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Study of oestrus cycle periodicity and oogenesis of adult albino rats: Response to hyperprolactinaemia induced by haloperidol

Savita Kuldip Kumar[1*], Pal Abhishek[2], Sahu Pratap Kumar[2], Tiwari Prashant[3]

[1]Department of Pharmacy, Smt. Vidyawati Group of Colleges, Jhansi- 284121, India

[2]School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar- 751003, India

[3]School of Pharmacy, Chouksey Engineering College, Bilaspur- 495004, India

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ABSTRACT

Objective: To investigate the possible effect of hyperprolactinaemia induced by HPL on oogenesis in female albino rats. **Methods:** The oestrus cycle of each rat was observed daily for 16 days at a regular interval of 24 hours including Sunday. Animals from each group were scarified 24 hr after last treatment (on 17th day) following the ethical procedure for histopathological examination ovaries were separated. **Results:** In our study we found that prolactin treatment at the diocstrous phase increased the number of apoptotic cells and significant changes in volume of corpus luteum and the number of steriodogenic cell per corpus luteum decreased and therefore resultant synthesis of oestrogen decreased. So, haloperidol possesses anticestrogenic activity which may be attributed to high serum prolactin levels induced by haloperidol in experimental animals. **Conclusion:** In this study intraperitoneall administration of haloperidol at doses of 1, 2 and 5 mg/kg/day for 16 days significantly produced hyperprolactinaemia in female albino rats as compared to control. Hyperprolactinaemia produced by haloperidol causes significant increase in periodicity of diocstrous phase and decreased the other phase significantly in a dose dependent manner.

1. Introduction

Neuroleptic drugs have been used for treatment of schizophrenia since the 1950's [1]. Typical neuroleptic drug treatment produces numerous adverse effects (tardive dyskinesia, vacuous chewing movements, facial Jerking etc). So, current neuroleptic research has an important goal to develop neuroleptic drugs (atypical) having minimum side effects [2]. Dopaminergic stabilizers may be conceptualized as drugs with normalizing effects on dopamine-mediated behaviours and neurochemical events [3]. (HPL) blocks D2 receptors on lactotrophs and thus remove the inhibitory influence (by dopamine) on prolactin (PRL) secretion [1,4]. Since prolactin (PRL) (a hormone produced by the anterior pituitary) was first identificated, the existence of hyperprolactinemic syndrome has been recognized. Main symptoms are galactorrhea, oligomenorrhea, amenorrhea and infertility in women and decreased libido and impotence in men [5]. Atypical antipsychotic-induced hyperprolactinemia can cause important clinical symptoms, particularly in young women and also in men, such as impotence, loss of libido, gynecomastia, anovulation and galactorrhea [6]. Hyperprolactinaemia or hyperprolactinemia (HP) is the presence of abnormally-high levels of prolactin in the blood. Normal levels are less than 500 mIU/L for women, and less than 450 mIU/L for men. Prolactin is a peptide hormone produced by the anterior pituitary gland primarily associated with lactation and plays a vital role in breast development during pregnancy. Hyperprolactinaemia may cause production and spontaneous flow of breast milk and

*Corresponding author: Savita Kuldip Kumar, Department of Pharmacy, Smt. Vidyawati group of colleges, Jhansi- 284121, India.
Tel: +91-7828865022,
Fax: 07753-302101
E-mail: ptc_ptc15@rediffmail.com
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disruptions in the normal menstrual period in women and hypogonadism, infertility and erectile dysfunction in men[7].

The hormone prolactin is downregulated by dopamine and is upregulated by oestrogen. A falsely-high measurement may occur due to the presence of the biologically-inactive macroprolactin in the serum. This can show up as high prolactin in some types of tests, but is asymptomatic [8]. The symptoms of hyperprolactinemia in women mainly result from changes in the release of gonadotropins and the consequent repercussions on ovarian function in female mice, haloperidol-induced hyperprolactinemia causes changes in the reproductive system, mainly in ovarian function and the endometrium [9]. Antipsychotic drug-induced sexual dysfunction is a common and problematic side effect, which may diminish quality of life and lead to treatment noncompliance. Up to date, there is still a scarcity of basic research regarding the chronic effects of most antipsychotic agents on sexual behavior [10]. Antipsychotic medications are a potential cause of hyperprolactinaemia and may be implicated in the development of pituitary adenomas [11]. During early life, prolactin (PRL) ingested by the pups through the milk participates in the development of neuroendocrine, immunological and reproductive systems. The present study tested whether a deficiency in PRL in the dam's milk during early lactation affected the offspring in terms of the maternal responsiveness in the sensitization paradigm and behavioral response to a novel environment in the offspring [12].

The levels of these hormones, however is controlled by the hypothalamic releasing hormone (HRL) and pituitary gonadotrophins. A feed back mechanism also operates whereby the pituitary releases gonadotrophins which are in turn controlled by estrogen and progesterone [13]. During schizophrenia treatment, PRL concentration can rise to ten times of normal level or above and female patients have amenorrhoea with or without galactorrhoea. In female rats, HPL induces hyperprolactinaemia and irregular oestrous cycle stage [13,14]. A number of studies have shown that antipsychotic and other drugs treatment induce oestrus cycle changes but their effect on the periodicity of oestrus cycle and oogenesis at different dose is yet to be studied. The work to be reported here has therefore been initiated to evaluate the hormonal dependent effect of HPL on the duration and periodicity of various phases of the cycle and oogenesis in the adult albino rats. Two meta-analyses have reported that men have an overall 40% greater chance of developing schizophrenia than women do. These and other findings have led to the suggestion that ovarian hormones may be protective against schizophrenia [15]. Loss of D2R caused a marked increase in serum prolactin levels, to higher levels in females compared to male KO mice. On the other hand, it produced a female-specific increase in circulating α MSH, and hypothalamic α MSH content[16].

2. Materials and methods

2.1 Animals

Female albino rats (3-4 months of age weighing 100-150 g) bred in the animal house facility of School of Pharmaceutical Sciences; Siksha 'O' Anusandhan University Bhubaneswar was used. The animals were housed under standard laboratory conditions, maintained on natural 12:12 hr light-dark cycle and given standard laboratory food and water *ad libitum*. All the observations were carried out between 9:00 to 11:00 am. The experimental protocols were approved by the Institutional Animal ethical Committee (1171/c/08/CPCSEA) and conducted according to the guidelines of CPCSEA.

2.2. Selection of animals for experiment

The vaginal smear of rat was examined daily 9:00 to 11:00 am for 15-16 days to select animals of regular cycle of four days. For the preparation, reading and reporting of the vaginal smear OECD guide line was followed [13].

2.3. Drug and treatment

Haloperidol (Serenac Searle, India) was diluted with distilled water. Drug was administered intraperitonealy (i.p.) in a constant volume of 0.5 mL/100 g of body weight of rat.

2.4. Treatment protocol

Animals in proestrus phase were divided into 4 groups of 6 animals each. First group received vehicle (saline 2 mL/kg/day), second group received HPL (1 mg/kg/day), third and fourth groups received 2 & 5 mg/kg /day of HPL respectively. The drugs were administered once daily (11:30 am) for 16 days.

2.5. Oestrus cycle assessment

Throughout the experimentation, the oestrus cycle of each rat was checked daily for 16 days at a regular interval of 24 hours including Sunday. Various stages of the oestrus cycle viz. proestrus, oestrus, metoestrus and dioestrus were characterized with a light microscope.

2.6. Histopathological examination of ovary for oogenesis

Animals from each group were scarified 24 hr after last treatment (on 17th day) following the ethical procedure. For histopathological examination ovaries were separated and stored in 10% formalin solution. Ovarian sample embedded in paraffin wax were used for serially section at 5 μm and stained with Heamatoxylin Eosin (HE) and mounted on glass slide for microscopic evaluation.

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2.7. Counting of ovarian follicles

Serial sections (5 μm) were prepared from single ovary (left) for counting follicles. A modified method [17] for differential follicle counts was used. Follicles type include primordial & primary (Primordial follicles were defined as an oocyte surrounded by a layer of squamous (flatted) granulose cells, primary follicles possessed on oocyte surrounded by a single layer of cuboidal granular cells. Secondary follicles (surrounded by more than one layer of cuboidal granular cells with no visible antrum). Early antral follicles (having emerging antral spaces), follicles with clear antral space, largest follicles with Zona pellucidand, oocytes with multy layered zona pellucida (mature preovulatory follicles). Follicles with clear visible nucleoli were counted. Light microscopy was used for morphological characterization of follicles (Figure 1). Starting with the first serial section that contained the ovarian tissue, every fifth serial section was scored for differential follicle numbers.

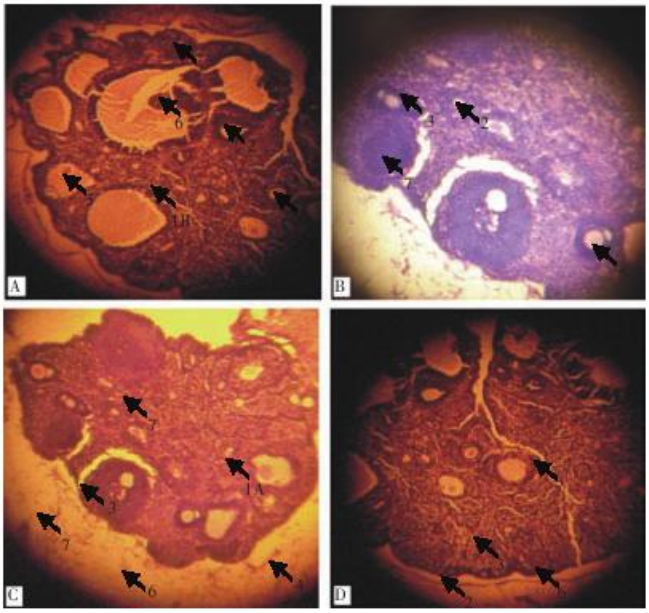

Figure 1. Histopathological examination of ovaries of control rates (A) and those treated with HPL (B,C,D, 1,2,5mg/kg/day).

Numbering as 1A– Primodial follicles, 1B–Primary follicles, 2–Secondary follicles, 3. Early antral follicles, 4. Follicles with clear antral space. 5. Large follicle with zona pellucida, 6. Oocyte with multilayered zona pellucida. 7. Corpus luteum .

2.8. Prolactin estimation

Blood sample for PRL assay were collected by cardiac puncture, plasma samples were separated by centrifugation, frozen and stored at −40 ℃ until used. Serum prolactin was estimated by Enzyme immune assay method [14]. Principle of assay was based on the competition between unlabelled rat prolactin and Acetycholinesterase (AChE) linked to rat prolactin (tracer) for limited specific rabbit anti-rat prolactin antiserum sites. The complex rabbit antiserum-rat prolactin (free prolactin or tracer) binds to the mouse monoclonal anti-rabbit antibody that is attached to the well. The plate was then washed and Ellman's reagent (enzymatic substrate for AChE and chromogen) was added to the wells. The AChE tracer acts on the Ellman's reagent to form a yellow compound. The intensity of the colour was determined by spectrophotometry and is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free rat prolactin present in the well during the immunological incubation. The observations were made at 410 nm wave length. Limit of quantification was 1 μg/mL. Data were recorded and analyzed with the help of curve fitting software.

2.9. Statistical analysis

All the values are expressed as mean ± SEM. The data were analyzed by using analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

3. Results

3.1. Prolactin estimation

Administration of Haloperidol significantly increased blood serum prolactin (PRL) as compared to control group animals. (Figure 2).

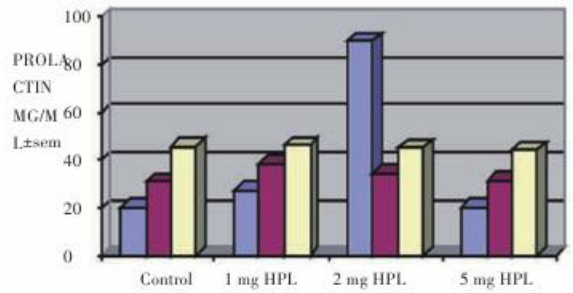

Figure 2. Effect of haloperidol (HPL) on blood serum prolactin level in albino rats.

Total number of animals in each group is 6 and data are expressed as mean ± SEM. ** $P<0.01$ as compared to control group.

3.2. Oestrus cycle assessment

Haloperidol (1, 2 & 5 mg/kg/day, i.p.) treatment resulted in significant increase in dioestrus duration and significant decrease in proestrus, oestrus and metoestrus duration (Table 1). It was revealed that the oestrus cycle of control animals was regular and normal. The various stages of the cycle viz. proestrus, oestrus, metoestrus and dioestrus revolve in (Figure 3,4). The percentage phase duration of dioestrus phase increased with increase in the dose of Haloperidol.

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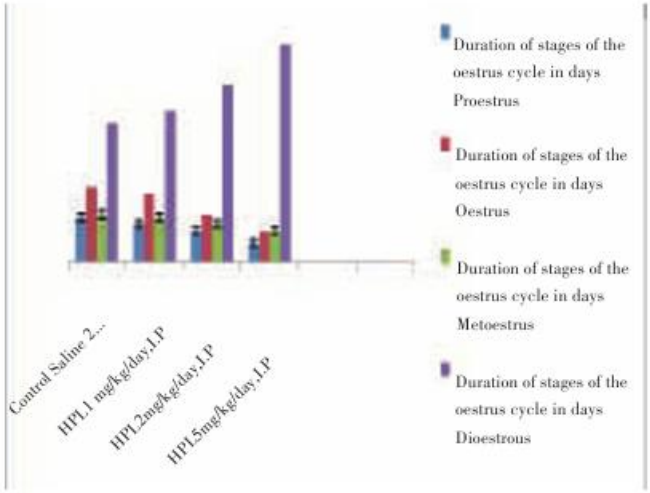

Figure 3. Effect of Haloperidol (HPL) on the duration of various stages of oestrus cycle in albino rats.

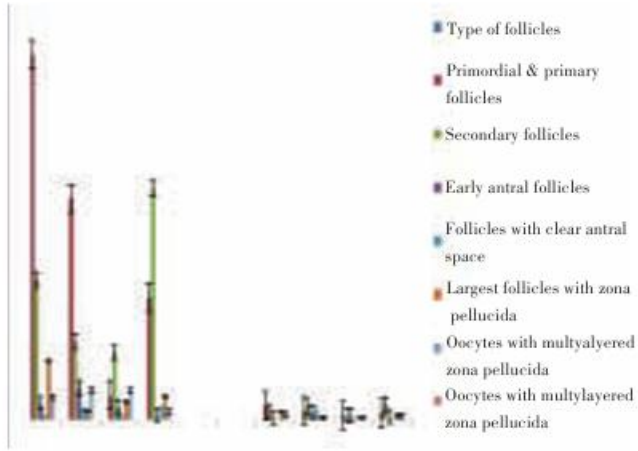

Figure 4. Effect of haloperidol (HPL) on the periodicity of oestrus cycle in albino rats.

P Proestrus, Eoestrus, M Metoestrus, D Dioestrus). Control group, (Receiving HPL 1 mg/kg/day), (Receiving HPL, 2 mg/kg/day) and (Receiving HPL, 5 mg/kg/day) for 16 days.

Table 1

Effect of Haloperidol (HPL) on the duration of various stages of oestrus cycle in albino rats.

Treatment and dose	Duration of stages of the oestrus cycle in days (Mean ± SEM)			
	Proestrus	Oestrus	Metoestrus	Dioestrous
Control Saline 2 ml/kg/day,i.p.	2.33 ± 0.22	4.00 ± 0.00	2.50 ± 0.00	7.33 ± 0.22
Haloperidol 1 mg/kg/day, i.p.	2.00 ± 0.00	3.66 ± 0.21	2.33 ± 0.22	8.00 ± 0.33
Haloperidol 2 mg/kg/day, i.p.	1.66 ± 0.22*	2.50 ± 0.22**	2.00 ± 0.22	9.33 ± 0.16**
Haloperidol5 mg/kg/day, i.p.	1.00 ± 0.00**	1.60 ± 0.22**	1.66 ± 0.00**	11.50 ± 0.44**

Observation period= 16 days,* $P<0.05$, ** $P<0.01$ as compared to control group.

Table 2

Effect of haloperidol (HPL) induced hyperprolectinaemia on ovarian follicle development (n=6 in each group).

Type of follicles	Control	1 mg / kg/day	2 mg / kg/day	5 mg / kg/day
Primordial & primary follicles	150 ± 5.79	90.83 ± 3.75**	10.16 ± 1.77**	50.16 ± 2.88**
Secondary follicles	57.50 ± 3.07	32.33 ± 5.83**	27.66 ± 1.76**	95.16 ± 6.18
Early antral follicles	6.50 ± 0.00	12.50 ± 2.67**	4.66 ± 1.20	1.33 ± 0.61
Follicles with clear antral space	2.50 ± 0.42	5.16 ± 4.38	2.33 ± 0.49	5.66 ± 0.80
Largest follicles with zona pellucida	24.16 ± 3.27	3.66 ± 0.76**	7.33 ± 0.73**	9.83 ± 2.04**
Oocytes with multylayered zona pellucida	9.16 ± 2.14	12.16 ± 0.72	12.00 ± 0.73	3.50 ± 1.17*

Data were expressed as Mean ± SEM, *$P<0.05$, **$P<0.01$, compared to control (Dunnett Comparison Test).

3.3. Oogenesis assessment

Histopathological examination of ovaries (Figure 1) were observed. At the dose 2 mg/kg/day of HPL showed maximum inhibitory effect on primordial and primary follicles (Table 2), one way ANOVA showed significant effect of HPL induced hyperprolactinaemia on secondary, early antral follicles and largest follicles with Zona pellucida ($P<0.01$) at the dose 1 mg/kg/day of HPL, mature oocytes with multilayered zona pellucida at the dose 5 mg/kg/day also effected significantly ($P<0.05$). Although follicles with clear antral space did not show significant effect at any dose level.

4. Discussion

Oestrus cycle is characterized as proestrus (epithelial cells – mostly rounded) oestrus (large cornified cell), metoestrus (large number of leucocytes with small number of non-nucleated epithelial cells) and dioestrus (mainly leucocytes) [18]. Observations associated with drug-induced hyper- or hypoprolactinemia in rat toxicology studies may be similar and include increased ovarian weight due to increased presence of corpora lutea. Hyperprolactinemia may be distinguished if mammary gland hyperplasia with secretion and/or vaginal mucification is observed. Reproductive toxicity study endpoints can differentiate hyper- from hypoprolactinemia based on their differential

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effects on estrous cycles, mating, and fertility. Although the manifestations of hyper- and hypoprolactinemia in rats generally differ from that in humans, mechanisms of drug-related changes in prolactin synthesis/release can be conserved across species and pathologically increased or decreased prolactin levels [19]. Drug-induced changes in prolactin signaling may obscure interpretation of preclinical toxicological endpoints. However, with informed consideration, classic hallmarks of hypo-/hyperprolactinemia can be recognized in short- and long-term rodent bioassays. Findings can be supported and expanded with additional in vivo and in vitro datasets [20].

Latent inhibition (LI) is a cross-species selective attention phenomenon manifested as poorer conditioning of stimuli that had been experienced as irrelevant prior to conditioning. Disruption of LI by pro-psychotic agents such as amphetamine and its restoration by antipsychotic drugs (APDs) is a well-established model of psychotic symptoms of schizophrenia. There is evidence that in schizophrenic women symptom severity and treatment response fluctuate along the menstrual cycle [21]. Hyperprolactinemia has short- and long-term consequences that can seriously affect quality of life: menstrual disturbances, galactorrhea, sexual dysfunction, gynecomastia, infertility, decreased bone mineral density, and breast cancer. Although many of these are definitively connected to elevated prolactin levels such as breast cancer [22].

The histological cyclic changes that occur in the vagina of normal animals are initiated and regulated by the hypothalamic ovarian pituitary (HPO) axis. During the oestrus cycle different phases appear due to changes in level of non-ovarian (prolactin, LH and FSH) or the ovarian (oestrogen and progesterone) hormone [23]. Histological changes in vaginal smear are influenced by changes in hormonal level in animal. It is observed by various workers that in normal animals, persistent dioestrus can be produced by testosterone, androsterone or progesterone, androstanedione [24-26], but there is no such study that hyperprolactinaemia produced by haloperidol (HPL) causes persistent dioestrus.

Prolactin is a polypeptide hormone secreted by the lactotroph cells of the anterior pituitary gland. The primary physiologic role of prolactin is the induction of lactation. Prolactin secretion is regulated via tonic secretion of dopamine in the tubero infundibular tract and the hypothalamohypophyseal vessels. Dopamine acts as a prolactin inhibiting factor on D2 receptors located on the surface of the pituitary lactotroph cells. Conventional (typical) antipsychotic agents like haloperidol inhibit dopamine action at D2 receptors and produce hyperprolactinaemia [27,28]. In present study hyperprolactinemia suppresses gonadotrophin releasing hormone (GnRH) pulsatile secretion from the hypothalamus and directly interferes with the pituitary action of the gonadotrophin luteinizing hormone (LH) and follicle stimulating hormone (FSH) on the gonads.

In our study we found that intraperitonial administration of haloperidol at doses of 1, 2 and 5 mg/kg/day for 16 days significantly produced hyperprolactinaemia in female albino rats. Hyperprolactinaemia produced by haloperidol (HPL) cause significant increase in dioestrus phase due to low level of oestrogen and decreased the other phases including estrous phase in a dose dependent manner. It is studied that prolactin treatment at the dioestrous phase induced a 13 fold increase the number of apoptotic cells and significant changes in volume of corpus luteum (38% decrease) and the number of steriodogenic cell per corpus luteum (70% decrease). Resultant synthesis of oestrogen decreased. So, haloperidol possesses antioestrogenic activity which may be attributed to high serum prolactin levels induced by haloperidol in experimental animals. The gonadotraphins, lutenising hormone (LH) and follicle stimulating hormone (FSH) are essential for the completion of follicular maturation and development of mature preovulatory grafian follicles. Higher concentration of PRL block the rise in FSH-R binding and progesterone production in cultured granulose cells [29]. This indicates that in ovaries decreased primordial, primary, secondary follicles, follicles with clear antral space and mature largest follicles with zona pellucida due to hyperprolactinemia confirmed by histopathological examination.

Conflict of interest statement

We declare that we have no conflict of interest

Acknowledgments

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