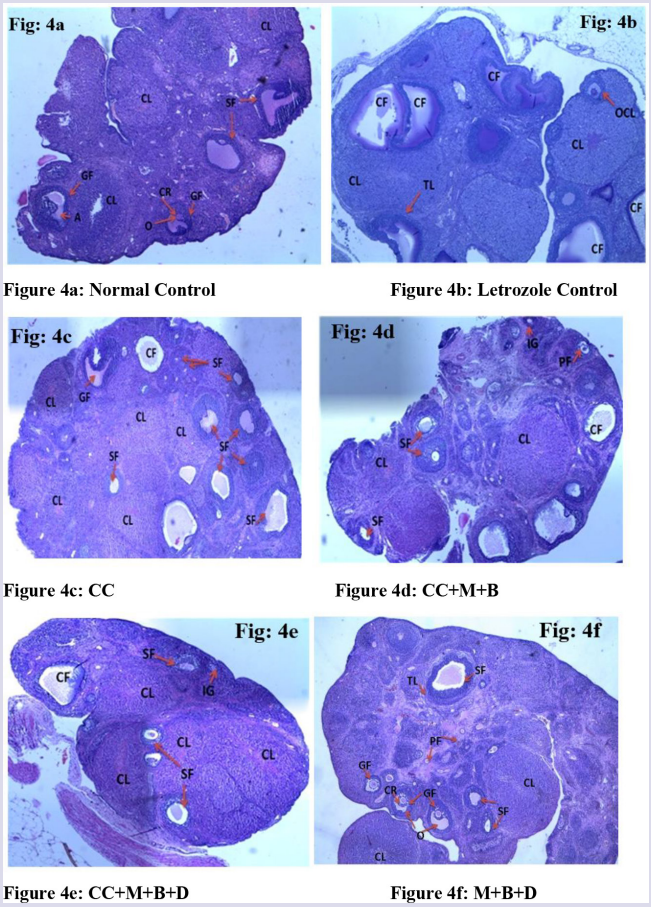
Table 4: Effect of treatments on catalase, SOD, LPO, glutathione peroxidase, glutathione-s-transferase levels.

Test Groups	Catalase (Units/ mg protein)	Super Oxide Dismutase (SOD) Units/mg protein	Lipid Peroxidation (LPO) Nmole / g Hb	Glutathione peroxidase (µg GSH utilized/mg protein/min)	Glutathone s-transferase (microgram/l)
Normal control	18.46±1.66	21.96±1.19	95.44±11.98	3.28±0.52	26.56±3.44
Letrozole control	15.47±0.28	18.37±0.31	115.47±0.38 (a*)	3.71±0.17	28.03±0.51
Clomiphene citrate	19.84±0.65	26.68±11.92	97.34±4.57	3.55±0.35	32.61±1.99
CC+M+B	17.21±2.89	18.97±3.45	109.14±10.24	3.32±0.33	23.21±3.88 (c**)
CC+M+B+D	18.39±3.05	19.06±2.27	111.04±12.79	3.68±0.48	24.65±1.7 (c*)
M+B+D	15.51±3.25	17.59±2.57 (c*)	96.43±14.16	3.59±0.71	28.43±2.75

All values listed as Mean± SE, (*) – $p \leq 0.05$, (**) – $p \leq 0.01$, (***) – $p \leq 0.001$.
a – compared with normal control group, c – compared with the Clomiphene citrate group.

no significant difference observed in estradiol levels. These findings are concurrent with earlier reported studies.[28] In the current study, rats treated with the combination of pure phytochemicals, Mimosine, Diosgenin and β-sitosterol shows recovery in imbalanced hormonal level after PCOS induction. This indicates the significant antiandrogenic effect of the treatment regimen with phytochemical combination. In the current study Mimosine, β-sitosterol and Diosgenin are administered in different combinations along with Clomiphene citrate (1mg/kg) as CC+M+B, CC+M+B+D and without clomiphene citrate as M+B+D (pure phytochemicals). The rats treated with the combination, M+B+D and those treated with combinations of phytochemicals with clomiphene citrate showed significant recovery, as compared with normal control group, in various altered physiological parameters associated with PCOS.

The treatment regimen of combination of pure phytochemicals, as evaluated in the study, is found to be effective in decreasing the elevated testosterone and LH levels and balancing the altered estradiol, progesterone and FSH levels in PCOS induced rats. Treatment with the combination of the pure phytochemicals resulted in significant recovery of other blood parameters like cholesterol, sugar and the levels of antioxidants like catalase, LPO, glutathione peroxidase and glutathione-s-reductase. In the current experimental study, PCOS induced rats treated with various combinations of phytochemicals showed restoration of lipid profile and profile of oxidative enzymes close to those of normal control rats. In histopathological evaluation, ovaries from PCOS induced rats after treatment with combinations of phytochemicals, showed corpus luteum, primordial follicles and secondary follicles in increasing number which is a positive sign of recovery of ovarian activity after PCOS induction.



Figures 4: Light Microscopic observation of Ovarian Tissue.
(Collage of multiple photomicrographs at 40X)
GF- graafian follicle, CF- cystic follicle, CL- corpus luteum, PF- primordial follicle, GLC- Granulosa lutein cells, TLC- Theca lutein cells, A- Antrum, SF- Secondary follicle, O-Ovum, IG- Interstitial gland, BV- Blood vessels, OCL – Corpus luteum with a retained ovum. (Luteinization proceeds without ovulation as the ovum still floats in follicular fluid), GLC- ovoidal shape granulosa lutein cells.

Corpus luteum is necessary for the secretion of progesterone, which regulates the menstrual cycle and supports the endometrial changes for implantation. The histological observations of the current study correlate well with the biochemical findings and corroborate the beneficial effects of treatment regimen with a combination of phytochemicals.[14,16,17,20,22]

The results of this study suggest that the treatment with a combination of Mimosine, β-sitosterol, Diosgenin is effective in the management of PCOS and helps in restoring the hormonal imbalance as well as other blood parameters that are altered in PCOS. The recovery of various parameters including hormonal levels after induction of PCOS in the rats of CC+M+B+D in noteworthy. The observations on this treatment group support the potential use of this treatment regimen with phytochemicals as an adjuvant with clomiphene citrate. This could not only reduce the dose of clomiphene citrate used but also avoid the undesirable effects of clomiphene citrate in PCOS patients. The current study, therefore, demonstrates the feasibility of considering a treatment regimen using a combination of phytochemicals as an alternate strategy in the management of PCOS.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Kim CH, Chon SJ, Lee SH. Effects of lifestyle modification in polycystic ovary syndrome compared to metformin only or metformin addition: A systematic review and meta-analysis. Sci Rep. 2020;10(1):7802. doi: 10.1038/s41598-020-64776-w, PMID 32385376.
2. Bency T, Rani S, Remya K, Rasheeb S, Azeen K. Polycystic ovarian syndrome: Therapeutic potential of herbal remedies. Int J Herb Med. 2016;4(5):91-6.
3. Teede J, Miss L, Deeks. Assessment and management of polycystic ovarian syndrome. 2011;195(6):65-112.
4. Abasian Z, Rostamzadeh A, Mohammadi M, Hosseini M, Rafieian-kopaei M. A review on role of medicinal plants in polycystic ovarian syndrome: Pathophysiology, neuroendocrine signaling, therapeutic status and future prospects. Middle East Fertil Soc J. 2018;23(4):255-62. doi: 10.1016/j.mefs.2018.04.005.
5. Laird M, Thomson K, Fenwick M, Mora J, Franks S, Hardy K. Androgen Stimulates Growth of Mouse Preantral Follicles *in vitro*: Interaction with Follicle-stimulating Hormone and With Growth Factors of the TGFβ Superfamily. Endocrinology. 2017;158(4):920-35. doi: 10.1210/en.2016-1538.
6. Dunaif A. Chang R. Franks S, Legro R. Polycystic ovarian syndrome. Springer Sci Buisness Media. 2008:1-355.
7. Mohammadi J, Saadipour K, Delaviz H, Mohammadi B. Anti-diabetic effects of an alcoholic extract of Juglans regia in an animal model. Turkish Journal of Medical Sciences. 2011;41(4):685-91. doi: 10.3906/sag-1004-802.
8. Oyebanji OG, Asaolu MF. Assessment of antioxidant status of women with polycystic ovarian syndrome. Asian Pac J Reprod. 2020;9(1):275523. doi: 10.4103/2305-0500.275523.
9. Ye HL, Jiang LP, Luo Y. Gastrointestinal side effects in type 2 diabetic patients treated with metformin: Interventions and traditional Chinese nursing. World Chin J Digestology. 2015;23(35):5591. doi: 10.11569/wcjd.v23.i35.5591.
10. Lorizio W, Wu AH, Beattie MS, Rugo H, Tchu S, Kerlikowske K, *et al.* Clinical and biomarker predictors of side effects from tamoxifen. Breast Cancer Res Treat. 2012;132(3):1107-18. doi: 10.1007/s10549-011-1893-4, PMID 22207277.
11. Liu S, Li X, Zheng Y, Teng B, Cao Y, Sun X. Study of Combined Use of Clomiphene Citrate and Gonadotropins on the Infertile Patients with PCOS. J Reprod Contracept. 2012;23(3):159-68. doi: 10.1016/S1001-7844(12)60025-X.
12. Sivakumar D, Chen L, Sultanbawa Y. A Comprehensive review on beneficial dietary phytochemicals in common traditional southern African leafy vegetables. Food Sci Nutr. 2018;6(4):714-27. doi: 10.1002/fsn3.643, PMID 29983933.
13. Muhammad G, Hussain MA, Jantan I, Bukhari SNA. *Mimosa pudica* L., a high-value medicinal plant as a source of bioactives for pharmaceuticals. Compr Rev Food Sci Food Saf. 2016;15(2):303-15. doi: 10.1111/1541-4337.12184, PMID 33371596.
14. Huq AM, Wai LK, Rullah K, Mohd Aluwi MFF, Stanslas J, Jamal JA. Oestrogenic activity of mimosine on MCF-7 breast cancer cell line through the ERα-mediated pathway. Chem Biol Drug Des. 2019;93(3):222-31. doi: 10.1111/cbdd.13404, PMID 30251480.
15. Dipe V. Gotardo A, Haraguchi M, Górniak. Mimosine and cyclophosphamide: A potential new combination therapy used to prevent tumor development. Braz Arch Biol Technol. 2012;55(6):871-6. doi: 10.1590/S1516-89132012000600010.
16. Jadhav M, Menon S, Shailajan S. *In vivo* evaluation of *Mimosa pudica* Linn. In the management of polycystic ovary using rat model. Int J Aplied Biol Pharm Technol. 2013;4(1):285-92.
17. Nair L, Menon S, Shailajan S. Baing M, Sane R. Reversed-phase high-performance thin-layer chromatographic quantification of mimosine from whole plant of *Mimosa pudica* Linn. J Planar Chromatogr Mod TLC. 2007; (1);20:49-51.
18. Saeidnia S. The story of beta-sitosterol- A review. Eur J Med Plants. 2014;4(5):590-609. doi: 10.9734/EJMP/2014/7764.
19. Paniagua-Pérez R, Madrigal-Bujaidar E, Reyes-Cadena S, Molina-Jasso D, Gallaga JP, Silva-Miranda A, *et al.* Genotoxic and cytotoxic studies of beta-sitosterol and pteropodine in mouse. J Biomed Biotechnol. 2005;2005(3):242-7. doi: 10.1155/JBB.2005.242, PMID 16192682.
20. Jesus M, Martins AP, Gallardo E, Silvestre S. Diosgenin: Recent highlights on pharmacology and analytical methodology. J Anal Methods Chem. 2016;2016, Article ID- 415629. doi: 10.1155/2016/4156293, PMID 28116217.
21. Ghosh S, More P, Derle A, Patil AB, Markad P, Asok A, *et al.* Diosgenin from *Dioscorea bulbifera*: Novel hit for treatment of type II diabetes mellitus with inhibitory activity against α-amylase and α-glucosidase. PLOS ONE. 2014;9(9):e106039. doi: 10.1371/journal.pone.0106039, PMID 25216353.
22. Balamurugan E, Manivannan J, Sivasubramanian J, Arunagiri P. Diosgenin prevents hepatic oxidative stress, lipid peroxidation and molecular alterations

in chronic renal failure rats. Int J Nutr Pharmacol Neurol Dis. 2013;3(3):289. doi: 10.4103/2231-0738.114870.

23. Uçkan K, Demir H, Turan K, Sarıkaya E, Demir C. Role of oxidative stress in obese and nonobese PCOS patients. Int J Clin Pract. 2022;2022:4579831. doi: 10.1155/2022/4579831, PMID 35685525.
24. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21(2):130-2. PMID 6490072.
25. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, J. and Pope. Selenium: Biochemical role as a component of glutathione peroxidase. Sci (N Y NY). 1973;179(4073):588-90. doi: 10.1126/science.179.4073.588, PMID 4686466.
26. Patterson BD, Payne LA, Chen YZ, Graham D B, Linda P, Yi-Zhu G. Plant Physiol. 1984;76(4):1014-8. doi: 10.1104/pp.76.4.1014, PMID 16663941.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3, PMID 36810.
28. Kafali H, Iriadam M, Ozardali I, Demir N. Letrozole-induced polycystic ovaries in the rat: A new model for cystic ovarian disease. Arch Med Res. 2004;35(2):103-8. doi: 10.1016/j.arcmed.2003.10.005, PMID 15010188.

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