INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-1, Jan-Mar-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 05th Jan-2013 *Revised: 12th Jan-2013* *Accepted: 12th Jan-2013*

Research article

IN VIVO EVALUATION OF *MIMOSA PUDICA* LINN. IN THE MANAGEMENT OF POLYCYSTIC OVARY USING RAT MODEL

Mamata Jadhav*[1], Sasikumar Menon [2] and Sunita Shailajan [3]

[*1] Ramnarain Ruia College, Matunga, Mumbai 400019

[2] Institute for Advanced Training and Research in Interdisciplinary Science194, Scheme No. 6, Road No. 15, Sion Koliwada, Sion (E), Mumbai 400 022

[3] Herbal Research Lab, Ramnarain Ruia College, Matunga, Mumbai 400019

ABSTRACT: This study aimed to evaluate the effect of *Mimosa pudica* Linn. (MP) in the treatment of polycystic ovary syndrome (PCOS) using a Letrozole induced PCOS rat model. PCOS was induced in albino wistar female rats by daily oral administration of Letrozole for 21 days. The low (250 mg/kg), mid (500 mg/kg) and high dose (1000 mg/kg) of MP was given orally to the PCOS induced rats for 15 days post Letrozole induction to determine the effective dose of MP in the treatment of PCOS. The biomarkers of ovarian function, plasma testosterone, estrogen and progesterone were analyzed to determine the fluctuations in sex steroid levels in PCOS induced rats. The plasma testosterone levels were found to be increased significantly in rats with PCOS whereas plasma estrogen and progesterone levels were significantly decreased. When compared with control, the PCOS induced rats showed characteristic ovary with high incidence of ovarian cysts with a diminished granulosa layer, significant number of atretic follicles and absence of corpora lutea. All the end points assessed were significantly improved after the treatment with mid and high dose of MP and achieved levels close to normal levels. The mid dose (500 mg/kg) and high dose (1000 mg/kg) of MP were found to be effective in the treatment of PCOS induced by Letrozole in rats. This effect of MP significantly reduced histopathological changes in ovary and endocrinological and biochemical changes induced by hyperandrogenism. Thus MP was found to have a good potential to be a very good alternative therapy in the treatment of PCOS.

Keywords: *Mimosa pudica* Linn.., Letrozole, Polycystic ovary syndrome, Clomiphene citrate

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women in their reproductive years. It is frequently associated with anovulatory fertility (Azziz, et al., 2009; Ehrmann, 2005). PCOS is defined as a syndrome of ovarian dysfunction along with the cardinal features of hyperandrogenism and polycystic ovary morphology (Bako, et al., 2005; Homburg, 2009). Though the exact pathophysiology of PCOS remains uncertain, increasing body of evidence suggests that an excess of ovarian androgen production, either genetically determined or due to extra-ovarian factors such as hyperinsulinaemia or disturbances of the hypothalamic-pituitary-ovarian axis is the main cause in the pathogenesis of PCOS (Archer and Chang, 2004; Goodarzi and Azziz, 2006). At present available drugs and hormonal therapies used in the management of PCOS display various side effects (Jadhav and Bhutani, 2005).

Mimosa pudica Linn.. (MP) from the family Leguminosae, is a prostate or suberect under shrub, is a native of tropical America (Brazil), naturalized nearly throughout the tropical and sub-tropical parts of India. It is found in Sub-Himalayan tracts, West Bengal, damper districts of Bihar and Orissa, Maharashtra and hot moist localities of southern states (Khare, 2004). MP is reported to be effective in the treatment of heavy menstrual blood loss (Menorrhagia), leucorrhoea and dysfunctional uterine bleeding. The plant is used in folk medicine and traditional systems of medicine in various disorders of female reproductive system (Khare, 2004; Sharma, et al., 2001). Therefore, the current study investigates the therapeutic efficacy of aqueous slurry of MP in the treatment of PCOS induced by chemical agent, Letrozole in the rat model.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

MATERIALS AND METHODS

Animals

Experiments were carried out after prior approval from Institutional Animal Ethics Committee (CPCSEA/315). Six-week-old female albino wistar rats (mean body weight, 180g) with 4 day regular estrus cycles were procured from Haffkine Biopharmaceuticals Limited., Parel, Mumbai. They were maintained (12 hours light/12 hours dark cycle) at 25 ± 2ºC and 45-55% of humidity. Animals were given free access to standard laboratory food (supplied by Amrut Feed) and water *ad libitum* (Kafali, et al., 2004; Sasikala and Shamila, 2009).

Plant material of MP

The whole plant of MP was collected from their natural habitat from Mumbai, Maharashtra state of India. The identification of the plant species was verified from Agharkar Research Institute, Pune. The voucher specimen number ARI 10-75 of 8/06/2010 was deposited in Herbal Research Laboratory of Ramnarain Ruia College, Matunga, Mumbai. The plant materials were washed thoroughly and dried at 37ºC ± 5 ºC before preparation of aqueous extract. The plant material was then powdered and stored in airtight bottles, until use. The aqueous slurry of plant material was prepared in 2.0 ml of distilled water before dosing.

Study Design

The study was conducted on 42 female albino wistar rats divided into seven groups of six animals each, including a control group that received vehicle only (1% aqueous solution of carboxymethyl cellulose (CMC) once daily p.o. The six treatment groups rats were administered with Letrozole (Femara® Manufactured by Novartis Pharma Stein AG, Stein, Switzerland) at concentrations of 1.0 mg/kg p.o. dissolved in 1% CMC (2.0 mL/kg) once daily. The treatment period was 21 days (Kafali, et al., 2004). Along with normal control group, Letrozole induced PCOS rats were divided into six treatment groups. The first PCOS induced group was left for natural recovery for 15 days post Letrozole treatment. The second PCOS induced group was given repetitive dose of 1.0 mg/kg daily clomiphene citrate (Fertomid-50 Manufactured by Cipla Ltd., Kumrek, Sikkim, India), a well known modern drug used in the treatment of polycystic ovary syndrome (Sasikala and Shamila, 2009). One of the PCOS induced group was sacrificed after 21 days of Letrozole treatment. The other three PCOS induced groups were given repetitive doses of 250 mg/kg (low dose), 500 mg/kg (mid dose) and 1000 mg/kg (high dose) of aqueous extract of MP once daily p.o. for 15 days post Letrozole induction. Ovarian and uterine weight changes and hormonal assay were carried out in PCOS induced rats and the results were compared with that of the group treated with Clomiphene citrate.

Blood sampling

At 24 hours post the last dose of treatment and after 18 hours fasting period, the rats were weighed and blood samples were collected by retino orbital puncture into different eppendorf tubes containing heparin sodium as an anticoagulant. The blood samples were centrifuged at 3000 rpm for 15 minutes and plasma was separated for hormonal assay. The plasma was stored in a freezer at -20 ± 2 ºC till further analysis.

Biochemical analysis

Plasma testosterone and estradiol were assayed by Competitive Chemiluminescent Immunoassay using automated instrument ADVIA Centaur, Bayer Diagnostics Europe Limited, Ireland. The testosterone was estimated using ADVIA Centaur TSTO kit and estrogen was estimated using ADVIA Centaur using E2-6 kit (Nini, et al., 2000; Yilmaz, et al., 2001; Leder, et al., 2002; Bandopadhyay, et al., 2003 and Yilmaz, et al., 2006). The plasma progesterone was assayed by Microparticle Enzyme Immunoassay (MEIA) using AxSYM analyzer – Abbott AxSYM® System, Abbott Japan (Abdulla, et al., 1983; Polly, et al., 2011). The plasma cholesterol levels were assayed by using method of Wybenga and Pileggi using Biolab Diagnostics Kit. The plasma testosterone levels, estrogen levels, progesterone levels and cholesterol levels were recorded after Letrozole induction of 21 days (i.e. on Day 22 of study period) and after plant treatment for 15 days (i.e on Day 37 of study period).

Tissue sampling

After blood sampling, the animals were sacrificed, ovaries and uterus were excised, cleaned of fat and weighed.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Histopathological examinations

The excised ovaries were fixed in Bouins fixative. Histopathalogical examinations of the ovaries were carried out using standardized histological methods. Sections of the ovary were cut from paraffin-embedded blocks. Histological examinations were carried out on hematoxylin-eosin stained sections using light microscopy.

Statistical analysis

Statistical analysis was performed using ANOVA followed by Dunnett's test as a multiple comparison test. $P < 0.001$, $P < 0.01$ and $P < 0.05$ were considered significant (Hayes, 1994). Values were expressed as mean ± SD.

RESULTS

Effect on testosterone levels

The plasma testosterone level in Normal control group was observed to be 235.82 ± 14.98 ng/dL on Day 22 and is maintained consistently up to 245.33 ± 13.84 ng/dL on Day 37 indicating the stable condition. After the treatment with Letrozole, the mean testosterone level in Letrozole control group significantly increased to 795.20 ± 24.10 ng/dL ($P < 0.001$). The testosterone level decreased from 716.37 ± 28.86 ng/dL to 255.42 ± 17.43 ng/dL ($P < 0.001$) after clomiphene citrate treatment. Significant fall of 268.00 ± 18.58 ng/dL and 270.50 ± 25.83 ng/dL ($P < 0.001$) were recorded when PCOS induced rats were treated with mid and high dose of MP respectively. Low dose of MP did not show any significant recovery of testosterone levels (Table 1.0).

Effect on estrogen levels

Estrogen level, which decreased significantly in PCOS induced rats from 55.83 ± 5.19 pg/mL to 29.88 ± 3.64 pg/mL ($P < 0.001$), increased significantly to 48.00 ± 5.48 pg/mL ($P < 0.001$) after clomiphene citrate treatment. Estrogen levels increased significantly to 51.83 ± 2.71 pg/mL and 50.50 ± 6.41 pg/mL ($P < 0.001$) after repetitive administration of mid and high dose of MP respectively. No significant effect was recorded after the treatment with low dose of MP (Table 1.0).

Effect on progesterone levels

Progesterone level, which significantly decreased in PCOS induced rats from 8.81 ± 0.81 ng/mL to 2.16 ± 0.82 ng/mL ($P < 0.001$) increased significantly to 7.02 ± 0.77 ng/mL ($P < 0.001$) after clomiphene citrate treatment. MP treatment also elevated progesterone levels to 6.71 ± 0.71 ng/mL with mid dose and 6.60 ± 0.81 ng/mL with high dose ($P < 0.001$). The low dose of MP did not show any significant changes in progesterone levels (Table 1.0).

Effect on cholesterol levels

The cholesterol levels increased significantly from 54.83 ± 4.54 mg/dL to 92.83 ± 9.54 mg/dL ($P < 0.001$) in PCOS induced rats. A decrease in cholesterol levels to 49.00 ± 4.65 mg/dL ($P < 0.001$) was observed after clomiphene citrate treatment. A repetitive administration of mid and high dose of MP also led to a significant decrease in cholesterol level to 66.67 ± 5.35 mg/dL and 65.83 ± 4.54 mg/dL ($P < 0.001$) respectively. No significant effect was found with low dose of MP (Table 1.0).

Effect on ovarian weights

The ovarian weight decreased significantly from 0.21 ± 0.02 mg to 0.13 ± 0.04 mg ($P < 0.001$) with treatment of clomiphene citrate in PCOS induced rats. When the PCOS induced rats were administered with low, mid and high dose of MP, the significant decrease in ovarian weights to 0.16 ± 0.02 mg ($P < 0.05$) and 0.15 ± 0.01 mg ($P < 0.01$) was observed with mid and high dose of MP respectively. MP treatment with low dose did not show any significant effect in restoring ovarian weights (Table 2.0).

Effect on uterine weights

The uterine weights which decreased from 1.71 ± 0.28 mg to 0.57 ± 0.09 mg ($P < 0.001$) in PCOS induced rats, increased significantly to 1.15 ± 0.18 mg ($P < 0.001$) after clomiphene citrate treatment. The repetitive administration of mid and high dose of MP also led to a significant increase in uterine weights to 1.08 ± 0.17 mg and 1.25 ± 0.11 mg ($P < 0.001$) respectively (Table 2.0).

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Table 1 Effect of various treatments on plasma Testosterone level (ng/dL), Estrogen level (pg/mL), Progesterone level (ng/mL) and Cholesterol level (mg/dL) in Letrozole induced PCOS rats (Mean± S.D., n=6)

Groups	Testosterone 22nd day	Testosterone 37th day	Estrogen 22nd day	Estrogen 37th day	Progesterone 22nd day	Progesterone 37th day	Cholesterol 22nd day	Cholesterol 37th day
Normal control	235.82 ± 14.98	245.33 ± 13.84	56.17 ± 3.66	55.83 ± 5.19	8.57 ± 0.71	8.81 ± 0.81	58.00 ± 7.43	54.83 ± 4.54
Letrozole Control	795.20 ± 24.10 b****	----	29.88 ± 3.64 b****	----	2.16 ± 0.82 b****	----	92.83 ± 9.54 b****	----
Natural recovery	781.10 ± 18.83 b****	759.73 ± 55.39 a*	34.67 ± 4.03 b****	31.17 ± 7.94 a*	3.42 ± 0.97 b****	3.01 ± 0.57 a*	96.00 ± 5.55 b****	87.33 ± 12.04 a*
Clomiphene citrate	716.37 ± 28.86 b****	255.42 ± 17.43 a****	27.33 ± 3.56 b****	48.00 ± 5.48 a****	3.45 ± 0.87 b****	7.02 ± 0.77 a****	96.83 ± 6.01 b****	49.00 ± 4.65 a****
MP 250 mg/kg	791.90 ± 25.21 b****	714.00 ± 113.94 a*	26.17 ± 2.40 b****	38.00 ± 11.14 a*	2.10 ± 0.76 b****	3.20 ± 1.09 a*	98.83 ± 2.14 b****	85.45 ± 5.01 a*
MP 500 mg/kg	737.30 ± 26.57 b****	268.00 ± 18.58 a****, c*	28.67 ± 3.88 b****	51.83 ± 2.71 a****, c*	2.97 ± 0.80 b****	6.71 ± 0.71 a****	98.67 ± 3.88 b****	66.67 ± 5.35 a****
MP 1000 mg/kg	796.15 ± 20.41 b****	270.50 ± 25.83 a****, c*	26.67 ± 3.08 b****	50.50 ± 6.41 a****, c*	2.12 ± 0.36 b****	6.60 ± 0.81 a****	96.67 ± 3.50 b****	65.83 ± 4.54 a****

Comparison with Letrozole control group NS^{a*}, *P*<0.05^{a**}, P<0.01^{a***}, P<0.001^{a****}
Comparison with Normal control group NS^{b*}, *P*<0.05^{b**}, P<0.01^{b***}, P<0.001^{b****}
Comparison with Modern drug group NS^{c*}, *P*<0.05^{c**}, P<0.01^{c***}, P<0.001^{c****}

Table 2 Effect of various treatments on Ovarian and Uterine weights (mg) in Letrozole induced PCOS rats (Mean± S.D., n=6)

Groups	Ovarian weights	Uterine weights
Normal control	0.14 ± 0.03	1.71 ± 0.28
Letrozole Control	0.21 ± 0.02 b****	0.57 ± 0.09 b****
Natural recovery	0.20 ± 0.02 a*	0.70 ± 0.15 a*
Clomiphene citrate	0.13 ± 0.04 a****	1.15 ± 0.28 a****
MP 250 mg/kg	0.20 ± 0.03 a*	0.75 ± 0.22 a*
MP 500 mg/kg	0.16 ± 0.02 a**, c*	1.08 ± 0.17 a****, c*
MP 1000 mg/kg	0.15 ± 0.01 a***, c*	1.25 ± 0.11 a****, c*

Comparison with Letrozole control group NS^{a*}, *P*<0.05^{a**}, P<0.01^{a***}, P<0.001^{a****}
Comparison with Normal control group NS^{b*}, *P*<0.05^{b**}, P<0.01^{b***}, P<0.001^{b****}
Comparison with Modern drug group NS^{c*}, *P*<0.05^{c**}, P<0.01^{c***}, P<0.001^{c****}

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Effect on ovarian histology

Ovary of Letrozole induced PCOS rats showed significant number of atretic ovarian follicles. Atretic secondary follicles were randomly interspread among normal follicles. In addition to marked atresia, descruption of the granulosa layer was also seen. Many cysts were found. Theca layer was found to be delineating, dying cells and the debris was collected in the antrum (Figure 1C and Figure 1D). When rats with PCOS were left for natural recovery, the recovery was not to its potential as compared to plant and Clomiphene citrate treatment as there were many cysts and atretic follicles were still found (Figure 1E). MP treatment with mid and high dose resulted in the ovarian tissue to show marked recovery of follicle with intact structure of granulosa layer and thecal layer. The ovary showed presence of well developed antral follicle with oocyte surrounded by Granulosa cells, Corona radiata, Cumulus oophorus and Thecal layer. It also showed a clear antrum which appeared to be without any cell debris. (Figure 1F, Figure 1G, Figure 1H, Figure I).

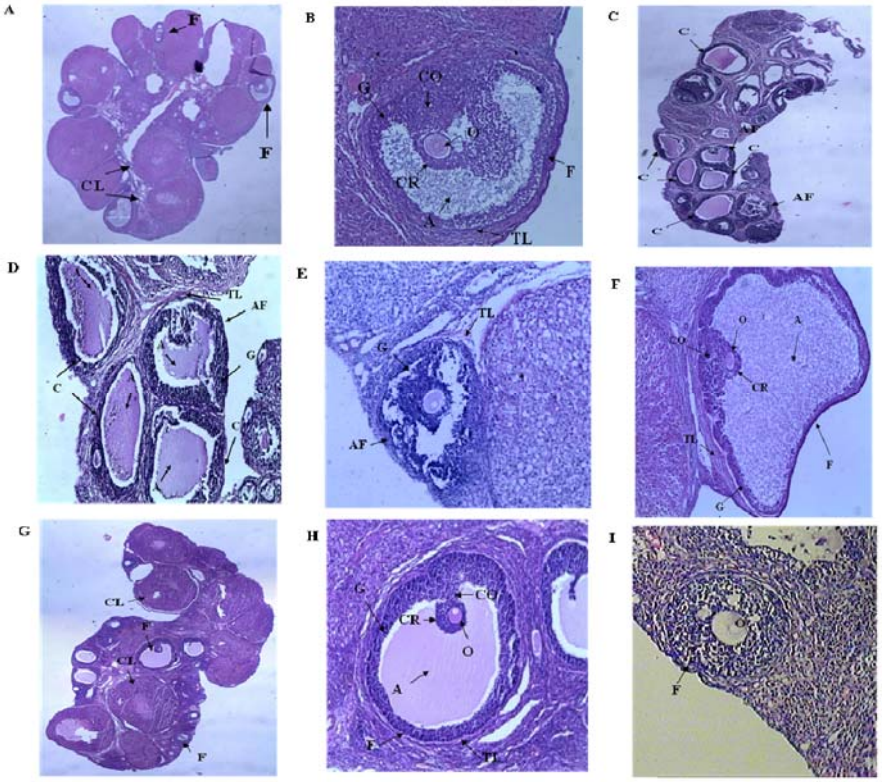

Figure 1 (A) Section of ovary from normal rat showing the presence of Antral follicles (F) and Corpus luteum (CL) (H&E, X4). **(B)** Section of ovary from normal rat showing the presence of Antral follicle (F) with antral cavity (A), Oocyte (O) surrounded by Granulosa cells (G), Corona radiata (CR), Cumulus oophorus (CO) and Thecal layer (TL) (H&E, X10). **(C)** Section of ovary from PCOS rat exhibiting many cystic follicles (C) with thin granulosa layer (H&E, X4). **(D)** Section of ovary from PCOS rat with Cystic degenerating follicle (C) with thin granulosa layer and Atretic follicle (AF) with degenerated granulosa layer (G), delineated thecal layer (TL) and the Antrum (A) filled with the cell debris and dying cells (H&E, X10). **(E)** Section of ovary from PCOS rat left for natural recovery exhibiting Atretic follicle (AF) with degenerating granulosa layer (G) and delineated thecal layer (TL) (H&E, X10). **(F)** Section of ovary from PCOS rat treated with Clomiphene citrate showing marked recovery with the presence of Antral follicle (F) with intact thecal layer (TL), Granulosa layer (G) and Oocyte (O) with Corona radiata (CR) and Cumulus oophorus (CO) (H&E, X10). **(G)** Section of ovary from PCOS rat treated with MP 500 mg/kg showing the presence of developing follicles (F) and Corpus luteum (CL) in the ovarian cortex (H&E, X4). **(H)** Section of ovary from PCOS rat treated with MP 500 mg/kg which indicate the presence of normal Antral follicle (F) with clear Antrum (A) and Oocyte (O) surrounded by Granulosa cells (G), Corona radiata (CR), Cumulus oophorus (CO) and Thecal layer (TL) (H&E, X10). **(I)** Section of ovary from PCOS rat treated with MP 1000 mg/kg showing the presence of normal developing follicle (F) with Oocyte (O) (H&E, X10).

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

DISCUSSION

In the present work, the PCOS induction after treatment with Letrozole was confirmed through polycystic ovarian morphology and plasma hormone profiles. PCOS is manifested primarily by anovulation and hyperandrogenism (Azziz, et al., 2009). Abnormal follicular maturation or acceleration of follicular atresia is reported with elevated intraovarian androgen levels. Therefore, intraovarian androgen excess resulting from either circulating hyperandrogenemia or abnormal steroidogenesis may result in abnormal follicular development and polycystic ovary. A deficiency in the activity of the aromatase enzyme is one of the many intra-ovarian disturbances thought to cause PCOS. Aromatase catalyzes the rate limiting step in the biosynthesis of estrogens from androgens. Letrozole is a non-steroidal inhibitor of the aromatase enzyme and causes androgen excess and promotes development of PCOS. In the current study, a PCOS rat model was established after treatment with Letrozole and it showed many histologic and biochemical findings consistent with human PCOS (Kafali, et al., 2004). The working of this model was confirmed by assaying plasma testosterone levels which were found to be increased significantly when compared with normal rats indicating hyperandrogenism. This coincides with the earlier experiments (Bhattacharya, et al., 2005; Kafali, et al., 2004; Baravalle, et al., 2006; Desai, et al., 2012; Rezvanfar, et al., 2012; Sasikala and Shamila, 2009; Maharjan, et al., 2011). Earlier findings have reported that being a non-steroidal aromatase inhibitor Letrozole blocks the conversion of testosterone to estradiol. This leads to the reduction in estrogen production (Kafali, et al., 2004; Maharjan, et al., 2011; Manneras, et al., 2007; Rezvanfar, et al., 2012; Sasikala and Shamila, 2009). Similar results were also seen in the present study. The PCOS induced rats showed decreased levels of estrogen. Repetitive administration of mid and high dose of MP led to a significant increase in estrogen levels and significant reduction in plasma testosterone levels. The plasma progesterone levels decreased significantly after induction of PCOS with Letrozole. This is in accordance with the earlier observations (Baravalle, et al., 2006; Kafali, et al., 2004; Rezvanfar, et al., 2012; Sasikala and Shamila, 2009). Increased progesterone levels were observed when the PCOS induced rats were repetitively administered with mid and high dose of MP. Women with PCOS are hyperandrogenemic which is associated with alterations in circulating lipids and lipoprotein levels resulting in dyslipidemia. Characteristically PCOS patients have elevated cholesterol levels (Desai, et al., 2012; Sasikala and Shamila, 2009). The patients with PCOS tend to be obese, probably due to high lipid and cholesterol content. Similar effects were seen after the induction of PCOS in rats in the current study. The PCOS induced rats showed elevated cholesterol levels which decreased significantly when the PCOS induced rats were treated with mid and high dose of MP.

Ovarian weight in PCOS induced rats was more than the normal rats which is in accordance with the earlier findings (Desai, et al., 2012; Maharjan, et al., 2010; Manneras, et al., 2007; Rezvanfar, et al., 2012; Sasikala and Shamila, 2009). The treatment with mid and high dose of MP prevented further increase in ovarian weight. In the present research work, the uterine weight was found to be decreased in PCOS induced rats. This coincides with the earlier findings (Kafali, et al., 2004). The uterine weights were returned to the normalcy when the treatment of mid and high dose of MP was given to the PCOS induced rats.

The biochemical results are also supported by Histopathological observations of light microscopy. It is reported that the histopathological study of PCOS induced rats show the formation of cysts in the ovary (Baravalle, et al., 2006; Kafali, et al., 2004; Rezvanfar, et al., 2012). The ovarian cortex shows the presence of atretic follicles and the formation of more than two cysts in the ovary. The cysts show the attenuated layer of granulosa cells and hyperplasia of thecal layer (Brawer, et al., 1986; Kafali, et al., 2004; Rezvanfar, et al., 2012). Atretic follicles exhibit massive degeneration and sloughing - off of the central granulosa layer into the antrum. Thus the follicles become atretic with the presence of dying cells and debris in the antrum. In PCOS condition the corpora lutea do not form or the number of corpora lutea are diminished indicating anovulation and the frequency of estrus cycle is almost nil in PCOS rats (Brawer, et al., 1986; Rezvanfar, et al., 2012; Sasikala and Shamila, 2009). In PCOS high local androgen concentrations are responsible for anovulation by direct effect on the ovary. Androgen-induced follicular atresia is thought to occur by entry of androgens into the granulosa layer of pre-antral follicles, where they bind to the cell receptors and cause the cell death. Androgens cause deterioration of follicles by increasing the number of pycnotic granulosa cells and degenerating oocytes (De Leo, et al., 1998).

The observations seen in the current research work when the rats were induced with PCOS by Letrozole also corroborate earlier findings. The histopathological observations of the MP treated group showed marked recovery of the ovarian tissue with the presence of normalized structure of antral follicle. The light microscopic observations also revealed the presence of many well defined antral follicles in the process of normalizing. The follicles showed normal granulosa layer with defined thecal layers. The follicles also showed the presence of a clear antrum free of any cell debris. The presence of corpora lutea was also seen after plant treatment suggesting that treatment with MP restored normal estrous cyclicity. The ovarian cortex showed the presence of many follicles in the various stages of development indicating normal oogenesis.

The effect of MP treatment with mid and high dose was found to be comparable with that of clomiphene citrate while no significant changes were observed with low dose of MP treatment in the normalization of various parameters in the PCOS induced rats.

CONCLUSION

In the present study, increased testosterone levels, decreased estrogen and progesterone levels, degenerated structure of follicles along with the presence of cysts in the ovarian tissue were apparent in Letrozole induced PCOS rats. MP treatment brought about significant recovery of testosterone, estrogen, progesterone levels and ovarian tissues in PCOS induced rats. MP also showed good anti-androgen effect by reducing elevated androgen levels. The anti-androgen and estrogenic effects of MP could prevent ovarian cell dysfunction in PCOS and improve fertility. The observed recovery of ovarian tissue as well as anti-androgen and estrogenic potential of MP may be responsible for its efficacy in the management of PCOS. Findings of the current study can provide a baseline data for designing further investigations on the therapeutic benefits of MP as an adjunct therapy in the management of PCOS. The use of MP can reduce the side effects of modern drug without compromising its therapeutic activity.

ACKNOWLEDGEMENTS

The authors sincerely thank all the research colleagues from TDM lab and herbal research lab, Ramnarain ruia college. The authors also acknowledge all the support provided by TDM lab.

REFERENCES

Altchek, A., Deligdisch, L. and Kase N. (2003). Diagnosis and management of ovarian disorders, 2nd edition, Elsevier science, USA.

Abdulla, U., Diver, M.J., Hipkin, L.J. and Davis, J.C. (1983). Plasma Progesterone levels as an index of ovulation. British Journal of Obstetrics and Gynecology, 90 (6): 543-548.

Archer, J.S. and Chang, R.J. (2004). Hirsutism and acne in polycystic ovary syndrome. Best Practice and Research Clinical Obstetrics and Gynaecology, 18(5): 737-754.

Azziz, R., Carmina, E., Dewailly, D., Kandarakis, E.D., Escobar-Morreale, H.F., Futterweit, W., Janssen, O.E., Legro, R.S., Norman, R.J., Taylor, A.E. and Witchel, S.F. (2009). The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete force report. Fertility and Sterility, 91(2): 456-488.

Azziz, R., Dewailly, D. and Owerbach, D. (1994). Clinical review 56: Nonclassic adrenal hyperplasia: current concepts. Clinical Endocrinology and Metabolism, 78: 810–815.

Bako, A.U., Morad, S. and Atiomo, W.A. (2005). Gynecological endocrinology Polycystic ovary syndrome: An overview. Reviews in Gynaecological Practice, 5: 115-122.

Bandopadhyay, S., Chakrabarti, J., Banerjee, S., Pal, A.K., Goswami, S.K., Chakravarty, B.N. and Kabir, S.N. (2003). Galactose toxicity in the rat as a model for premature ovarian failure: an experimental approach readdressed. Human Reproduction, 18(10). 2031-2038.

Baravalle, C., Salvetti, N.R., Mira, G.A., Pezzone, N. and Ortega, H.H. (2006). Microscopic characterization of follicular structures in Letrozole – induced polycystic ovarian syndrome in the rat. Archives of Medical Research, 27: 830-839.

Bhattacharya, D., Mandal, S.K., Mukherjee, S. and Pradhan, M. (2005). Clinical correlation with biochemical status in polycystic ovarian syndrome. Journal of Obstetrics and Gynecology, 55 (1).

Brawer, J.R., Munoz, M., Farookhi, R. (1986). Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat, Biology of Reproduction, 35: 647-655.

De leo, V., Lanzetta, D., D'antona, D., Marca, A. and Morgante, G. (1998). Hormonal effects of Flutamide in young women with polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism, 83(1): 99-102.

Desai, B.N., Maharjan, R.H. and Nampoothiri, L.P. (2012). Aloe barbadensis Mill. formulation restores lipid profile to normal in a letrozole-induced polycystic ovarian syndrome rat model. Pharmacognosy Research, 4(2): 109–115.

Ehrmann, D.A. (2005). Polycystic ovary syndrome. The New England Journal of Medicine, 352(12): 1223-36.

Goodarzi, M.O. and Azziz, R. (2006). Diagnosis, epidemiology and genetics of the polycystic ovary syndrome. Best Practice and Research in Clinical Endocrinology and Metabolism, 20(2): 193-205.

Hayes, W. (1994). Principles and methods of Toxicology. 3rd edition, Raven press, New York, 521-550.

Homburg, R. (2009). Androgen circle of polycystic ovary syndrome. Human Reproduction, 24(7), 1548-1555.

Jadhav, A.N. and Bhutani, K.K. (2005). Ayurveda and Gynaecological disorders. Journal of Ethnopharmacology, 97: 151-159.

Kafali, H., Iriadam, M., Ozardali and Demir, N. (2004). Letrozole induced polycystic ovaries in the rat: a new rat model for cystic ovarian disease. Archives of Medical Research, 35: 103-108.

Khare, C.P. (2004). Encyclopedia of indian Medicinal Plants. Springer.

Leder, B.Z., Leblanc, K.M., Longcope, C., Lee, H., Catlin, D.H. and Finkelstein, J.S. (2002). Effects of oral androstenedione administration on serum terstosterone and estradiol levels in postmenopausal women. The Journal of Clinical Endocrinology and Metabolism, 87(12). 5449.

Maharjan, R., Nagar, P.S., and Nampoothiri, L. (2010). Effect of Aloe Barbedensis Mill. Formulation on Letrozole induced polycystic ovarian syndrome rat model. Journal of Ayurveda and Integrative Medicine, 1(4): 273-279.

Manneras, L., Cajander, S., Holmang, A., Seleskovic, Z., Lystig, T., Lonn, M. and Elisabet. (2007). A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. Endocrinology, 148: 3781-3791.

Nini, A.F., Riggs, B.L., Atkinson, E.J., O'Fallon, W.M., Eastell, R. and Khosla, S. (2000). Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. Journal of Clinical. Investigation, 106(12). 1553-1560.

Polly, Z.A., Begum, S., Ferdousi, S., Begum, N., Ali, T. and Begum, A. (2011). Relationship of FEF25-75, Pefr and SVC with Estrogen and Progesterone level in postmenopausal women. Journal of Bangladesh Society and Physiology, 6 (2): 116 – 121.

Rezvanfar, M.A., Rezvanfar, M.A., Ahmadi, A., Shojaei-Saadi, H.A., Baeeri, M., Abdollahi, M. (2012). Molecular mechanism of a novel Selenium based complementary medicine which confers protection against hyperandrogenism induced polycystic ovary, Theriogenology, 78: 620-631.

Sasikala, S.L. and Shamila, S. (2009). A novel ayurvedic medicine-Ashokarishtam in the treatment of Letrozole induced PCOS in rat. Journal of Cell and Tissue Research, 9(2): 1903-1907.

Sasikala, S.L. and Shamila, S. (2009). Unique rat model exhibiting biochemical fluctuations of Letrozole induced polycystic ovary syndrome and subsequent treatment with allopathic and ayurvedic medicines. Journal of Cell and Tissue Research, 9(2): 2013-2017.

Sharma, P.C., Yelna, M.B. and Dennis, T.J. (2001). Database on Medicinal plants used in Ayurveda. Central Council for Research in Ayurveda & Siddha, 2.

Yilmaz, B., Canpolat, S., Sandal, S., Akpolat, N., Kutlu, S., Ilhan, N. and Kelestimur, H. (2006). Paint thinner exposure inhibits testosterone synthesis and secretion in a reversible manner in the rat. Reproductive. Toxicology ,22(4): 791-796

Yilmaz, B., Kutlu, S., Canpolat, S., Sandal, S., Ayar, A., Mogulkoc, R. and Kelestimur, H. (2001). Effects of paint thinner exposure on serum LH, FSH and testosterone levels and hypothalamic catecholamine contents in the male rat. Bioogical and Pharmaceutical Buletin, 24 (2). 163-166.