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Neuroleptic drug-induced hyperprolactinemia and associated neurochemical, hematological and histological changes in rats

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ABSTRACT

The study aims to validate the dose of haloperidol (HPL) and sulpiride (SPD) needed to induce hyperprolactinemia in both male and female albino rats and to evaluate the neurochemical, hematological and histological changes in the anterior pituitary gland, adrenal gland, and spleen. HPL (1, 2 and 5 mg/kg/day) and SPD (20 and 40 mg/kg/day) significantly ($p<0.05$) increased the serum prolactin (PRL) level. They showed hypertrophic reversible changes in the cells of the anterior pituitary gland. Unlike SPD, HPL showed dose-dependent hyperprolactinemia. So the highest dose of HPL and a lower dose of SPD were used for further study. HPL 5 mg/kg/day for 16 days and SPD 20 mg/kg/day for 28 days significantly decreased dopamine concentration in brain homogenate. They also cause an increase in total leukocyte count (TLC) and a decrease in red blood cell (RBC) count and hemoglobin (Hb) concentration. In addition, Spleen shows signs of infection or inflammation. HPL (5 mg/kg/day) for 16 days and SPD (20 mg/kg/day) for 28 days may be used as experimental models to induce hyperprolactinemia in both male and female rats. The decrease in dopamine level, changes in hematological parameters and spleen inflammation can be used as the markers of hyperprolactinemia.

Keywords: Haloperidol, sulpiride, hyperprolactinemia, dopamine, animal model.

1. INTRODUCTION

Prolactin hormone stimulates milk production. Post-parturition, prolactin produces an instant effect on breast directly. Prolactin-releasing hormone (PRH), which enhances the concentration of prolactin in the blood is secreted. On the contrary, prolactin-concentration is brought down by Prolactin Inhibiting Hormone (PIH, Dopamine) wherein an elevated level of prolactin also inhibits the release of prolactin. Prolactin secretion is directly related to sleep, i.e. its level rises during sleep, irrespective of timing i.e. night or day.

Emotional stress also increases the production of prolactin (Waugh & Grant, 2014). The decrease in the release of prolactin is provided by several neurotransmitters and more than 20 hormones, but yet dopamine released from the hypothalamus plays a significant role. Prolactin production is inhibited by the dopaminergic agonist. On the other hand, dopaminergic antagonists such as the antipsychotic drugs stimulate the production of prolactin (Craig & Stitzel, 2006). Additionally, neuropeptides (small protein-like molecule peptides, used by neurons to communicate with each other) like oxytocin, thyrotropin-releasing hormone (TRH), vasoactive intestinal polypeptide (VIP), peptide histidine methionine (PHM) promote prolactin (PRL) secretion (Bali et al. 2014). There are a number of studies on antipsychotic drugs that have a great impact on human endocrine function in day to day practice. There are various drugs like phenothiazines, haloperidol, risperidone, sulpiride, levosulpiride, amisulpride, paliperidone, molindone, zotepine, and metoclopramide etc. which are accountable for the elevation of PRL that reduce hypothalamic dopamine secretion and pituitary activation and result in hyperprolactinemia (Voicu et al. 2013; Laszczynska et al. 2001; Canuso et al. 2002; Lee & Kim, 2006; Fell et al. 2004). The effects of hyperprolactinemia include the development of impotence in men. In the case of a female, it results in the development of amenorrhea and infertility. Furthermore, an enhanced level of circulating prolactin leads to a decrease in the production of testosterone and 17- estradiol in testes and ovaries respectively (Craig & Stitzel, 2006). The consequence of hyperprolactinemia is galactorrhea, amenorrhea, impotence and azoospermia and gynecomastia (Marken et al. 1992). Dopamine stimulates lactotroph cells of the anterior pituitary gland and incites a tonic suppression on prolactin secretion (Halbreich et al. 2003; Crosignani, 2006). Dopamine antagonists (antidopaminergic drugs) like haloperidol, sulpiride, amisulpride etc. block dopamine (D_2) receptors. Prolonged injection of HPL and SPD significantly increase striatal D_2 receptor binding (Fox et al, 1994). Hyperprolactinemia is reported in schizophrenic patients taking antipsychotic drugs. However, few animal models are available for such studies. Therefore, an attempt has been made to validate the experimental model of HPL and SPD induced hyperprolactinemia in albino rats. Neurochemical, hematology analysis and histopathological changes were observed to support the validation of the animal model.

2. MATERIALS AND METHODS

Animals for the experiment

The study was performed after the approval of study protocol by the institutional animal ethics committee of SOA University (Regd. No. - 1171/C/08/CPCSEA). For the experiment, both male and female Wistar albino rats of similar weight 120±5 g were randomly selected and were well-nourished in the animal house of School of Pharmaceutical Sciences, "Siksha O Anusandhan" (Deemed to be University), Bhubaneswar. As per the norms of CPCSEA, all the required necessary conditions were maintained, the room temperature was kept at 22±2°C, and the animals were fed with adequate food. On the day of experimentation, the animals were subjected to laboratory conditions one hour prior to performing the experiment. Daytime hours (08:00 to 16:00 hrs) were preferred for the experiment.

Drug and treatment

The study protocol involves intraperitoneal (i.p.) administration of HPL (Himedia Laboratories Pvt. Ltd.) at dose of 1 mg/10ml/kg/day, 2 mg/10ml/kg/day and 5 mg/10ml/kg/day daily for a period of 16 days and SPD (Unimed Technologies Ltd. Panchmahal, India) at dose of 20 mg/10ml/kg/day and 40 mg/10ml/kg/day daily for a period of 28 days. The drugs were diluted with distilled water prior to their administration.

Haloperidol-induced hyperprolactinemia

Animals were divided into 8 groups with 6 animals per group. The first group contains the male rat while the second group contains female rats. These two groups were administered vehicle (saline 2 ml/kg/day). The third group (male) and the fourth group (female) were administered with HPL (1 mg/kg/day). Similarly, the fifth group (male) and sixth group (female) were given HPL (2 mg/kg/day). The seventh group (male) and eighth group (female) were administered HPL (5 mg/kg/day). Intraperitoneal administration of HPL was done once daily (8 to 10 am) for a period of 16 days (Tiwari et al. 2019b; Kumar et al. 2013). On the 17th day, blood was collected by cardiac puncture for assessment of prolactin level (PRL), and the animals were sacrificed to isolate anterior pituitary for histopathology studies.

Sulpiride-induced hyperprolactinemia

The animals taken for the experiment were divided into 6 groups with 6 animals per group. The first group (male) and the second group (female) were administered with vehicle (saline 2 ml/kg/day). However, the third group (male) and the fourth group (female)

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were administered with SPD (20 mg/kg/day). Similarly, the fifth group (male) and sixth group (female) were administered with SPD (40 mg/kg/day). The drugs were intraperitoneally administered once daily (8 to 10 am) for 28 continual days (Tiwari et al. 2019a; Mostafapour et al. 2014). On the 29thday, blood was collected by cardiac puncture for evaluation of prolactin (PRL) level, and the animals were sacrificed to isolate anterior pituitary for histopathology studies.

Dose selection

One dose for each HPL and SPD were selected for estimation of prolactin and DA, and also for hematological and histopathological studies. The animals were dosed for specified days after which the blood was collected by cardiac puncture and animals were sacrificed for collection of brain, adrenal gland, and spleen to study the histopathology.

Prolactin estimation

Blood serum samples were separated by centrifugation, frozen and stored at -4^{0}c until use. The concentration of PRL hormone was determined by well-established enzyme immune assay method using rat prolactin kit (Kumar et al. 2013; Pansini et al. 1985). All measurements were done by enzyme-linked immunosorbent assay (ELISA) kits as per the procedures recommended by the manufacturer (Erba Lachema, Czech Republic, www.erbalachema.com).

Dopamine Estimation

The entire brain of a rat was collected and weighed. Homogenization of brain tissues was performed for about one minute in HCl-butanol (1:10) mixture. These homogenized samples were then subjected to centrifugation at 3000 rpm for about 10minutes. One ml of aliquot supernatant phase was removed followed by its addition to centrifuge tube comprising 2.5ml hexane and 0.3ml of 0.1M HCl. The dopamine assay was then performed by taking 0.2 ml of the aqueous phase. The temperature was maintained at 0^{0}C. Then, 0.05 ml 0.4 M HCl, and 0.1 ml of sodium acetate buffer of PH 6.9 were added to 0.5 ml of an aqueous phase. This was followed by addition of 0.1 ml iodine solution in 0.1M ethanol for oxidation. This reaction was then interrupted for 2 minutes by addition of 0.1ml of sodium sulfate solution. After 1.5 minutes, 0.1ml of acetic acid was added. This solution was then subjected to heating for a duration of 6minutes. Then the sample was cooled. When the temperature of the sample was dropped down to room temperature, excitation and emission spectra was performed with spectrofluorimeter at 330-375 nm (Manikkoth et al. 2016).

Hematological evaluation

Hematology studies were performed in drug-induced hyperprolactinemic rats (HPL 5 mg/kg/day and SPD 20 mg/kg/day) by estimating the red blood cell count (RBC), Total leukocyte count (TLC), hemoglobin count (Hb), packed cell volume (PCV), platelet count, mean corpuscular volume (MCV) etc. Measuring the weight of spleen in treated groups as well as the control group was also done (Mishra & Mohanty, 2010).

Histopathological examination of the anterior pituitary gland, adrenal gland, and spleen

Animals from each group were sacrificed after 24 hr of last treatment (on 17th and 29th days) by following ethical standards. Anterior pituitary gland, adrenal gland, and spleen were separated and stored in 10% formalin solution for histopathological examination. Anterior pituitary gland, adrenal gland and spleen sample embedded in paraffin wax were used for a serial section at 5 µm and stained with hematoxylin-eosin (HE) and then mounted on a glass slide for microscopic evaluation (Duhan et al. 1991).

Statistical analysis

The data obtained by the various parameters in the study were presented as mean ± sem. One-way analysis of variance (ANOVA) followed by Tukey's-test was applied for statistical analysis.

3. RESULTS

Prolactin estimation

The control group normal serum prolactin (PRL) level was 10.95±0.45ng/mL in male rats and 9.59±0.68ng/mL in female rats. After prolonged administration of HPL (1 mg/kg/day) for 16 days treated group serum PRL level significantly ($p<0.05$) increased to 19.94±1.83 ng/mL in male rat and 18.91±0.53 ng/mL in female rats. Administration of HPL (2 mg/kg/day) significantly ($p<0.05$) increased the serum PRL level to 20.92±0.95 ng/mL in male rats and 19.2±0.53ng/mL in female rats. Similarly, administration of HPL (5 mg/kg/day) significantly ($p<0.05$) raised the serum PRL level to 25.10±0.35 ng/mL in male rats and 22.05±0.35 ng/mL in female

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rats (Fig. 1). Whereas, administration of SPD (20 mg/kg/day) significantly ($p<0.05$) raised the serum PRL level to 17.16.10±1.04 ng/mL in male rats and 19.86±0.37 ng/mL in female rats. Likewise, administration of SPD (40 mg/kg/day) significantly ($p<0.05$) raised the serum PRL level to 17.17±0.62 ng/mL in male rat and 18.18±2.91 ng/mL in female rats.

Dopamine estimation

The dopamine (DA) level in control group was 38.24±0.04 units/g in the rats. Experimental data suggested that HPL (5 mg/kg/day for 16 days) showed a significant decrease in dopamine (DA) level to 11.52±0.007 units/g and SPD (20 mg/kg/day for 28 days) exhibited a significant decrease in DA level to 14.146±0.03 units/g (Fig. 2).

Hematological evaluation

Study revealed that HPL (5 mg/kg/day) for 16 continuous days and SPD (20 mg/kg/day) for 28 continuous days significantly decrease Hb count and RBC count and a significant increase in PCV, TLC count, platelet count, MCV, and spleen weight as compared to the control group (Table 1).

Histology of Control, HPL and SPD treated groups

Anterior pituitary gland

Histological examination of anterior pituitary gland showed dilated capillary, neuronal cell damage, irregular acidophil, and minor basophil cells in both HPL and SPD treated groups (Fig. 3).

Adrenal gland

Adrenal gland of HPL (5 mg/kg/day) and SPD (20 mg/kg/day) treated rats showed normal cytoarchitecture with well-developed cortex and medulla (Fig. 4).

Spleen

No irregularities were observed in control. Traces of loosely packed red pulp (RP) and white pulp (WP) was also observed. The white pulp consisted of aggregates of lymphocytes with eccentrically placed blood vessels (BV). There is a presence of collapsed interlobular matrix. The higher number of collapsed melano-macrophages indicated infection/inflammation due to the HPL and SPD. Infected spleen showed a muddy appearance with loose connective tissue (Fig. 5).

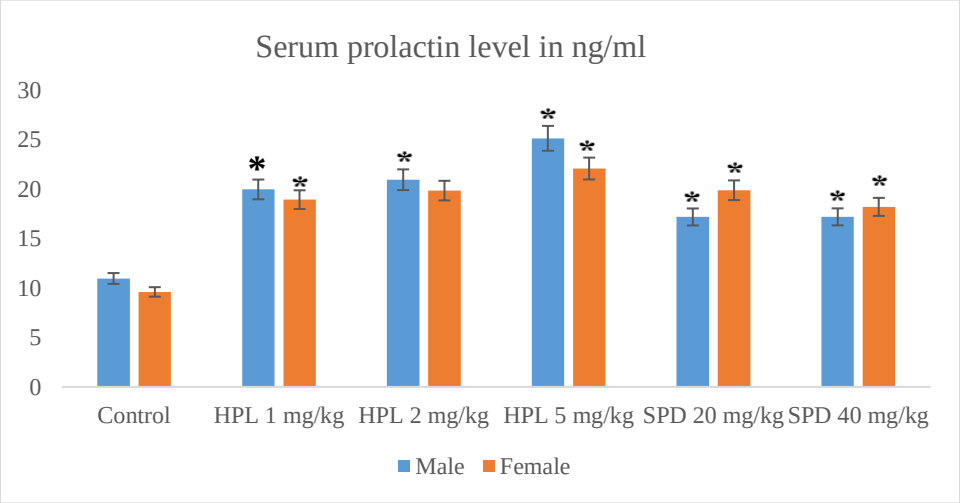

Fig. 1 - Effect of HPL and SPD on blood serum prolactin level in albino rats. Data are expressed as mean ± SEM n = 6. $p<0.01$ as compared to the control group (Tukey's-test).

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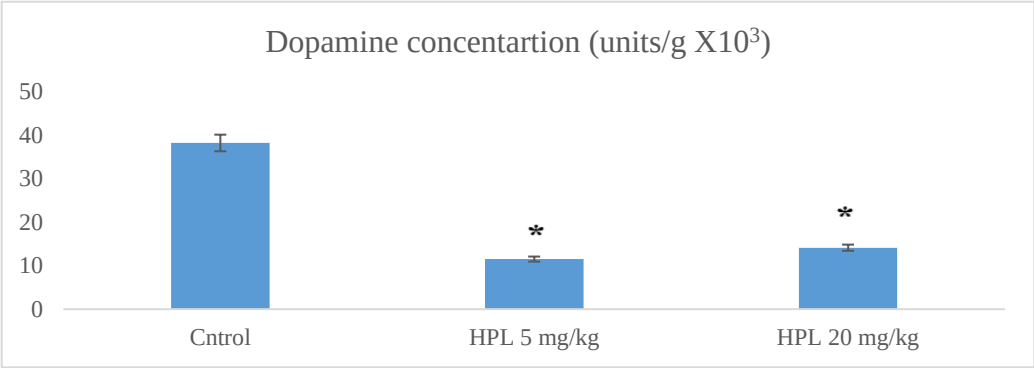

Fig. 2 - Effect of HPL and SPD on brain neurotransmitter levels (dopamine) in albino rats. Data are expressed as mean ± SEM. n = 6. $p<0.01$ as compared to the control group (Tukey's-test).

Table 1. Effects of haloperidol (HPL) and sulpiride (SPD) exposure on hematological parameters of the albino rat. (n=6).

Treatment (dose mg/kg/day)	Red Blood Cell Count x10³cell/µL	Total leukocyte Count x10³cell/µL	Hemoglobin(g Hb/DL)	PCV	Platelet	MCV	Spleen weight in grams
Control (normal saline)	7.32±0.00	3.71±0.00	14.38±0.00	43.42±0.00	1.40±0.00	59.37±0.00	0.77±0.00
HPL 5 mg/kg	6.17±0.00 *	6.94±0.00*	12.60±0.00*	44.84±0.00*	2.11±0.00*	61.77±0.00*	0.88±0.00*
SPD 20 mg/kg	6.22±0.00 *	6.83±0.00*	12.67±0.00*	44.70±0.00*	2.05±0.00*	61.61±0.00*	0.87±0.00*

All the values are mean ± SEM of six individual observations (one way ANOVA followed by Tukey's-test). * Indicates significance from control at $p<0.05$ level.

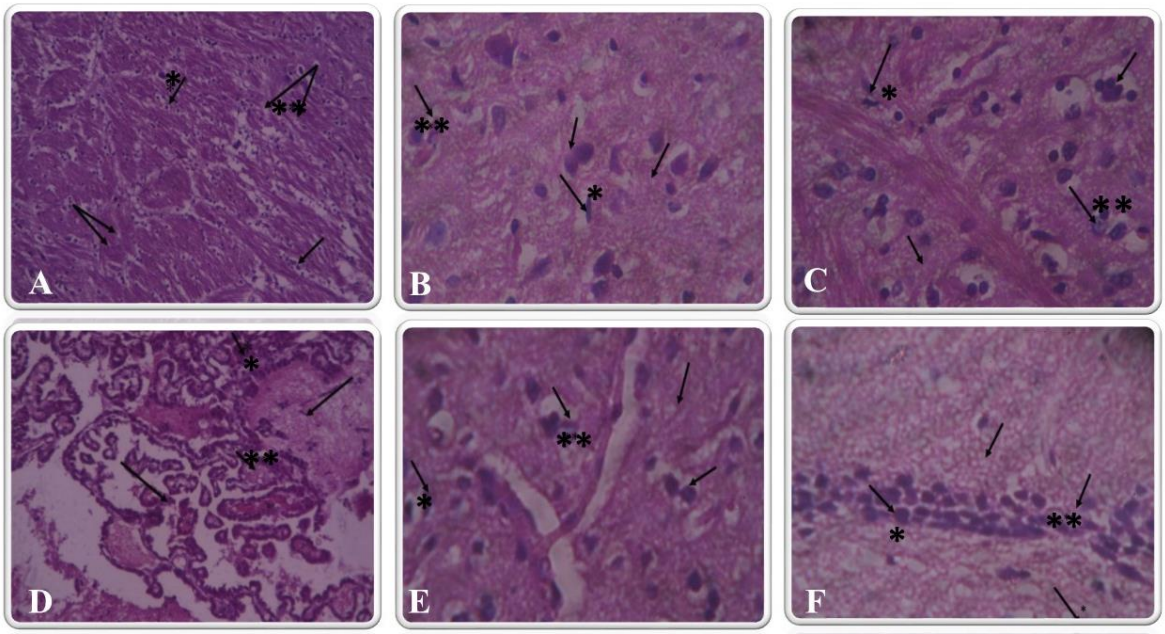

Fig. 3 - A represents a control group showing normal capillary (single arrow) neuronal fibers (double arrow) regular acidophil cell (single star) and basophils cell (double star). **B** (HPL 1 mg/kg/day)**, C** (HPL 2 mg/kg/day), **D** (HPL 5 mg/kg/day), **E (**SPD 20 mg/kg/day) and **F (**SPD 40 mg/kg/day) treated groups showing dilated capillary, injured neuronal fibers, irregular acidophil cell (single star) and minor basophils cells (double star). (HE, 100x).

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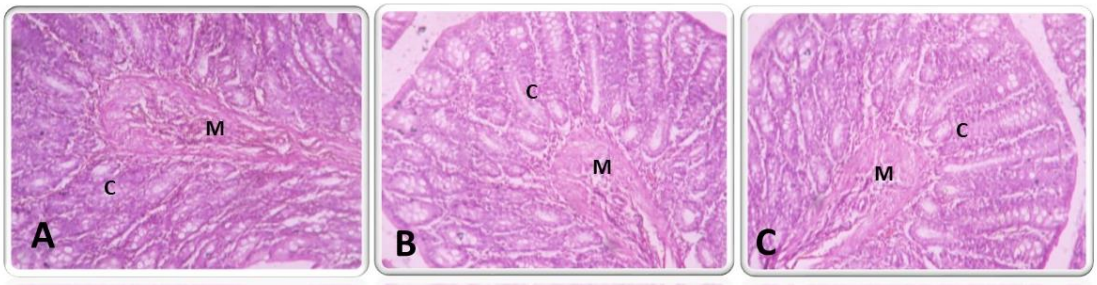

Fig. 4 - A Control group). **B** HPL (5 mg/kg/day) for 16 days and **C** SPD (20 mg/kg/day) for 28 days. All showing normal cytoarchitecture. M-Medulla and C-Cortex. (HE, 100x).

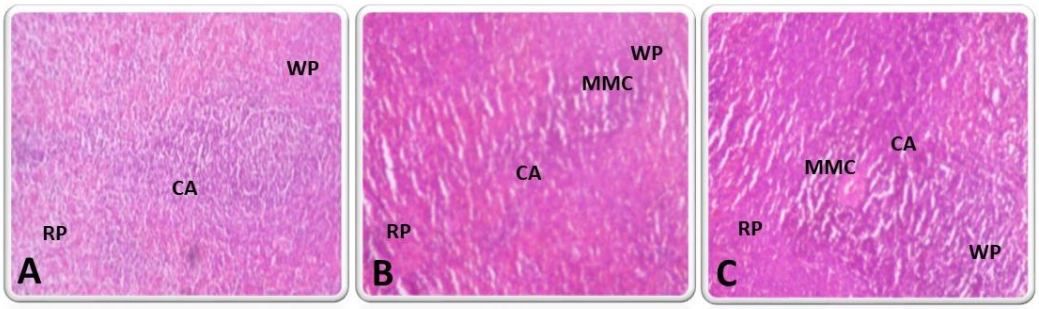

Fig. 5 - A Control group signifies normal cytoarchitecture. **B** HPL (5 mg/kg/day) for 16 days and **C** SPD (20 mg/kg/day) for 28 days showing collapsed interlobular matrix (HE, 100x).
RP-Red pulp, WP-White pulp, CA-Central artery and MMC-Melano macrophage center.

4. DISCUSSION

Antipsychotics, which can also be referred to as neuroleptics, are the class of compounds which possess a strong affinity towards various sub-class of dopamine receptors. They have the ability to bind with the dopamine receptors and block dopamine receptors, but that, in turn, leads to enhancement of the level of prolactin (hyperprolactinemia) which is a major disadvantage of neuroleptic drugs (La Torre & Falorni, 2007).

Several drugs apart from neuroleptic drugs are like monoamine oxidase inhibitors (MOAI), selective serotonin reuptake inhibitors (SSRI), and some tricyclic antidepressants also have the potential in causing hyperprolactinemia. Elevation in prolactin levels along with prokinetic effect is brought about by various other compounds such as H2-receptor antagonists, antihypertensive drugs, cholinomimetics, estrogens, anti-androgens, opiates, and anticonvulsants. Nevertheless, post autologous blood stem-cell transplantation, conditioning, and chemotherapy seem to present the development of hyperprolactinemia. Although the changes in the prolactin level are observed less frequently than seen in case of critical damage to the hypothalamus-pituitary-gonad/thyroid axis (Li et al. 2016; Molitch, 2005).

It is also witnessed that administration of HPL results in maximum elevation of prolactin level. SPD showed a marked increase in serum prolactin level. Amongst the atypical antipsychotics, Risperidone works as an effective elevator of prolactin. Olanzapine and quetiapine have a very little effect on the development of hyperprolactinemia. Clozapine and aripiprazole, on the other hand, have a negligible impact towards rising the prolactin level (Haddad & Wieck, 2004; Hannigan et al. 1997). Neuroleptics enhance the release of prolactin in human beings and animals. This prolactin enhancement tendency can be closely associated with a clinical neuroleptic potency which in turn can be related to the strength of dopamine receptor blocker (Clemens et al. 1974; Crooke, 1938).

We have earlier reported that intraperitoneal administration of haloperidol for 16 days at doses level of 1, 2 and 5 mg/kg/day significantly produced hyperprolactinemia in female albino rats (Kumar et al. 2013). Mostafapour et al found that on the administration of sulpiride at a dose of 20 mg/kg/day intraperitoneally for a period of 28 days in rats showed a remarkable rise in serum PRL level (Mostafapour et al. 2014). Van Coppenolle et al, administred sulpiride daily intraperitoneally at a dose of 40 mg/kg body weight in male wistar rats for 30 and 60 days and they found a significant rise in plasma PRL levels in all groups of animals (Van Coppenolle et al. 2001). So there is a need to validate one dose for HPL and SPD to induce hyperprolactinemia both in male and female rats.

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The current study of hyperprolactinemia screening method was validated by prolonged administration of HPL 1, 2 and 5 mg/kg/day continuously for 16 days and SPD 20 and 40 mg/kg/day continuously for 28 days. We also evaluated the neurochemical, hematological and histopathological changes associated with hyperprolactinemia in albino rats. Hyperprolactinemia is mostly seen in women but also observed in men and even in adolescence and childhood. So, we have selected both male albino rats and female albino rats to confirm the serum prolactin level in control and treated group. The results clearly indicated that HPL (1, 2 and 5 mg/kg/day for 16 days) and SPD (20 and 40 mg/kg/day for 28 days) induced hyperprolactinemia and significantly increased serum PRL level in treated groups as compared to control group. However, HPL showed a dose-dependent effect. Hence, we opted for HPL 5 mg/kg/day for further study. Whereas, administration of SPD (20 and 40 mg/kg/day) did not show any dose-dependent increase in serum prolactin level. So, we selected SPD 20 mg/kg for further study. Both HPL and SPD (irrespective of doses) showed reversible hypertrophic (Lucca et al. 2014; Paparrigopoulos et al. 2007) changes in the anterior pituitary gland. This might have contributed to the hyperprolactinemia. HPL (5 mg/kg/day) for 16 days and SPD (20 mg/kg/day) for 28 days were administered to albino rats for dopamine estimation, hematological analysis and histopathological examination of adrenal gland and spleen.

On administering a high dose of neuroleptic drugs for a longer duration of time, structural changes in the brain is brought about. Such changes result in the development of neuroinflammation. Hence, they are regarded as inflammation markers in the brain and peripheral region (Mondelli & Howes, 2014; Al-Amin et al., 2013). Histological examination of anterior pituitary gland demonstrated dilated capillary, neuronal cell damage, irregular acidophil, and minor basophil cells (figure-3). These changes are associated with hyperprolactinemia (Mahmood, 2014). In addition, there is also a significant increase in PRL level due to prolonged administration of HPL (1, 2 and 5 mg/kg) for 16 continuous days and SPD (20 and 40 mg/kg/day) for 28 continuous days (figure 1). So HPL and SPD can be used as an experimental model to induce hyperprolactinemia.

The previous study revealed that administration of HPL increases the levels of serum prolactin (PRL), follicle-stimulating hormone (FSH), testosterone, adrenocorticotropic hormone (ACTH), gonadotropins (Mueller et al. 1976; Brambilla et al. 1975). It is also witnessed that HPL increased the thyroid production of T4 plasma concentration, but did not have an influence on T3 plasma concentration in the wistar rats (Samadi et al. 2016). Likewise, administration of SPD also increase levels of serum prolactin (PRL), dehydroepiandrosterone (DHEA) androsterone, progesterone, testosterone, and etiocholanolone and decrease the levels of 17-beta-estradiol (E2), progesterone P4, FSH[14,] (Mostafapour et al. 2014; Baptista et al. 1997; Ruiz et al. 1984; Ahmadi et al. 2013; Mancini et al. 1975). Our study with respect to prolactin is in agreement with earlier studies.

HPL (5 mg/kg/day for 16 days) and SPD (20 mg/kg/day for 28 days) induced hyperprolactinemia and reduced dopamine level in brain significantly. In the management of PRL secretion, dopamine (DA) plays a significant role (Fitzgerald & Dinan, 2008; Ben-Jonathan & Hnasko, 2001). Obtained results confirmed the inverse relationship between dopamine and prolactin. The histological study shows well- developed medulla in control as well as treated groups (figure-4). So, HPL and SPD may not have any significant effect on the synthesis of dopamine, adrenaline, and noradrenaline. The decrease in dopamine level in our study may be attributed to some other cause (Foucart et al. 1987; Mitchell, 1994).

The mechanism of action of neuroleptic drugs is blockage of dopamine receptors in the nervous system. Neuroleptic drugs are found to inhibit the dopamine-sensitive adenylate cyclase pathway, enhance firing rate of dopamine neurons, act on dopaminergic neurons and cause a decrease in the production as well as the release of dopamine, and affect the overall level of dopamine. In agreement with this, induction of depolarization block (or depolarization inactivation) in DA neurons by the neuroleptic drugs followed by a decrease in the release of DA may be a possible mechanism underlying these effects (Seeman et al. 1975).

Many antipsychotic drugs may adversely affect the hematopoietic system (Lubran, 1989; Mishra et al. 2010). Drug-induced hematological abnormalities may be due to the toxicity of the drug, immunologic mechanism, and inborn errors of metabolism etc., (Stubner et al. 2004). Neuroleptic drugs associated with hematological irregularities and linked pathology has presently developed into a subject of apprehension (Mishra et al. 2010). So, a preclinical attempt has been made to confirm the hematological changes (RBC, TLC, Hb, PCV, MCV, platelet etc.) in normal and treated groups. There was a significant increase in TLC and decrease in RBC and Hb count which may be attributed to spleen damage or infection or inflammation (Podolec et al. 1979).

The spleen is a lymph node-like organ possessing hematopoiesis function. Control group showed no irregularities. It also showed loosely packed red and white pulp (RP & WP). The white pulp consists of aggregates of lymphocytes with an eccentrically placed blood vessel (BV). The red pulp consists of sinusoid spaces (SS), lymphocytes (LYM) and red blood cells (figure-5). The white pulps are with a darker staining mantle zone and a lighter staining germinal zone, collapsed interlobular matrix, a higher number of collapsed melano-macrophages indicating infection due to HPL and SPD induced hyperprolactinemia (Elalfy et al. 2017). So, the spleen was degraded and damaged in HPL and SPD treated groups as indicated by muddy appearance. The hematological changes are also in agreement with this.

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HPL and SPD induced hyperprolactinemia are associated with a decrease in DA concentration in the brain, changes in hematological parameters (RBC, TLC, Hb, MCV etc.), and spleen infection. There is a need to overcome these adverse effects in schizophrenic patients. This can be possible only if we have suitable pharmacological screening models to induce hyperprolactinemia which can be successfully used to screen potential drugs against neuroleptic drug-induced hyperprolactinemia and associated changes. Thus, the HPL and SPD induced hyperprolactinemia animal model can fill this gap. The decrease in dopamine level, changes in hematological parameters (increase in TLC and decrease in RBC and Hb) and spleen inflammation are the markers of hyperprolactinemia.

5. CONCLUSION

HPL (5 mg/kg/day) for 16 days and SPD (20 mg/kg/day) for 28 days may be used as experimental models to induce hyperprolactinemia in both male and female rats. The Decrease in dopamine level, changes in hematological parameters (increase in TLC and decrease in RBC and Hb) and spleen inflammation can be used as the markers of hyperprolactinemia.

Conflict of interest

We declare that we have no conflict of interest.

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Ethical approval

The Animal ethical guidelines are followed in the study for experimentation. The study was performed after the approval of study protocol by the institutional animal ethics committee of SOA University (Regd. No. - 1171/C/08/CPCSEA).

Data and materials availability:

All data associated with this study are present in the paper.

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