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Mifepristone (RU486) induces polycystic ovarian syndrome in female Wistar rats with features analogous to humans

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Summary

Numerous inducers of polycystic ovarian syndrome (PCOS) at different doses have been proposed in several experimental animals but there is no consensus on an appropriate dose(s) that should ideally reproduce the key biochemical and clinical features of PCOS similar to those of humans. Therefore, this study was aimed at investigating an appropriate dose(s) for the induction of PCOS in female Wistar rats. Twenty-four female albino rats (190.00 ± 13.00 g) with 4-5 days of estrus cyclicity were completely randomized into 4 groups (A - D) of six animals each. Animals in group A (control) were subcutaneously administered 0.2 ml of pure olive oil, while those in groups B, C and D were subcutaneously administered same volume corresponding to 5.0, 7.5 and 10.0 mg of mifepristone in olive oil for 9 days starting from the day of estrus (Day 1). The estrus cycle, serum testosterone (T), estradiol (E), prolactin (Pr), follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P), insulin (Is), weight of the animals, fasting blood glucose (FBS) and ovarian morphology were monitored/ evaluated/ examined. The 5.0 mg of mifepristone extended the estrus stage for four days, increased ($p<0.05$) the levels of serum E, P, Pr, FSH, T, triacylglycerides (TAG), and total cholesterol (TC) as well as decreased the concentrations of LH and high density lipoprotein-cholesterol (HDL-C). There was no significant difference ($p<0.05$) in the Is concentration, animal body weights and FBS at day 10 in rats administered 5.0 mg of mifepristone. The 7.5 mg of mifepristone produced irregular estrus cycle, increased Pr, TAG, T, and TC concentrations and FBS whereas it decreased E, P, HDL-C, and LH. The Is, FSH and body weights of the animals were not significantly altered at 7.5 mg of mifepristone. The 10.0 mg of mifepristone produced irregular estrus cycle, increased the levels of E, TAG, Is, LH, T as well as decreased the levels of P and HDL-C. The levels of Pr, FSH, TC, body weights and FBS were not significantly altered at this dose. There was no ovarian follicular growth and atresia in the 5.0 and 7.5 mg mifepristone-treated rats whereas the 10 mg of mifepristone produced these histopathological features. Overall, the study concluded that subcutaneous administration of mifepristone (RU486) induces polycystic ovarian syndrome in rats through deprivation of progesterone with the 10 mg producing majority of the key biochemical and clinical features associated with PCOS in humans. The study, therefore, recommends the subcutaneous administration of mifepristone (RU486) on daily basis for 9 days as a good model for inducing PCOS in animals.

Keywords: PCOS, mifepristone, biochemical features, ovarian morphology, estrus cyclicity

Introduction

Polycystic ovarian syndrome (PCOS) is a condition of ovarian dysfunction characterized by cardinal features of hyperandrogenism and polycystic ovary morphology. According to the 2003 Rotherdam Concensus, PCOS is a syndrome associated with the presence of any two of these three criterion: (a) Menstrual disturbance-oligomenorrhoea/anovulation; (b) Clinical and/ or biochemical signs of hyperandrogenism like acne, hirsutism etc., after other causes of hyperandrogenism have been ruled out, and (c) Ultrasound appearance of polycystic ovary as polycystic adnexae (Rotherdam ESHRE/ASRM 2004; Franks, 2005). It is one of the most common endocrine disorders of women that affect 4-10% of those in reproductive age of 21-45 years (Strowitzki et al., 2010; Teede et al., 2010). It is thought to be one of the leading causes of female subfertility (Goldenberg and Glueck, 2008). The precise cause of polycystic ovarian syndrome is unknown; however, it is considered to be a complex multi-genetic disorder

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caused by disordered gonadotropin release and dysregulation of steroidogenesis (Pagan et al., 2006).

PCOS indirectly leads to reduction in fertility as a consequence of dysfunctional follicular maturation and ovulation, miscarriages, dysregulation of reproductive hormones including hypersecretion of Lutenizing hormone and hyperandrogenism causing acne and hirsutism (Goodarzi et al., 2011; Pasquali et al., 2011). Women with PCOS often exhibit non-reproductive metabolic abnormalities such as obesity, hyperinsulinemia, insulin resistance, dyslipidemia, increased risk of cardiovascular diseases and type 2 diabetes (Lobo and Carmina, 2000; Goodarzi et al., 2011; Pasquali et al., 2011). Furthermore, PCOS consist of chronic anovulation, menstrual disturbance, hyperandrogenism, polycystic ovaries and metabolic syndromes (Jonard and Dewailly, 2004; Dipankar et al., 2005; Dabadghao et al., 2007).

Previously described rodent PCOS models have used androgens, estrogens, aromatase inhibitors, antiprogesterones RU486, exposure to light, and genetic manipulations to induce PCOS-like characteristics. The antiprogesterone or antiprogestine RU486 is a synthetic steroid showing high affinity for progesterone (and glucocorticoid) receptors with potent antagonistic but no agonistic activity (Baulieu, 1991). Mifepristone, an antiprogesterone, binds to the progesterone receptor thereby prevent the binding of endogenous progesterone. It does not activate a true biological response to progesterone, but has both weak anti-glucocorticoid and anti-androgenic activity (Baird, 2000).

Studies on the administration of mifepristone RU486 in the induction of PCOS as reported by Lakhani et al. (2006) revealed that treatment of female rats with 2 mg of mifepristone for 7-9 days did not significantly affect the body weight and serum FSH. Furthermore, studies by Sánchez-Criado *et al.* (1993) and Ruiz *et al.* (1996) reported that the administration of 2 mg/100g of body weight of mifepristone to female rats increased serum LH and testosterone. Serum insulin also tended to be elevated, but not significantly, and the ovaries of the animals were characterized by increase in the abundance of follicular cysts and arrested follicular growth. However, these studies did not determine the clinical/biochemical aspects such as lipid profile and serum concentrations of other reproductive hormones such as progesterone, prolactin and estradiol. Ruiz et al. (1996) and Sánchez-Criado *et al.* (1993) also reported that administration of 4 mg of mifepristone RU486 produced distorted estrus cycle, increased levels of LH, T, E, and Pr, and decreased FSH concentration but this model did not account for the metabolic disturbances associated with PCOS. Rats treated with 2.0 and 4.0 mg of antiprogesterone RU486 exhibited some endocrine and ovarian morphological features similar to human PCOS, due to lack of progesterone actions; however, for RU486 administration to be validated as a useful PCOS model, metabolic disturbances require further detailed assessment (Walters et al., 2012a). Furthermore, due to the ethical and logistic limitations on human experimentation, suitable animal models that mimic all or most PCOS traits are indispensable.

A range of animal models, including rodents, sheep, and nonhuman primates, have been employed to study the pathogenesis of PCOS (Abbott et al., 2005; Walters et al., 2012a; Franks, 2012; Padmanabhan and Veiga-Lopez, 2013), but information is scanty on appropriate dose(s) that can produce key biochemical and clinical features of PCOS that are analogous to those in humans. Therefore, in this study, we set out to comprehensively assess reproductive, endocrine, and metabolic features associated with PCOS at 5.0, 7.5 and 10.0 mg doses of mifepristone RU486 (higher than previously reported) with a view to recommend the best dose that could produce virtually all the essential features of PCOS in female Wistar rats that are analogous to those found in humans.

Materials and Methods

Experimental animals

Female albino rats (*Rattus norvegicus*) of Wistar strain weighing 190.00 ± 13.00 g were

obtained from the Animal House of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.

Assay kits

The assay kits for prolactin, progesterone, testosterone, insulin, estradiol, follicle stimulating and luteinizing hormones were products of Monobind Inc., Lake Forest, Calabasa, USA. Total cholesterol, triglyceride and HDL cholesterol kits were made by Randox Laboratory, Co-Atrim, United Kingdom, while Accu-chek Active strip and glucometer were products of Roche Diagnostic GmbH, Sandhofer Strasse, Germany. Pure olive oil was a product of Andalu-Cia, Goya en Espana, Sevilla, Spain, while Mifepristone was made by Ranbaxy Laboratories Limited, New Delhi, India.

Animal grouping and induction of polycystic ovarian syndrome

Twenty-four female Wistar rats housed in separate cages under standard laboratory conditions (temperature: 22±3^{o}C; photoperiod: about 12h/12h light/dark cycle; humidity: 35 - 40%) were maintained on rat pellets (Vital Feeds, Grand Cereals, Jos, Nigeria) and water *ad libitum*. The rats were completely randomized into four groups of six animals each as follows:

Group I – (Control) received 0.2 ml of the vehicle only (pure olive oil)

Group II – received 5.0 mg of mifepristone

Group III– received 7.5 mg of mifepristone

Group IV– received 10.0 mg of mifepristone

The mifepristone was prepared in pure olive oil (vehicle). The administration was done subcutaneously once daily for 9 consecutive days starting from their estrus cycle. The animals were sacrificed 24 hours after the last dose. This study was carried out following ethical approval from the University Ethical Research Committee of University of Ilorin, Ilorin, Nigeria.

Assessment of estrus cyclicity

Estrus cycle stage was determined daily in the animals between 10:00 – 11:00 hours by light microscopic examination of vaginal smears as described by Walters et al. (2012a). Briefly, the rats were held at the thorax, ventral surface uppermost, whilst providing lumbar support as far as possible. Vaginal secretions were collected using a cotton-tipped swabs softened with a drop of saline. About 1-2 inches of the swab was inserted into the vagina of the female rats and the end was rotated through 2-3 revolutions (to allow the cotton tip pick up an adequate load of cells). The swab was withdrawn and the tip of the cotton rolled along the length of a glass slide. The dried smear was dipped 3 to 5 times in 70% alcohol in order to "fix it". The slides were then stained with 0.5% of aqueous methylene blue solution, rinsed in tap water and examined with an Olympus light microscope under ×10 objective lens with the condenser lens switched off (Drury and Wallington, 1980). A digital camera, SAMSUNG ES-95 (SAMSUNG Corporation Digital Camera, Gyeonggi-do, Republic of Korea) was used to capture the images.

Determination of fasting blood glucose

The determination of fasting blood glucose was done after fasting (without food, but water) the animals for 18 hours on days 0, 5, and 10 using Accu-chek glucose strip and glucometer as previously described by Yakubu et al (2014).

Preparation of serum

The procedure described by Yakubu et al. (2008) was used to prepare the serum. Briefly, under ether anesthesia, the veins, after being slightly displaced (to prevent blood contaminating the interstitial fluid), were cut with a sterile scapel blade and 5 ml of the blood was collected into clean and dry centrifuge tubes. The blood was then left undisturbed for 10 minutes to clot at room temperature. The tubes were thereafter centrifuged at 894 x *g* for 15 minutes using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals,

Essex, England). The sera were later aspirated with Pasteur pipettes into clean, dry, sample bottles and used within 12 hours of preparation for the hormonal assay.

Determination of biochemical/metabolic parameters

The procedures described in the instruction manual of the assay kits were adopted for the determination of insulin, prolactin, estradiol, testosterone, progesterone and TAG (Tietz, 1995), luteinizing hormone (Goldstein and Kosasa, 1975), follicle stimulating hormone (Wide, 1981), total cholesterol (Richmond, 1973), and HDL-C (Friedewald et al., 1972). The body weight of the animals was determined on days 0, 5 and 10 using a weighing balance.

Histological examination of ovaries

The left ovary from a representative of each group was excised and fixed in 10% formalin for histological examination as described by Drury and Wallington (1980). Briefly, the ovaries were dehydrated in ascending grades of ethanol (70%, 90% and 95%), cleaned in xylene and processed to paraffin blocks, sectioned (5 μm thick) and stained with hematoxylin and eosin dyes. The slides were examined using light microscope (OLYMPUS, Model CX21FSI, Philippines).

Statistical analysis

Data were expressed as means of six replicates ± SEM. Data from test groups were compared with their respective controls and differences considered significant at $P < 0.05$ using one way Analysis of Variance and Duncan's Multiple Range Tests.

Results

Mifepristone produced widely varying degrees of alterations in the biochemical/clinical parameters, and the ovarian histology investigated/examined in the present study. Specifically, the 5.0 and 10.0 mg of mifepristone significantly increased ($p<0.05$) the concentration of estradiol whereas the 7.5 mg decreased it (Table 1). Administration of 5.0 and 7.5 mg of mifepristone did not significantly ($p>0.05$) alter the levels of insulin in the animals whereas 10.0 mg of mifepristone elevated ($p<0.05$) the level of the hormone. The serum FSH levels of the animals increased ($p<0.05$) following the administration of 5.0 mg whereas that of the gonadotropin was not significantly altered ($p>0.05$) at 7.5 and 10.0 mg of mifepristone.

Furthermore, the 5.0 and 7.5 mg of mifepristone significantly ($p<0.05$) decreased the level of LH whereas the 10.0 mg increased it by 84.89%. Again, all the doses of mifepristone elevated testosterone contents in the animals whereas progesterone was only increased ($p<0.05$) by the 5.0 mg of mifepristone. The 7.5 and 10.0 mg of mifepristone significantly ($p<0.05$) reduced the progesterone content of the animals. In addition, the prolactin concentration was elevated ($p<0.05$) at 5.0 and 7.5 mg of mifepristone whereas the 10.0 mg did not significantly ($p>0.05$) alter the prolactin concentration of the animals (Table 1).

The 5.0, 7.5, and 10.0 mg of mifepristone significantly ($p<0.05$) reduced the levels of HDL-C whereas the concentration of triglycerides dose-dependently increased ($p<0.05$) with the 10.0 mg of mifepristone producing an elevation of four-fold the control value (Table 2). The total cholesterol content was significantly ($p<0.05$) elevated at 5.0 and 7.5 mg of mifepristone whereas the 10.0 mg produced total cholesterol levels that compared favorably ($p>0.05$) with the olive oil treated control animals (Table 2).

Mifepristone administration at all the doses (5.0, 7.5 and 10.0 mg) did not significantly ($p>0.05$) alter the body weights of the female rats throughout the period of exposure (Table 3). In addition, the 5.0 mg of mifepristone did not produce a definite pattern of effect on the blood glucose levels of the animals throughout the period of exposure (Table 4). In specific terms, the blood glucose increased ($p<0.05$) at day 5 whereas it compared favorably ($p>0.05$) with their respective controls by day 10. Furthermore, the 7.5 mg of mifepristone significantly ($p<0.05$)

Table 1: Effects of administration of mifepristone on serum hormone concentration of female rats

Groups	Estradiol (Pg/ml)	Insulin (mIu/ml)	Follicle Stimulating Hormone (mIu/ml)	Lutinizing Hormone (mIu/ml)	Testosterone (ng/ml)	Progesterone (ng/ml)	Prolactin (ng/ml)
Control	1348.50± 28.00ab	118.95± 1.44a	2.75± 0.38a	2.25± 0.14a	0.035± 0.00a	33.00± 0.58a	1.80± 0.12a
5mg Mifepristone	1410.00± 11.55c	122.00± 1.16a	4.40± 1.50b	1.10± 0.18b	8.60± 0.12c	53.00± 2.60b	3.78± 0.01b
7.5mg Mifepristone	1310.00± 5.77b	120.50± 0.87a	2.40± 0.23a	1.10± 0.00b	2.80± 0.75b	23.00± 1.16c	2.13± 0.04b
10.0mg Mifepristone	1397.50± 1.44c	127.50± 1.44b	2.85± 0.38a	4.16± 0.67c	7.50± 0.17c	20.80± 3.29c	1.90± 0.06a

Data are mean of six determinations ± SEM. Test values with superscripts different from the control for each parameter are significantly different (P<0.05).

Table 2: Effects of administration of mifepristone on serum lipid profile of female rats

GROUPS	Total cholesterol (mmol/ dL)	HDL cholesterol (mmol/ dL)	Triglycerides (mmol/ dL)
Control	0.19±0.02[a]	2.13±0.30[a]	0.17±0.03[a]
5.0 mg Mifepristone	0.31±0.05[b]	1.62±0.43[b]	0.48±0.20[b]
7.5mg Mifepristone	0.33±0.06[b]	1.57±0.40[b]	0.69±0.05[b]
10.0mg Mifepristone	0.21±0.02[a]	0.87±0.15[b]	0.75±0.07[b]

Data are mean of six determinations ± SEM. Test values with superscripts different from the control for each parameter are significantly different (P<0.05).

Table 3: Effects of administration of mifepristone on body weight of female rats

	Body Weight (g)		
GROUPS	Day 0	Day 5	Day 10
Control	189.00±1.16a	188.00±4.41 a	193.00±6.25 a
5.0 mg Mifepristone	196.00±3.22 a	196.00±3.84 a	189.00±2.90 a
7.5mg Mifepristone	194.00±3.18 a	195.00±4.06 a	189.00±2.91 a
10.0mg Mifepristone	189.00±7.83 a	190.00±6.57 a	189.00±5.46 a

Data are mean of six determinations ± SEM. Test values with superscripts different from the control for each parameter are significantly different (P<0.05).

Table 4: Effects of administration of mifepristone on fasting blood glucose level of female rats

	Fasting Blood Glucose (mg/dL)		
GROUPS	Day 0	Day 5	Day 10
Control	54.67±1.01[a]	53.33±6.69[a]	57.00±1.50[a]
5.0 mg Mifepristone	54.00±4.51[a]	63.33±4.10[b]	56.00±5.51[a]
7.5mg Mifepristone	52.33±4.84[a]	66.00±3.51[b]	67.00±2.52[b]
10.0mg Mifepristone	54.33±4.81[a]	55.67±0.24[a]	58.00±1.00[a]

Data are mean of six determinations ± SEM. Test values with superscripts different from the control for each parameter are significantly different (P<0.05).

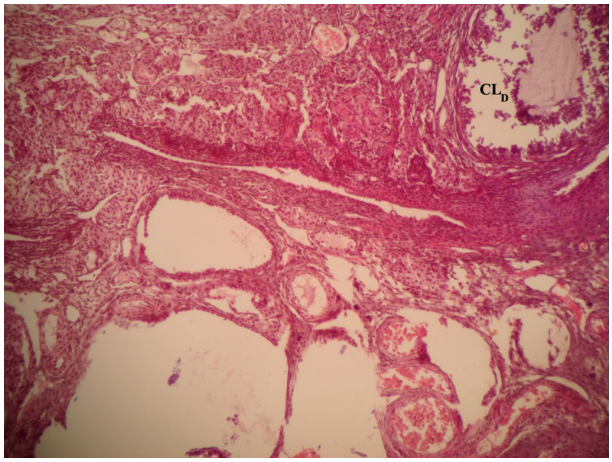

Plate 1: Cross section of the ovary of rats injected olive oil (×400; H&E). CL_D – Developing corpus luteum

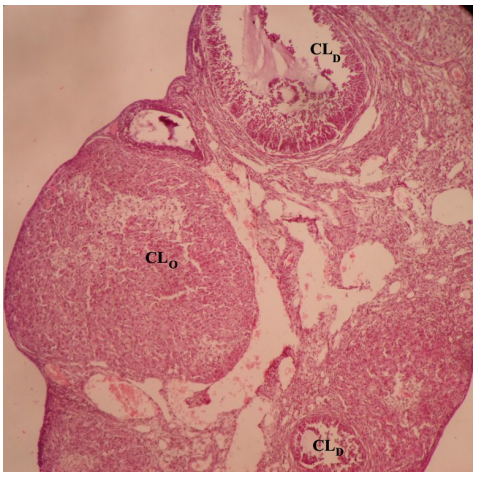

Plate 2: Cross section of the ovary of rat injected 5.0 mg of mifepristone (×400; H & E). CL_O – Old corpus luteum, CL_D – Developing corpus luteum

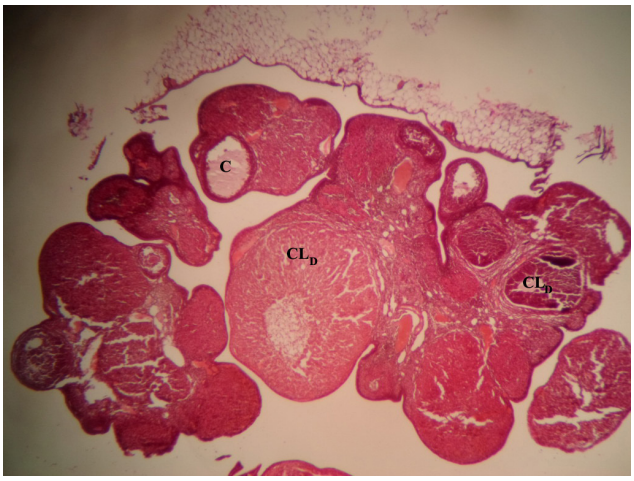

Plate 3: Cross section of the ovary of rat administered 7.5 mg of mifepristone (×400;H & E). C- Cysts, CL_D – Developing corpus luteum

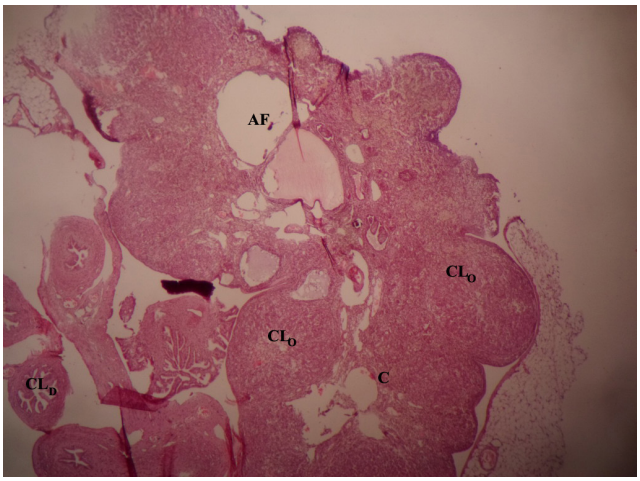

Plate 4: Cross section of the ovary of rat administered 10.0 mg of mifepristone (×400;H & E). C- Cysts, CL_O – Old corpus luteum, CL_D – Developing corpus luteum AF – Atretic follicle

increased the blood glucose level of the animals at the days of intervention whereas the 10.0 mg of mifepristone did not significantly ($p>0.05$) alter the blood glucose level of the animals throughout the period of administration (Table 4).

The daily vaginal cytology of the rats after induction revealed an alteration in the estrus cycle compared to the consistent 4-5 days of estrus cyclicity in all the rats given varying doses of mifepristone. There was persistent and extended days of the estrus stage evident by the presence of cornified epithelial cells in rats administered 5.0 mg of mifepristone for a period of 4-5 days during the induction while those administered 7.5 and 10.0 mg of mifepristone produced irregularity in their cycle which differed from the consistent stages in their estrus cycle observed before induction.

There was no ovarian follicular growth and atresia in the 5.0 and 7.5 mg of mifepristone-treated rats whereas the 10 mg of mifepristone produced significant number of atretic ovarian follicles and ovarian cysts. There were degenerated follicle and

decreased corpora lutea with few fresh ones growing in animals administered 10 mg of mifepristone when compared to those given 5.0 and 7.5 mg of mifepristone (Plates 1- 4).

Discussion

PCOS presents with many clinical symptoms such as oligomenorrhea, hyperandrogenism, disrupted folliculogenesis, insulin resistance and chronic annovulation which lead to metabolic and reproductive dysfunction especially infertility (Yakubu and Ibiyo, 2013). Although, studies have been reported previously on induction of PCOS with mifepristone, these studies did not reveal adequate and comprehensive account of the metabolic disturbances associated with the use of mifepristone to induce PCOS in animals at doses higher than 4.0 mg. Thus, this study provides a more comprehensive evaluation of endocrine and metabolic features associated with the induction of PCOS at 5.0, 7.5 and 10 mg doses of mifepristone that are yet to be previously reported in open scientific literature.

Mifepristone RU486, an antiprogestagen, is a synthetic steroid with high affinity for progesterone (and glucocorticoid) receptors and potent antagonistic but no agonistic activity. The compound is a 19-nor-steroid with substitutions at positions C11 and C17 (17 beta-hydroxy-11 beta-[4-dimethylamino phenyl] 17 alpha-[1-propynyl]estra-4,9-dien-3-one) (Baulieu, 1991). Therefore, the underlying pharmacological properties of mifepristone validate its use in the induction of PCOS, and rats treated with RU486, due to lack of progesterone action, show numerous endocrine and ovarian morphological features similar to human PCOS (Walters et al., 2012a). The decreased level of progesterone in rats administered 7.5 and 10.0 mg of mifepristone might be a consequence of the administered drug repressing the synthesis of endogenous progesterone and/or acting as a competitive inhibitor of the endogenous progesterone. Furthermore, increased follicular cyst, many old corpora lutea and a few new developing ones in rats administered 10 mg of mifepristone in the current study might have accounted for the significantly decreased progesterone concentration. Such reduction in the levels of serum progesterone due to the administration of 7.5 and 10.0 mg of mifepristone may impede ovulation, and conception in the female rats. The disrupted hormonal features and the ovarian histology at 10.0 mg of mifepristone are consistent with the biochemical/clinical features of PCOS in humans. The elevated levels of estradiol in rats administered 5.0 and 10.0 mg of mifepristone may be attributed to feedback inhibition of GnRH secretion by estrogens and progesterone. Such feedback inhibition of GnRH prevents the mid-cycle surge of LH and ovulation.

Follicle stimulating hormone is the central hormone of mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life (Simoni and Nieschlag, 1995). FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells (Kumar et al., 1997). The absence of an effect on FSH in rats administered 7.5 and 10.0 mg of mifepristone might be a result of the various doses of mifepristone exerting its inhibitory effect on the anterior pituitary gland and or the hypothalamus since the secretion of FSH is regulated by the gonadotropin releasing hormone secreted by the hypothalamus. In synergy with FSH, estradiol stimulates granulosa cell proliferation during follicular development (Telefo et al., 1998). The absence of an effect on the estradiol content of animals administered 7.5 mg of mifepristone RU486 may be due to the unaltered levels of FSH in the animals. The increased level of FSH in rats administered 5.0 mg of mifepristone might have accounted for their elevated estradiol levels, the reason would be that the release of FSH affects the enzyme, aromatase, which induces the conversion of testosterone to estradiol in the granulosa cells. Prolactin initiates breast development by inducing lobuloalveolar growth of the mammary gland and also stimulates lactogenesis. High prolactin levels tend to suppress the ovulatory cycle by

inhibiting the secretion of both follicle-stimulating and gonadotropin releasing hormones (GnRH) (Fitzgerald and Dinan, 2008). It is known that prolactin inhibits follicular development (Dorrington and Gore-Langton, 1982; Kalison et al., 1985). Therefore, an increase in prolactin level might have disrupted ovulation and promoted acyclicity of the estrus cycle which is one of the major symptoms of PCOS in animals including humans.

Maturation of pre-ovulatory follicles and ovulation are under the combined and balanced influences of ovarian and extra-ovarian hormones. Imbalances or alterations in these hormones lead to irregularity in the ovarian functions and duration of estrus cycle (Shivalingappa et al., 2002). The vaginal cytology of rats administered 5.0 and 7.5 mg of mifepristone revealed persistent estrus phase which was confirmed by the presence of predominantly cornified epithelial cells while rats that received 10 mg of mifepristone had total disruption of their estrus cycle and this might be a consequence of the doses of mifepristone on follicular maturation, thus causing delay in ovulation and leading to follicular atresia. Furthermore, high level of serum testosterone following the administration of 10 mg of mifepristone would inhibit ovulation and is consistent with the findings by Sanchez-Criado et al. (1993) who reported that androgen may be involved in the failure of ovulation in the mifepristone RU486-treated rats. Endocrine disruption, such as hyperandrogenism, the most consistent feature of women with PCOS (Abbott et al., 2002), was reported in all the animals administered with various doses of mifepristone with 10 mg of mifepristone exhibiting more of the features analogous to humans. Hyperandrogenism, one of the primary features of human PCOS, is associated with LH hypersecretion (Walters et al., 2012a), which was prominent in animals given 10.0 mg of mifepristone as their testosterone and LH level were significantly elevated compared to those administered 5.0 and 7.5 mg of mifepristone. This increased serum testosterone content in animals administered 10.0 mg of mifepristone might be due to absence of or deprived progesterone action at these doses and the mifepristone having high affinity for progesterone receptors thereby inducing an inappropriate feedback on gonadotropin secretion. This feedback results in a stimulation effect of estrogen on LH secretion and a concomitant suppression of FSH secretion (Ruiz et al., 1996).

In this study, 10.0 mg of mifepristone induced some ovarian morphologic features of PCOS, including fewer corpora lutea and an increased occurrence of atretic cyst-like follicles, although not the classic polycystic appearance when compared to animals that received 5.0 mg and 7.5 mg of RU486. The onset of follicular atresia that was prominent in animals administered 10.0 mg of mifepristone might be attributed to the elevated testosterone in these animals. The anti-folliculogenic action of the elevated levels of LH (Hillier, 1990) and the absence of progesterone actions on follicular development (Taya et al., 1981; Richards and Bogovich, 1982) in the final steps of follicular rupture (Iwamasa et al., 1992) are additional features that are probably involved in the effects of RU486 on ovulation in rats administered 10.0 mg of mifepristone.

The alterations in the biochemical parameters were also supported by histoarchitectural changes in the present study. It is reported that the histopathological study of PCOS-induced rats revealed the formation of cysts in the ovary (Kafali et al., 2004; Baravalle et al., 2006; Rezvanfar et al., 2012). The ovarian cortex shows the presence of atretic follicles and the formation of more than two cysts in the ovary (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 are not formed or the number of corpora lutea is diminished indicating anovulation, and the frequency of estrus cycle is almost nil (Brawer et al., 1986; Sasikala and Shamila, 2009; Rezvanfar et al., 2012). In PCOS, high serum androgen concentrations are responsible for anovulation by direct effect on the ovary. Androgen-induced follicular atresia, as seen in rats administered 10.0 mg of mifepristone, 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 cell death (De Leo et al., 1998; Walters et al. 2012b).

Women with PCOS often exhibit non-reproductive metabolic abnormalities such as obesity, metabolic syndrome, hyperinsulinemia, insulin resistance, dyslipidemia with an increased risk of cardiovascular disease and type 2 diabetes (Lobo and Carmina, 2000; Goodarzi et al., 2011; Pasquali et al., 2011). Insulin resistance refers to the insensitivity of tissues (such as skeletal muscle, liver, kidney, and adipose tissue) to insulin action, with the weaker glucose utilization of body after insulin action resulting in hyperglycemia (Saruc et al., 2003; Straczkowski et al., 2003). The elevated level of insulin in animals given 10.0 mg of mifepristone is in accordance with the report that women with PCOS have a higher prevalence and a greater degree of hyperinsulinemia (Dunaif et al., 1987; Conway et al., 1990) and insulin resistance (Dunaif et al., 1989; Rajkhowa et al., 1994; Morales et al., 1996). Also, the increase in the insulin concentration of animals administered 10.0 mg of mifepristone might be due to elevated level of triacylglycerides which can block the insulin-receptor binding sites thereby preventing insulin from binding accordingly. Also, increased triacylglyceride level and low HDL cholesterol in animals given 10.0 mg of mifepristone in this study also supports the report that at least one abnormal lipid level is seen in 70% of women with PCOS (Legro et al., 2001) and that the pattern of dyslipidemia found in the PCOS patient often features elevated triglycerides and low HDL-C (Holte et al., 1994). The dyslipidemia following the administration of 10.0 mg of mifepristone in the present study is in accordance with reported changes in lipid content of PCOS animals. This consistently emphasizes that the 10.0 mg of mifepristone could be adopted as a better model for inducing PCOS in female Wistar rats.

Conclusion

Overall, the available evidence in the present study suggests that all the doses (5.0, 7.5 and 10.0 mg) of mifepristone produced widely varying features of PCOS. The 10.0 mg-induced PCOS most as evidenced from menstrual disturbance (irregular estrus cycle); clinical and biochemical characteristics (increased testosterone levels and estradiol, decreased progesterone levels, unaltered prolactin and follicle stimulating hormone levels, low HDL cholesterol and high triacylglyceride contents) and the ovarian histological changes (appearance of degenerated follicles accompanied with ovarian cysts). This study, therefore, recommends the subcutaneous administration of 10 mg of mifepristone RU486 for inducing majority of the key features of PCOS that are similar to those in humans.

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