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Intra Vaginal Drug for Treatment of Polycystic Ovary Syndrome

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Abstract---*Polycystic ovary syndrome is the ordinary also the most controversial endocrinological syndrome affecting 4-10 percent of women. The Polycystic ovary syndrome PCOS is an endocrinopathy characterize by the augmented insulin resistance. The vaginal mucosal cavity is a viable, secure, highly attractive drug delivery site and highly versatile in drug absorption, metabolism, and removal. Logically, metformin was introduced to determine the extent to which hyperinsulinemia affects the condition's pathogenesis. Additionaly, sustained contact with the vaginal mucosa of a delivery system can be accomplished more effectively than at the other absorption sites such as intestinal mucosa or rectum. For the women with PCOS who are not overweight the Metformin is an active ovulation induction agent and provides few benefits for an ovulatory infertility such as clomiphene over other first-line therapies. Metformin should be given to women with the PCOS undergo in vitro fertilization to decrease their risk.of ovarian hyperstimulation syndrome. Limited evidence signifies that metformin may be a suitable alternative to oral contraceptive pill (OCP) in the treatment hyperandrogenic symptoms of PCOS, including hirsutism and acne.*

Keywords--- *Polycystic ovary syndrome (PCOS), Endocrinopathy, Metformin, Hirsutism, Insulin resistance, Clomiphene, hyperinsulinemia, In vitro fertilization*

I. INTRODUCTION

Polycystic ovary syndrome with clinical signs of abnormal menstrual cycles, hirsutism, and acne is characterized by ovulation, hyperandrogenism and infertility. An estimated 5-10% of women with reproductive age are exaggerated by the disease. The anovulatory infertility is one of the most ordinary complaints of the women having polycystic ovary syndrome. They also have a similar increase in the incidence of cardiovascular risk factors to the metabolic syndrome[1]. By the age of 40, type 2 diabetes or reduced glucose tolerance will have up to 40 percent. Therefore, polycystic ovary syndrome is a major health issue and may be a major health problem that affects young women today.

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among young women, with about one in five women having ovaries with an ultrasound polycystic appearance and nearly half of those with polycystic ovaries meeting PCOS diagnostic criteria [1]. In contrast, PCOS is associated with low follicle stimulating hormone (FSH) levels and elevated luteinizing hormone (LH) levels. The high level of LH activates the release ofdihydroepiandrosterone sulphate (DHES) testosterone and estrogen. This ultimately leads to ovary cyst development [2].

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PCOS-related medical symptoms vary from diagnosis of cystic ovaries to menstrual irregularities associated with lack of ovulation or anovulation, hirsutism, obesity, and insulin resistance, further increasing the risk of breast cancer [3]. MTF-HCl is widely recommended by physicians for the treatment of PCOS due to its superior therapeutic efficacy compared to the other drugs among different treatment modalities [4]. It is currently understood that the pathophysiology of the polycystic ovary syndrome (PCOS) is associated with hypothalamic-pituitary-ovarian axis disruption [5]. While oral pharmacotherapies such as metformin, flutamide, and clomiphene citrate are used for its treatment, but severe side effects with long period of treatment render them unattainable.

Recently, the role of elevated level of insulin resistance or insulin has been implicated in the pathogenesis of PCOS. The human ovary consists of mainly four layers. The outermost layer of ovary is known as germinal epithelium and ovarian cortex comprises of ovarian follicles and stroma in between them[1], [3]. In addition, cortex also contains corpus luteum derived from the follicles. Presence and activation of insulin receptors (IRs) in both follicular and stromal sections of the human ovary trigger steroidogenesis mainly testosterone by theca cells indicates the critical role of the insulin in PCOS [6]. Furthermore, the theca cells over-express other receptors such as lipoprotein receptors (low-density lipoprotein (LDL) & high-density lipoprotein (HDL)), LH receptors, P450 side-chain cleavage (P450scc), 3b-hydroxysteroid dehydrogenase (3b-HSD), stereoidogenic acute regulatory (StAR) protein, and cytochrome P450c17 (CYP-17)[7]. All of these molecules have reported to augment steroidogenesis. The Insulin activates steroidogenesis in the theca cells by numerous molecular pathways, either through cross-talk with the LH-induced cAMP accumulation, that further activates PI3K activity or cAMP-independent actions of LH[7]. Moreover, the insulin dependent up-regulation of the LDL-receptor transcriptional activity through intracellular mechanisms which consist of PI3K-, PKA-, and MAPK-signaling pathways has also suggested to contribute to steroidogenesis[8]. On other hand, chronic low-grade inflammation[9][10]**,** angiogenesis[11], heavy exercise [12] and oxidative stress also contribute in the pathogenesis of PCOS.

Current treatment modalities for PCOS include metformin hydrochloride, clomiphene citrate, combination of clomiphene citrate and metformin hydrochloride, hormonal therapy, rosiglitazone, and pioglitazone. However, resistance of first line drug, clomiphene citrate [16]and long-term treatment regimen of other drugs with lesser clinical output encouraged for developing effective therapy in treatment of PCOS. Oral metformin hydrochloride is broadly used by the gynaecologists in the management of PCOS for the induction of ovulation. Metformin hydrochloride restrain the GnRH secretion by stimulating the hypothalamic AMP-activated protein kinase. Furthermore, metformin hydrochloride inhibits the ovarian gluconeogenesis and in this way, attenuates the ovarian androgen production [5]. More amusingly, metformin hydrochloride alters the effects of insulin on the production of ovarian androgen, growth of endometrium and theca cell hyperplasia. Metformin hydrochloride reduces the expression of inflammatory marker, CRP (C-reactive protein) in PCOS. In addition, metformin hydrochloride reduces angiogenesis through Erk1/2/Erk5 and NF-kappaB pathways by enhancing the antiangiogenic thrombospondin-1 level. Metformin hydrochloride remains stable at acidic pH as compared to the basic pH. Though, due to the hindered oral bioavailability (50-60%) and lactic acidosis side effect, metformin hydrochloride is administered in the range of 500 mg to 1500 mg/day over 21 days to induce ovulation. These limitations involve the metformin hydrochloride administration through an alternate route without compromising with established clinical

benefits. It was recognized that the intravaginal administration of metformin hydrochloride by modified cationic niosomes combined with thermosensitive gel offered greater therapeutic profile in the treatment of PCOS in terms of reduced dose-dose regimen and better clinical output compared to oral metformin.

Vaginal delivery of drugs offers avoidance of first pass metabolism, steady drug levels, administration of lower doses, and reduction in the occurrence and harshness of drug targetability, gastrointestinal side effects, high bioavailability, and self-medication. The use of metformin for women having polycystic ovary syndrome has stimulate an incredible amount of interest. Metformin hydrochloride (MTF-HCl) is a widely suggested therapeutic option accessible for the PCOS management. Though, in spite of favourable physicochemical properties, the oral administration of the MTF-HCl is connected with the impaired bioavailability (50-60%), frequent dosing (500 mg 2–3 times a day) and lactic acidosis in PCOS that eventually decreases the compliance of patient. Due to oral route of administration, these drugs exhibit large number of intolerable systemic side effects. Therefore there is a need to for the better drugs which target the PCOS core, axis of hypothalamus-pituitary-ovarian (HPO) and standardize the wide spectrum of PCOS anomaly. The aim of this paper is to provide an intravaginalmucoadhesive tablet of Met-HCl for the treatment of PCOS, exhibiting the higher bioavailability, reduction in the frequency of dose, more retention time and high patient compliance. The mucoadhesive tablet of metformin is administered intravaginally, and after the insertion, the granules of the tablet get melted into vaginal fluid and provide longer retention time of the drug in the vagina.

II. MATERIALS AND METHODS

2.1 Preparation and optimization of mucoadhesive tablets of metformin hydrochloride

The mucoadhesive tablets of Met-HCl were tested under both official and non-official tests for tablet as prescribed in Indian Pharmacopoeia (**Indian Pharmacopoeia, 2014**)[31] like hardness test, weight variation test, friability test, dissolution test and drug content analysis.

2.2 Weight variation test

For the weight variation test, 20 mucoadhesive tablets of the metformin hydrochloride 500mg, 1000mg, and 1500mg were randomly taken, separately weighed and then the average weight was calculated. Subsequently, each tablet's weight was compared with the average weight and % error was calculated. "As specified in Indian Pharmacopoeia, not more than two tablets should lie outside the allowed limit of 5% deviation for tablets weighing 250 mg or more".

2.3 Hardness test

Hardness test was conducted using Pfizer Hardness Tester on 5 tablets of each dosage group and then average hardness was calculated.

2.4 Friability test

For friability test, 20 tablets from each dose of metformin hydrochloride 500mg, 1000mg and 1500mg was weighed and then placed in a Roche Friability test apparatus that was rotating at 25 rpm and dropping the tablets by a distance of 6 inches with each revolution. After 4 min, the tablets were taken out and weighed again.

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$$\% \text{ Friability} = W_0 - W/W_0 \text{ X } 100$$

Where, W_0= Initial weight of tablet

W= Weight of tablet after revolution

2.5 Drug content analysis

Drug content analysis of mucoadhesive tablets of Metformin hydrochloride 500mg, 1000mg and 1500mg was carried out by using U.V/Visible spectrophotometry (Shimadzu 1800, Kyoto, Japan)[32]. The calibration curve of the metformin hydrochloride was formed in distilled water that followed the Beer Lambert's Law and exhibited the "linear regression equation of y= 0.0886x +0.0936 R^2= 09846". For drug content analysis, 3 mucoadhesive tablets from each dose of metformin hydrochloride 500mg, 1000mg and 1500mg were taken crushed and drug was extracted out in the distilled water. The filtered samples' absorbance of were taken at 232 nm and from the slope equation the amount of metformin hydrochloride was calculated.

2.6 Dissolution test

Dissolution test was carried out on 5 bilayer tablets of Metformin hydrochloride by using USP dissolution test apparatus type 2. About 900 ml of simulated vaginal liquid (pH~4.2) was taken as a dissolution medium which was maintained at 50 rpm and 37°C, as suggested for dissolution testing of the vaginal products (Indian Pharmacopoeia, 2014)[31]. At predetermined intervals of time, 5 ml sample was removes and replaced with the fresh dissolution medium to sustain sink conditions. The samples were then filtered through 0.22-µm membrane filter (MDI, Ambala, India**)** and the absorbance of each filtrate was then measured at 232 nm by utilizing a UV/Visible Spectrophotometer (Shimadzy, 1800, Japan). The optimized compositions of metformin hydrochloride intravaginalmucoadhesive tablets are given in table 1, 2 and 3.

Table-1: Optimized composition of intravaginalmucoadhesive tablet containing 500mg of metformin hydrochloride with total weight of each tablet 662mg.

Sr. No.	Ingredients	Quantity (mg)
1	Metformin hydrochloride	500
2	Hydroxypropyl methyl cellulose	100
3	Starch	25
4	Mannitol	25
5	Starch paste (10% w/v)	as required
6	Magnesium stearate	6
7	Talc	6

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Table-2: Optimized composition of intravaginalmucoadhesive tablet containing 1000mg of metformin hydrochloride with total weight of each tablet 1173mg.

Sr. No.	Ingredients	Quantity (mg)
1	Metformin hydrochloride	1000
2	Hydroxypropyl methyl cellulose	100
3	Starch	25
4	Mannitol	25
5	Starch paste (10% w/v)	as required
6	Magnesium stearate	11.5
7	Talc	11.5

Table-3: Optimized composition of intravaginalmucoadhesive tablet containing 1500mg of metformin hydrochloride with total weight of each tablet 1683mg.

Sr. No.	Ingredients	Quantity (mg)
1	Metformin hydrochloride	1500
2	Hydroxypropyl methyl cellulose	100
3	Starch	25
4	Mannitol	25
5	Starch paste (10% w/v)	as required
6	Magnesium stearate	16.5
7	Talc	16.5

2.7 Storage condition

The mucoadhesive tablet should be stored at 20-25°C in an airtight container or Store at room temperature, in a dry place to protect the mucoadhesive tablets from sunlight and moisture.

2.8 In-vivo study

The therapeutic efficacy of formulated metformin hydrochloride mucoadhesive tablets was tested in vivo in experimental animal model polycystic ovary syndrome (PCOS). Institutional Animal Ethics Committee (IAEC) (1201/a/08/CPCSEA) duly accepted the experimental protocol for animal study. In short, 24 Wistar female rats (180-220 g) were subdivided into four groups of six animals each with 4-5 days of estruscyclicity (n= 6). On

standard laboratory chow and water *ad libitum*, rats were maintained. Both animal experiments are performed in compliance with the guidelines of CPCSEA (Committee on Experimental Animal Control and Supervision), Ministry of Forestry and Culture, Government of India, India

2.9 Induction of polycystic ovary syndrome (PCOS)

Mifepristone (RU-486, 20 mg/kg/day, *p.o.)* was managed for 13 days in female wistar rats to induce PCOS that mimicked standard PCOS parameters as seen in women **(Oakley O, 2011)** as per the experimental protocol given in the **Table 4.**

Table 4: Experimental Protocol

Sr. No	Groups	Treatment
1	Normal control	Untreated
2	PCOS control	Mifepristone (20mg/kg; *p.o.*) for 13 days
3	PCOS+Met-HCl (Orally)	Mifepristone (20mg/kg/day; *p.o.*) for 13 days+ Met-HCl (500mg/day for next 7 days), followed by Met-HCl (1000mg/day for next 7 days) followed by Met-HCl (1500mg/day for next 7 days)
4	PCOS+Met-HCl (Intravaginally)	Mifepristone (20mg/kg; *p.o.*) for 13 days+ Met-HClmucoadhesive tablet (500mg/day for next 7 days), followed by Met-HClmucoadhesive tablet (1000mg/day for next 7 days) followed by Met-HClmucoadhesive tablet (1500mg/day for next 7 days)

2.10 Estimation of hormonal levels in serum

The therapeutic efficacy of Met-HCl's optimized mucoadhesive tablet has been evaluated by vaginal route in PCOS rats and compared to PCOS rats treated with pure drug solution oral gavages. Eventually, the rats were killed and blood was isolated as well as ovarian tissues. Blood was processed as an anticoagulant in microcentrifuge tubes of sodium citrate (10% w / v). Serum was isolated by centrifugation (4500 rpm for 15 minutes) and processed for further study at -20◦C. Until histopathology study, uterus and ovary tissues were weighed separately and set in a 4 percent paraformaldehyde solution. Competitive Enzyme Linked Immunosorbent Assay (ELISA) was used to assess serum concentration of estrogen, progesterone, and testosterone in blood samples while serum FSH and LH levels were calculated using non-competitive ELISA assay.

2.11 Histopathological evaluation of ovaries (Hematoxylin and eosin staining method)

Histopathological examination of the isolated ovary tissues using standardized hematoxyl-eosine staining technique.

2.12 Statistical analysis

All the results have been expressed as mean ± standard error of means (S.E.M.). Using Graph Pad Prism Version-5.0 software, the data were analyzed statistically. The *p*-value < 0.05 was examined to be statistically important.

III. RESULTS

3.1 Charaterisation of intravaginalmucoadhesive tablet of metformin hydrochloride:

The tailored intravaginalmucoadhesive tablets of metformin hydrochloride 500mg, 1000mg and 1500mg have successfully qualified the various optimization parameters. Evalution of preformulation parameters of granules of metformin hydrochloride is shown in **table 5**. The mucoadhesive tablets of Met-HCl have successfully qualified the weight variation test. Results indicated that no tablet was lying outside the allowed limit of deviation. Hence, mucoadhesive tablets of metformin hydrochloride 500mg, 1000mg and 1500mg showed the weight variation results with weight loss percentile 0.18±0.11, 0.20±0.23 and 0.21±0.14 respectively. The average hardness of mucoadhesive tablets of metformin hydrochloride 500mg, 1000mg and 1500mg was estimated to be 6.4±1.34, 6.1±1.07 and 6.6±1.87 kg/cm2 respectively (Table 6). Furthermore, friability test was performed and the weight loss of tablet of Met-HCl of 500 mg dose, 1000mg and 1500mg was noticed 0.23% ± 0.009, 0.20% ± 0.007 and 0.14% ±0.012 respectively (**Table 6**), which lies in the allowed limit of 1% as specified in Indian Pharmacopoeia, 2014. The drug content of tablet of Met-HCl of 500 mg dose, 1000mg and 1500mg was noticed 99.94%±2.13, 98.87% ±5.78 and 98.33%±4.56 respectively (Table 6).

Table 5: Evalution of preformulation parameters of granules of metformin hydrochloride (Met-HCL) (n=3, ± S.D.)

Sr. No.	Charaterisation parameters	Met-HCL (500mg)	Met-HCL (1000mg)	Met-HCL (1500mg)
1	Bulk volume (g/ml)	0.464±0.034	0.512±0.053	0.487±0.026
2	Tapped volume (g/ml)	0.547± 0.32	0.631± 0.48	0.453± 0.18
3	Hausner's Ratio (HR)	1.32±0.70	1.56± 0.12	1.29± 0.09
4	Carr's Index (% CI)	18.72± 0.72	16.94± 0.19	19.21± 0.25
5	Angle of repose (θ)	27.31±1.09	25.30±1.24	26.40±0.096

Table 6: Characterization of compressed intravaginalmucoadhesive tablet of metformin hydrochloride (Met-HCL) (n=3, ± S.D.)

Sr. No.	Charaterisation parameters	Met-HCL (500mg)	Met-HCL (1000mg)	Met-HCL (1500mg)
1	Weight variation (%)	0.18±0.11	0.20±0.23	0.21±0.14
2	Hardness (kg/cm^2)	6.4±1.34	6.1±1.07	6.6±1.87
3	Friability (%)	0.23% ± 0.009	0.20% ± 0.007	0.14% ±0.012
4	Drug content (%)	99.94±2.13	98.87±3.78	98.33±4.56

Intravaginalmucoadhesive tablet of Met-HCl of 500 mg dose released 90.72% of drug within 6 hours in simulated fluid of vaginal (pH~4.2). Continuance to this, the 1000 mg intravaginalmucoadhesive tablet of Met-HCl released 85.35% of drug within 8 hours. Correspondingly, 1500 mg Met-HCl bearing intravaginalmucoadhesive tablet released 98.66% of drug within 6 hours. Hence, in vitro drug release data demonstrated that all three types of intravaginalmucoadhesive tablet bearing Met-HCl released the therapeutic moiety within 8 hours (Table 7) (Figure1).

Table 7: Dissolution testing of intravaginalmucoadhesive tablet of metformin hydrochloride (Met-HCL) (n=3, ± S.D.)

Sr. No.	Dissolution Time (in hours)	Met-HCL (500mg)	Met-HCL (1000mg)	Met-HCL (1500mg)
1	0.25	16.9	23.36	19.16
2	0.5	16.9	39.24	38.26
3	1	29.88	52.11	62.46
4	2	40.32	66.96	82.22
5	4	56.16	63.18	91.93
6	6	90.72	85.32	98.66
7	8	90.72	85.35	98.66

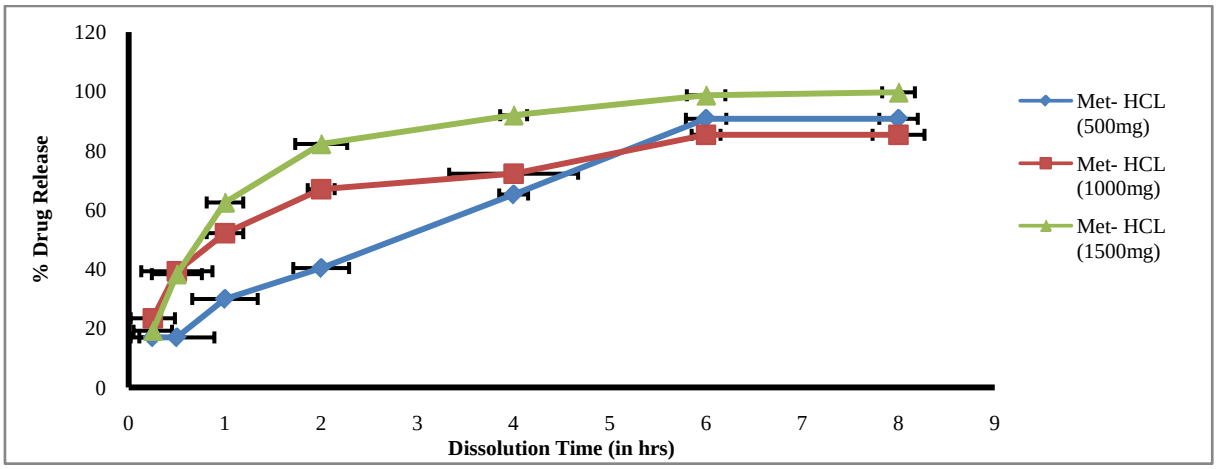

Figure 1: Dissolution testing of intravaginalmucoadhesive tablet of metformin hydrochloride.

3.2 Histopathological evaluation of ovaries (Hematoxylin and eosin staining method)

Histological examination of the isolated ovaries from mifepristone PCOS rats illustrated several ovarian modifications, such as ovarian stroma occupied by fluid-filled cysts with an abnormally thin layer of granulose with hypertrophied theca layer. Intravaginal administration of tailored mucoadhesive tablet of Met-HCl markedly

reversed the normal ovarian histology with development of normal follicles and few cysts (Figure 2).

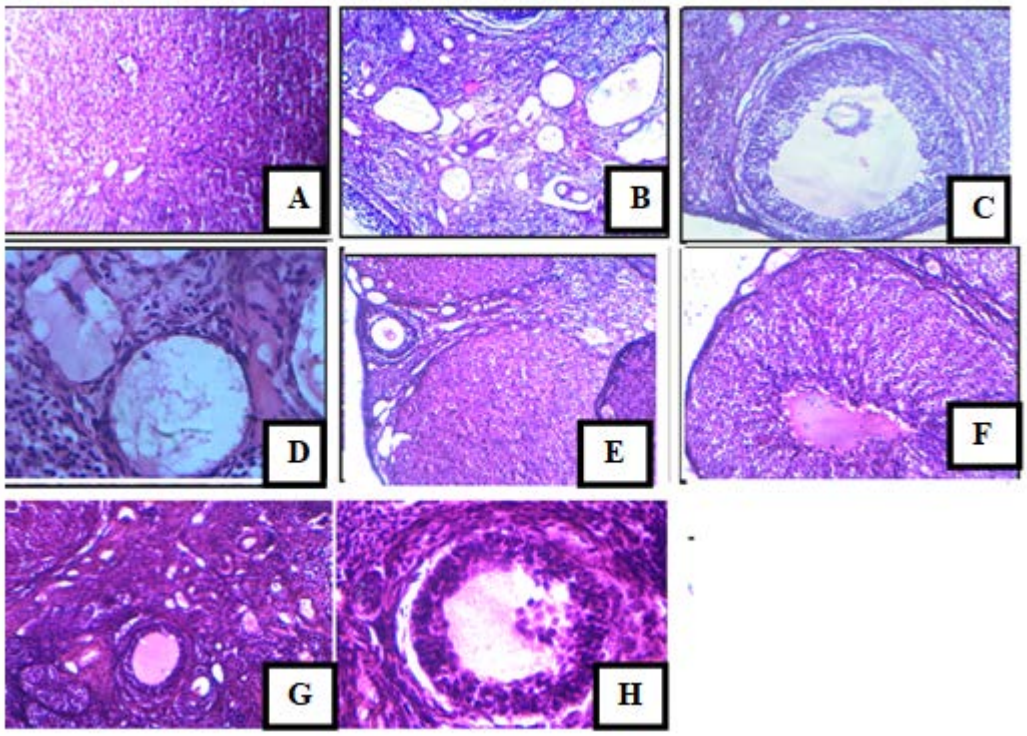

Figure-2: Photomicrographs of ovarian sections (5µm):

A: Represents normal ovaries

B-D: Mifepristone treated ovaries indicating abundant follicular cysts and arrested follicular development (B-X10, C-X40 and D-X100 magnification

E-F: Mifepristone treated ovary followed by oral suspension of metformin administration with its basement membrane depicting intact ovarian surface epithelium. Healthy follicles have not been observed along with atretic follicles. E-X10, F-X40 magnification

G-H: Mifepristone treated ovary followed by vaginal metformin administration indicating development of healthy developing follicle with its oocyte and well defined granulosa cells. G-X10, H-X40 magnification

3.3 Estimation of hormonal levels in serum

Administration of mifepristone (RU-486, 20 mg/kg/day, *p.o.)* for 13 days in the female wistar rats significantly enhanced the levels of serum testosterone, estrogen and luteinizing hormone along with significant decline in progesterone and follicle stimulating hormone levels confirming PCOS pathology. Intravaginal administration of tailored mucoadhesive tablet of Met-HCl markedly improved the mifepristone-induced hormonal imbalance **(Table 8)**.

Table-8: Measurement of hormonal levels in serum [(n=6, ± S.D.) p<.05 [a] vs. PCOS control (one way ANOVA) vs. normal control; p<.05 [b]]

Sr. No	Hormones	Normal control	PCOS control	PCOS + Met-HCL (orally)	PCOS+ Met-HCL (intravaginally)
1	Progesterone (ng/ml)	35±1.25	11±1.67[a]	38±2.1 [b]	41±1.24 [b]
2	Estradiol (pg/ml)	502±2.45	704±2.86[a]	538±2.65[b]	558±1.98[b]
3	Testosterone (ng/ml)	2.4±1.09	4.5±1.00[a]	3.4±1.11[b]	2.9±1.32[b]
4	LH (mIU/ml)	10±0.97	21±1.9[a]	17±0.98[b]	13±1.00[b]
5	FSH (mIU/ml)	27±1.43	17±1.45[a]	29±1.54[b]	26±1.20[b]

IV. CONCLUSION

With the support of results obtained in present paper, it may be concluded that mifepristone administration in female wistar rats produces the signs of PCOS. The tailored mucoadhesive tablet of Met-HCl through intra vaginal route of administration exhibited the comparable or equivalent preclinical benefits when compared with Met-HCl administered through oral route. This indicates that intra vaginal route of administration may be used as an alternative way for delivery of Metformin-HCl in the treatment of PCOS without compromising with clinical benefits.

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