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Research Article

A seed extract of *Mucuna pruriens* reduced male reproductive endocrine disruptions in rats induced by chlorpromazine

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Abstract

Current research aims to assess the therapeutic impact of *Mucuna pruriens* seed extract on *PROTAMIN* (*PRM*) *I* and *II* gene expression and hormones in chlorpromazine-induced endocrine disruptions and reproductive toxicity in male rats. Thirty male Wistar rats were categorized into five groups: the negative control group, rats that received distilled water for 52 days; the induction group, rats that received (20 mg/kg) of chlorpromazine for 52 days; and three treatment groups that were pretreated with chlorpromazine (similar to the induction group) that received a low, medium, and high dose of *Mucuna pruriens* (500, 1000, and 2000 mg/kg, respectively). Serum samples were collected to measure testosterone, FSH (follicular stimulating hormone), LH (luteinizing hormone), and prolactin serum levels using the ELISA technique. Tissue samples were collected to measure *PRM I* and II gene expression and for histopathological study. The *PRM I* and II genes were significantly downregulated in the chlorpromazine-treated group. These genes were also significantly upregulated in *Mucuna pruriens*-treated groups. *The Mucuna pruriens*-treated groups revealed a significant rise in serum LH, testosterone, and FSH concentrations, decreased serum prolactin, and improved histology of testicular damage compared to the induction group. In conclusion, the endocrine disruption and hormonal changes induced by chlorpromazine improved when *Mucuna pruriens* was administered, improving the impairment in gene expression and hormones.

Keywords

chlorpromazine, *Mucuna pruriens*, *PRM I*, *PRM II*, endocrine disruption, reproductive toxicity

Introduction

Endocrine-disrupting chemicals (EDC) are exogenous substances that possess the potential to disrupt hormonal balance and normal endocrine system functioning, which may have a negative impact on both the reproduction and health of humans and animals (Ghosh et al. 2022); EDCs could target hormonal systems, sex differentiation, and reproductive development (Casals-Casas and Desvergne 2011); and EDCs interfere with the synthesis and release of hormones in the body (Gupta et al. 2010). Furthermore, EDC can stimulate or antagonize natural hormones, disrupting the body's normal functions (Christiansen et al. 2012).

EDCs include synthetic antipsychotic drugs, which are used for the management of psychosis (Shahnazaryan et al. 2019). Psychiatric patients who chronically use these drugs have been known to experience high-risk reproductive problems (Kaar et al. 2020; Oyovwi et al. 2021). Chlorpromazine is a first-generation antipsychotic drug; psychotic patients under chlorpromazine treatment develop infertility

problems (Croubels and Daeseleire 2012) because it interacts with dopaminergic receptors in the anterior pituitary gland, leading to notable psycho-neuroendocrine alterations; these changes include disruption of reproductive hormones, abnormal protamine expression, and modified spermatogenesis (Soliman et al. 2014; Ilgin 2020).

Protamines (PRM) are the most common nucleoproteins in mature sperm that replace histones during spermiogenesis, provide DNA packaging in sperm, and chromatin condensation of sperm, which protects the genetic integrity of the paternal genome as it passes through the male and female reproductive tracts (Moritz and Hammoud 2022). PRM is one of the molecular markers of fertility; the correlation between abnormal protamine levels and infertility is interesting because abnormal protamine expression has negative effects on sperm, such as low sperm counts, decreased sperm motility and morphology, lower fertilization ability, and increased sperm chromatin damage (Pardede et al. 2020).

Mucuna pruriens, a member of the Fabaceae family, is widespread worldwide in tropical and subtropical regions (Lampariello et al. 2012). It is commonly used nutritionally and therapeutically, especially in Ayurveda, the ancient Indian medical system. *Mucuna pruriens* may be found in food or in supplement form. It contains many nutrients and bioactive compounds that benefit human health (Pathania et al. 2020). The seeds of *Mucuna pruriens* are rich in levodopa as well as protein, lipids, dietary fiber, carbohydrates, minerals, flavonoids, phenolics, tannins, and phytic acid. Additionally, these seeds are sources of niacin and ascorbic acid (Oyinloye et al. 2023). *Mucuna pruriens* is a potent medicinal herb traditionally utilized in tropical regions, especially in India and China, for the alternative management of male infertility. Studies have shown that *Mucuna pruriens* can enhance male fertility by restoring mitochondrial membrane potential, lowering reactive oxygen species (ROS) levels, controlling apoptosis, controlling unspecific ROS generation, and reactivating the antioxidant defense system (Singh et al. 2013; Khamees et al. 2018). It also helps manage stress (Shukla et al. 2010), reduces lipid peroxidation and sperm DNA damage, influences the HPG (hypothalamus-pituitary-gonadal) axis, increases hormone levels due to the active component, levodopa, in its seeds, and increases the level of dopamine, which plays an important role in mediating male sexual behavior and function (Acevedo-Rodriguez et al. 2018).

The current study aims to evaluate the effect of *Mucuna pruriens* seed extracts on the PRM I and II gene expressions in chlorpromazine-induced endocrine disruptions and reproductive system damage in male rats.

Materials and methods

Materials

All compounds and kits used in this study were pure: chlorpromazine (99.98% purity) (Sanofi, French), *Mucuna pruriens* seeds (Echemi, China), *PRM I*, *PRM II* (Alph DNA, Canada), LH, FSH, testosterone, and prolactin ELISA Kit (MyBioSource, USA). A botanist from the Department of Pharmacognosy—College of Pharmacy—Mustansiriyah University verified *Mucuna pruriens* seeds.

Extraction procedures

100 grams of the *Mucuna pruriens* seed were defatted using 399 ml of acetone by shaking for 48 hours at room temperature (25 ± 2 °C). The solution was then filtered, and the solid material was taken. The defatted materials were extracted using equal proportions of water: ethanol solution (1:1 ratio) with 0.1% ascorbic acid (1500 ml) by shaking for 12 hours; the resultant solution was filtered, and the filtrate was obtained and dried. The dried powder was further examined after dissolving in hot water using TLC (TLC plates were run in *n*-butanol-*n*-propanol-water-acetic acid (3:3:2:1), and spots were visualized by ninhydrin reagent (300 mg dissolved in 100 ml *n*-butanol and 3 ml acetic acid). Yielding is presented in Table 1 (Misra and Wagner 2004, 2007).

Table 1. Results of the extraction of *Mucuna pruriens* seed.

Compound	Quantity referenced to the total material (%)
L-Lysine	0.03
L-Cystine	0.14
Glycine	0.18
4-amino butyric acid	0.32
L-Serine	0.41
Alanine	0.43
Tryptamine	1.43
L-DOPA	1.92
Glutathione	3.9
L-glutamate	4.8

All materials were compared to their respective reference samples (Sigma-Aldrich, Germany).

Animal housing

Male Wistar rats weighing 150–200 g were used. The rats were housed in large, comfortable cages. For 12 days, they were allowed to acclimate in a controlled environment, including temperature and humidity. They were also allowed food and water ad libitum.

Study design

Thirty male rats were allocated randomly into five groups (n = 6). The groups were characterized as follows: Group 1 (negative control) rats received distilled water orally by gastric gavage for 52 days. Group 2 (induction group) rats received 20 mg/kg chlorpromazine orally by gastric gavage once daily for 52 days (Opeyemi et al. 2021).

Group 3 (low-dose *Mucuna pruriens*) induction with chlorpromazine (20 mg/kg, orally) once daily for 52 days, starting in day 23, rats treated with *Mucuna pruriens* at a dose of 500 mg/kg orally by gastric gavage once daily till day 52. Group 4 (medium-dose

Mucuna pruriens) induction with chlorpromazine (20 mg/kg, orally) once daily for 52 days, starting in day 23, rats treated with *Mucuna pruriens* at a dose of 1,000 mg/kg orally by gastric gavage once daily till day 52. Group 5 (high-dose *Mucuna pruriens*) induction with chlorpromazine (20 mg/kg, orally) once daily for 52 days, starting on day 23, rats treated with *Mucuna pruriens* at a dose of 2,000 mg/kg orally by gastric gavage once daily till day 52 (Muthu and Krishnamoorthy 2011), as illustrated in Fig. 1.

Study settings

The experiment started on 11 October 2023, and ended on 14 December 2023, in the animal house of the Pharmacology and Toxicology Department at the College of Pharmacy—Mustansiriyah University.

Analytical procedure

Each testis was separated into two parts: one for histopathological study and preserved in 10% neutral buffered formalin (NBF) to protect the tissue structure from autolysis; the second part of testicular tissues was preserved in TRIzol and stored at -20 °C for gene expression assays.

To measure *PRM I* (F: GGCGAGATGCTCTTGAAGTC, R: TAGCACCATGGCCAGATACC) and *PRM II* (F: GATCCTGTGAAGCCTCTTGC, R: TCCTGACCTCCTGGCACTAT) gene expression using the real-time quantitative polymerase chain reaction (RT-PCR) method. This method involved testicular tissue isolation, total RNA extraction using TRIzol and purification, and complementary DNA (cDNA) synthesis using a one-step genomic DNA (gDNA) removal and a cDNA synthesis kit. Total cDNA (24 µL) was amplified in real-time PCR (including 10 µL of Perfect Start Green qPCR Super Mix with 2 µL of primers prepared from forward and reverse solutions and 4 µL of cDNA and 4 µL of nuclease-free water). The amplification conditions were pre-denaturation at 94 °C for 30 sec by one cycle, denaturation at 94 °C for 10 sec by 35 cycles, annealing at 58 °C for 15 sec by 35 cycles, extension at 72 °C for 20 sec by 35 cycles, and melting curve

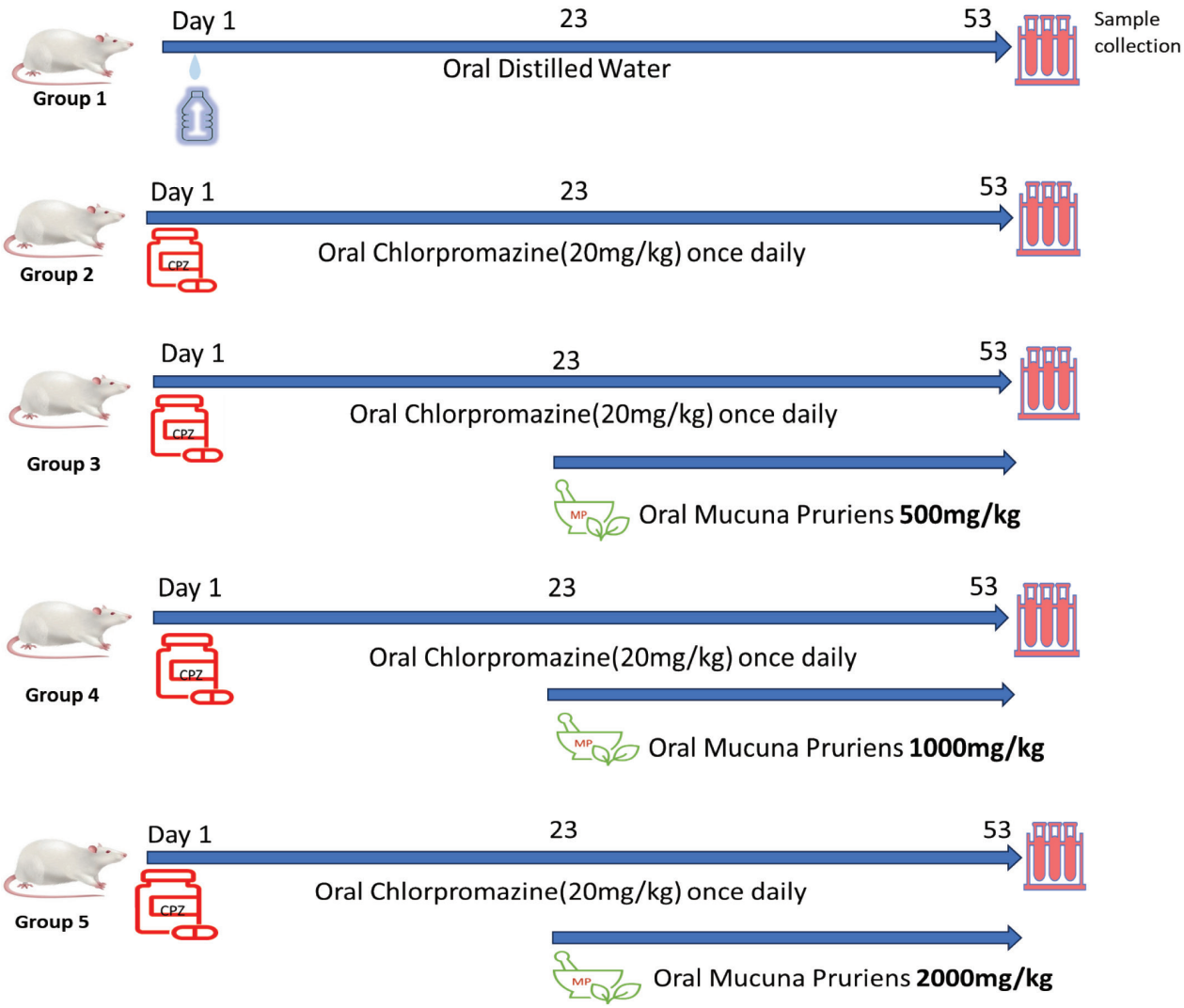

Figure 1. Flow chart of the study.

70 °C–95 °C for 1 sec by one cycle. The $2^{-\Delta\Delta ct}$ method is the relative quantification method most frequently found in software packages for qPCR experiments. This method uses the threshold cycle (CT) information generated from a qPCR system to calculate relative gene expression in target and reference samples, using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (F: CAACGGATACATTGGGGGTA, R: AGAACATCATCCCTGCATCC) as a reference gene (Fraga et al. 2014; Hussein et al. 2024a; Hussein et al. 2024b).

For measurement of LH, FSH, testosterone, and prolactin levels, first blood samples were withdrawn from the heart apex (left ventricle) by a 5 ml syringe with gauge 23 needles, then put in the gel tubes and left for 15 minutes to clot at room temperature, then centrifuged for 15 minutes at 3000 RPM. The collected serum was divided into portions in Eppendorf tubes (1.5 ml). They were stored at -20 °C (Ongaro et al. 2021). The kits utilize a sandwich ELISA technique, which includes two antibodies with distinct antigen epitopes. Each plate was pre-coated with capture antibody; the plates were filled with serum samples to perform a specific antigen-antibody reaction (incubation with enzyme-conjugated antibody). Subsequently, a washing-out process was performed to eliminate the unbounded antibodies. Ultimately, the substrate is added to generate a calorimetric signal that can be detected using the plate reader (Sakamoto et al. 2018; Al-Mousawi et al. 2020).

Ethical considerations

The study was approved by the Research Ethical Committee of the College of Pharmacy, Mustansiriyah University, approval number 32, reference number 121, date: 23 June 2023.

Statistical analysis

Variables followed a normal distribution and were analyzed using ANOVA with a *post-hoc* Tukey test. For the data's non-normal distribution, the Kruskal-Wallis and post hoc Dunn tests were employed to compare groups. The data are displayed as the average value plus or minus the standard error mean (SEM). The data underwent analysis using GraphPad Prism 10.2, which was utilized to generate graphs and figures. $P \leq 0.05$ was regarded as statistically significant.

Sample size

For sample size computation, program G Power was utilized (Faul et al. 2007) based on Cohen's principles (Charan and Kantharia 2013). The groupings in the table were generated using random integers. The animals were housed in well-marked containers and provided with tail tags to minimize any potential confusion (Festing 2006).

Results

Effect of *Mucuna pruriens* seed extracts on PRM I and PRM II gene expression in rats exposed to chlorpromazine

Fig. 2 illustrates the average expression levels of PRM I and PRM II genes following the administration of chlorpromazine and *Mucuna pruriens*. The PRM I gene expression was significantly downregulated (0.75 ± 0.11 fold) in the induction group treated with chlorpromazine compared to the control group. Meanwhile, this effect was significantly improved when treated with *Mucuna pruriens* seed

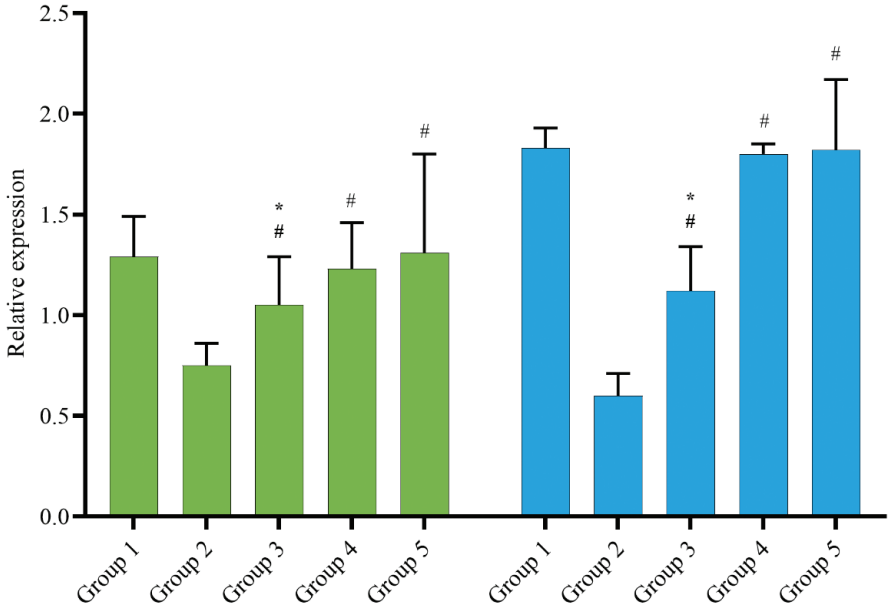

Figure 2. Effect of *Mucuna pruriens* on *PRM I* and *PRM II* gene expression of rats exposed to chlorpromazine. Data were stated as mean ± SEM. * Indicates a significant difference ($p \leq 0.05$) against control (group 1); # Indicates a significant difference ($p \leq 0.05$) against induction (group 2).

extract. When treated with 500 mg of *Mucuna pruriens*, the mean *PRM I* fold of expression (1.05 ± 0.24 fold) was significantly higher than the induction group. Moreover, when treated with 1000 mg of *Mucuna pruriens*, there are no significant differences compared to the control group. Also, when treated with 2000 mg of *Mucuna pruriens* (1.31 ± 0.49 fold), there are no significant differences compared to the control group.

Regarding the PRM II gene expression, there was a significant downregulation (0.60 ± 0.11 fold) of PRM II in the induction group compared to the control group. When treated with 500 mg of *Mucuna pruriens*, the mean PRM I fold of expression (1.12 ± 0.22) showed a significant difference compared to the induction group. Similarly, the groups that were given 1000 mg and 2000 mg of *Mucuna pruriens* significantly upregulated (1.80 ± 0.05 and 1.82 ± 0.35 fold, respectively) compared to the induction group, which was not statistically significant compared to the control group.

Effect of *Mucuna pruriens* on the sex hormones of rats exposed to chlorpromazine

A significant decrease (0.37 ± 0.02 IU/L) in serum FSH concentration was observed in the induction group compared to the control group (0.80 ± 0.01 IU/L). All tested groups of *Mucuna pruriens* showed significantly higher FSH levels than the induction group. In contrast, only a high-dose group of *Mucuna pruriens* showed no significant difference compared to the control group (Fig. 3A).

A significant decrease (0.85 ± 0.03 IU/L) in serum LH concentration was observed in the induction group compared to the control group. All tested groups of *Mucuna pruriens* showed significantly higher LH levels than the induction group. In contrast, both medium- and high-dose groups of *Mucuna pruriens* showed no significant difference compared to the control group (Fig. 3B).

A significant decrease (9.53 ± 0.3 nmol/L) in serum testosterone levels was observed in the induction group compared to the control group. All tested groups of *Mucuna pruriens* showed significantly higher testosterone levels than the induction group. In contrast, all doses of *Mucuna pruriens* showed no significant difference compared to the control group (Fig. 3C).

A significant increase (1.63 ± 0.01 IU/L) in serum prolactin levels was observed in the induction group compared to the control group. All tested groups of *Mucuna pruriens* showed significantly lower prolactin levels than the induction group. In contrast, all doses of *Mucuna pruriens* showed no significant difference compared to the control group (Fig. 3D).

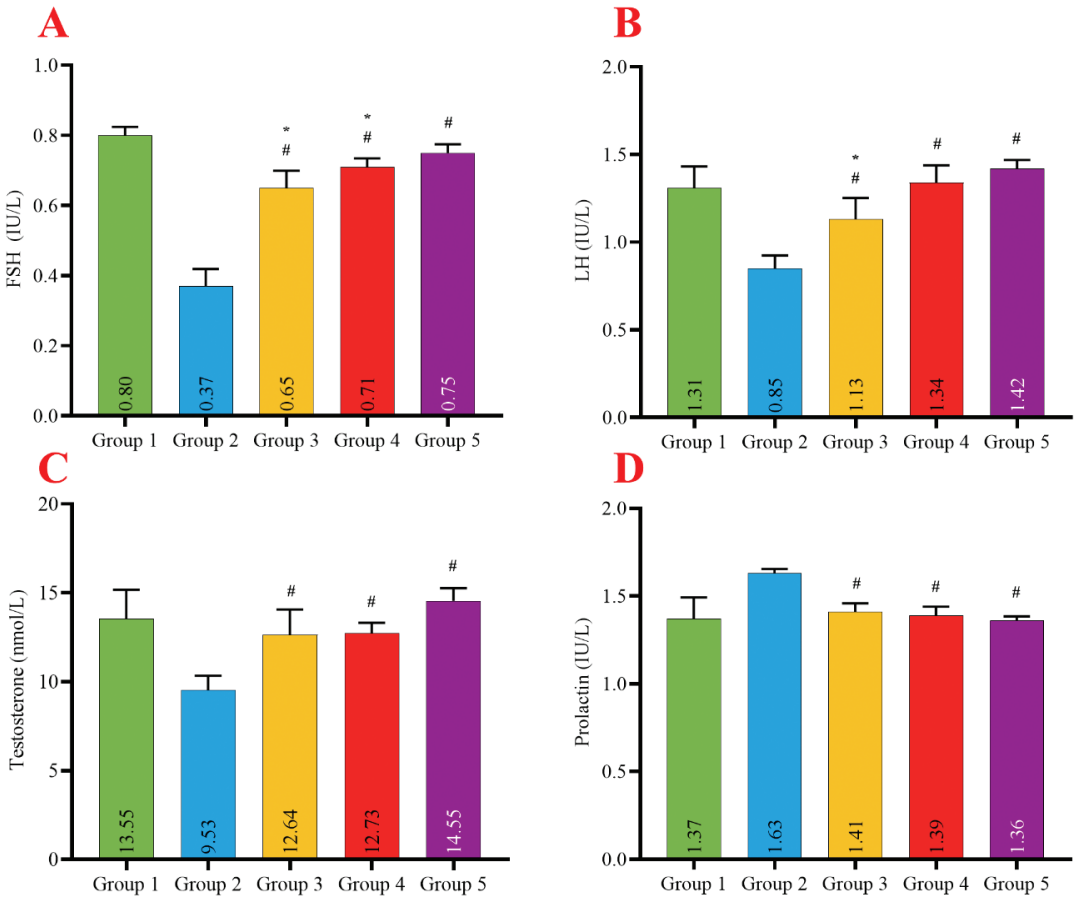

Figure 3. Effect of *Mucuna pruriens* seed extract on sex hormones of rats exposed to chlorpromazine. Data were stated as mean ± SEM. * Indicates a significant difference ($p \leq 0.05$) against control (group 1); # Indicates a significant difference ($p \leq 0.05$) against induction (group 2).

Effect of *Mucuna pruriens* on the testicular histology of rats exposed to chlorpromazine

The histopathological figures of the testicle in the control group revealed a normal appearance with a uniform shape and regular outline of seminiferous tubules, a normal appearance of germinal epithelial cells, normal testicular interstitial vascular elements, and normal cytoarchitecture. The magnification of seminiferous tubules showed a normal appearance of luminal spermatozoa cell contents, normal primary with secondary spermatocytes, normal spermatogonium cells, myoid cells, and normal interstitial Leydig cells (Fig. 4A).

The histopathology of the induction group testicle revealed severe toxic orchitis characterized by moderate atrophy of seminiferous tubules associated with little patches of spermatogenic cells due to arrested spermatogenesis and an irregular outline of tubules. The magnification of figures revealed a few spermatogonium cells with severe degeneration and necrosis of other types of germinal epithelial cells with marked nuclear pyknosis, and the lumen of tubules showed necrotic tissue (Fig. 4B).

Treatment with 500 mg of *Mucuna pruriens* revealed a normal appearance with slightly normal-sized seminiferous tubules, a normal appearance of germinal epithelial cells, and a normal testicular interstitium. The magnification of the figures revealed normal spermatogenesis with a normal appearance of spermatogonium cell lines, normal primary lines with secondary spermatocyte lines, and normal maturation stages of spermatids (Fig. 4C).

Treatment with 1000 mg of *Mucuna pruriens* revealed a normal appearance with the normal size of seminiferous tubules, the normal appearance of germinal epithelial cells, and a normal testicular interstitium. The germinal epithelium showed a normal appearance of the spermatogonium cell line, a few normal primaries with a secondary spermatocytes and Sertoli cells, and a normal maturation stage of spermatids. Few figures revealed the normal size of seminiferous tubules with mild microcirculation, vascular congestion with dilation, interstitial edema, and thickening of the testicular interstitium (Fig. 4D).

Treatment with 2000 mg of *Mucuna pruriens* revealed a normal appearance and size of seminiferous tubules, a normal appearance of germinal epithelial cells, and a normal testicular interstitium. The magnification of the figures revealed the normal appearance of the spermatogonium cell line, the normal primary line with a secondary spermatocytes line, and the normal maturation stage of spermatids. Few figures revealed slight hypertrophy of seminiferous tubules, with a normal appearance of germinal epithelial cells and little figures of microcirculation within the cell line of spermatogonium cells and normal testicular interstitium (Fig. 4E).

Discussion

Certain medications are considered EDCs, capable of disrupting endocrine homeostasis and exacerbating male infertility problems (Czarnywojtek et al. 2021), including antipsychotic drugs; these drugs commonly modulate dopamine and serotonin and could impact male sexual function and spermatogenesis (Ilgin 2020).

The present study demonstrates a notable reduction in the *PRM I* and *PRM II* gene expressions in the group treated with chlorpromazine compared to the control. These results agree with previous studies (Opeyemi et al. 2021; Oyovwi et al. 2021). This study demonstrated the anti-fertility effects of chlorpromazine by changing protamine, which is important in chromatin condensation.

Male infertility is linked to changes in *PRM I* and *PRM II* protamine expression. Protamines are post-meiotic nuclear proteins; during the late haploid phase of spermatogenesis, histones are replaced by protamines, which play a role in preserving the DNA of sperm and condensing the head of the sperm (Yoshida et al. 2018). The protamination of sperm chromatin is essential for compacting the nucleus, which is necessary for sperm motility. This process also protects DNA from free radicals and preserves the sperm genome from oxidation and harmful molecules present in the female reproductive system (Steger et al. 2001; Ghassan et al. 2020; Pardede et al. 2020).

FSH and testosterone regulate the mechanism of chromatin condensation during spermiogenesis (Cho et al. 2003). So, a decreased FSH level enhanced the sperm chromatin decondensation rate and affected sperm morphology. Deficits in testosterone signaling also led to chromatin packaging loss during spermiogenesis, revealing protamine level loss in the sperm and testis (Gill-Sharma et al. 2011).

In this study, groups that were treated with *Mucuna pruriens* demonstrated highly significant *PRM I* and *PRM II* gene expression up-regulation; this could be attributed to the seed of *Mucuna pruriens* containing L-Dopa as its active substance, which stimulates GnRH (gonadotropin-releasing hormone) secretion and enhances male reproductive function. This, in turn, stimulates the secretion of LH and FSH from the pituitary gland anterior portion; elevated FSH, LH, and testosterone serum levels stimulate spermatogenesis (Misra and Wagner 2007; Singh et al. 2013; Waheed et al. 2020). L-Dopa in *Mucuna pruriens* has been demonstrated to possess an antioxidant capacity. It has the potential to reduce apoptotic germ cells in chlorpromazine-treated male rats. Along with flavonoids, tannins, and other contents of the *Mucuna pruriens* seed, it has been admitted as the principal phenolic compound that serves as a main antioxidant for scavenging free radicals (Polterait 1997).

The current research's outcomes also stated that chlorpromazine treatment produced psycho-neuroendocrine changes. These changes are shown by neurochemical imbalance, hormonal alteration, induced hypothalamic and pituitary neuronal degenerations, and testicular endocrine and exocrine derangements.

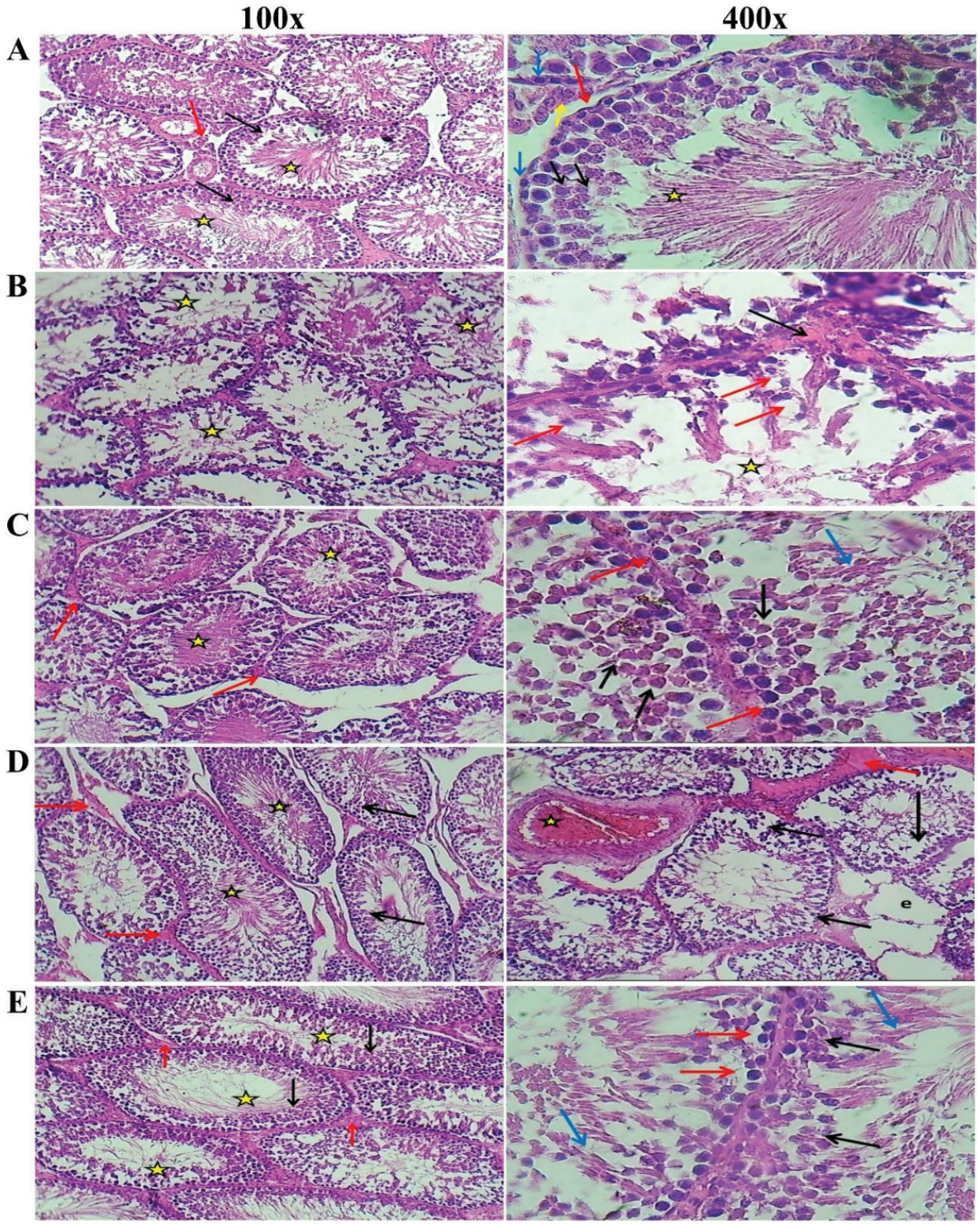

Figure 4. Histopathological sections of tests, (**A**) control group; (**B**) induction group; showing severe orchitis characterized by reduced size seminiferous tubules with irregular outline content, little patches of spermatogenic cells in 100× (asterisk), line of spermatogonium cells with severing degeneration and necrosis of other types of germinal epithelial cells (red arrow), and luminal necrotic tissue (asterisk) at 400×; (**C**) group 3; (**D**) group 4; (**E**) group 5. H and E stain (100× and 400× magnification).

Hormones preserve and regulate the male reproductive system (Oyovwi et al. 2021). Hormonal analysis showed that serum levels of FSH, LH, and testosterone concentrations were significantly decreased. In contrast, serum prolactin concentrations were significantly increased in the induction group treated with chlorpromazine compared to the control group. The current study also reported that the concentrations of testosterone serum, LH, and

FSH in treated *Mucuna pruriens* seed extract groups were significantly improved compared to the induction group. Furthermore, the prolactin concentration in groups treated with *Mucuna pruriens* was significantly decreased, as shown in previous studies (Ben-Jonathan and Hnasko 2001; Zamani et al. 2015; Opeyemi et al. 2021; Oyovwi et al. 2021).

Dopamine D2 receptors on lactotroph cells in the anterior pituitary gland are inhibited by chlorpromazine. Dopamine is crucial for prolactin secretion and tonic inhibition, and its inhibition by chlorpromazine leads to increased prolactin levels in rats. Elevated prolactin levels then suppress the release of GnRHs from the hypothalamic axis. This lowers LH, testosterone, and FSH levels, which are responsible for hypogonadism, decreased libido, and infertility in males (Melmed and Jameson 2014). Hyperprolactinemia causes Leydig cell damage, so it decreases the levels of testosterone and causes oxidative stress in the arcuate nucleus of the hypothalamus and nitration of tyrosine hydroxylase (the rate-limiting enzyme in the production of dopamine), which leads to dopamine release and progressive loss (MohanKumar et al. 2011). *Mucuna pruriens* seeds have a lot of L-Dopa and its byproducts. Giving these seed extracts to infertile males significantly increased dopamine levels in their blood (Shukla et al. 2009).

The histopathological study of testicular tissue in the induction group treated with chlorpromazine revealed damage to the seminiferous tubular and spermatogonium cells with severing degeneration and germinal epithelial cell necrosis with nuclear pyknosis, which agreed with previous studies (Vidal and Whitney 2014; Dutta et al. 2021; Oyovwi et al. 2021).

This study demonstrated a significant decrease in the level of reproductive hormones associated with increased degenerative lesions in the testes and oxidative stress. Chlorpromazine decreased the stimulation of GnRH, so the reduction in serum LH with a consequent reduction in testosterone levels was associated with an increased index of abnormal sperm and degeneration of the seminiferous tubules that cause arrest in the maturation of sperm from hypo spermatogenesis to absent spermatogenesis. Also, a reduction in serum LH levels directly affects Leydig cells, leading to hypertrophy of these cells (Faccio et al. 2014).

Oxidative stress plays an important role in reproductive disorders. Spermatozoa are highly vulnerable to oxidative stress due to the high content of polyunsaturated fatty acids (PUFA) in the plasma membrane. Excessive ROS and its metabolites damage lipids, proteins, and sperm DNA either directly or indirectly through the activation of sperm caspases and the production of endonuclease (Faccio et al. 2014; Takalani et al. 2023). Also, it can disrupt Leydig cells' steroidogenic capacity and the germinal epithelium's capacity to differentiate normal spermatozoa (Aitken and Roman 2008).

Interestingly, the testis sections in treatment groups with *Mucuna pruriens* show improved histo-morphologic integrity of the testis. *Mucuna pruriens* can potentially increase FSH, LH, and testosterone levels by activating the pituitary-testicular axis and improving Leydig cell damage. It also contains many bioactive constituents that act as antioxidants that suppress ROS production and increase free-radical scavenging before they interact with the plasma membrane of sperm, reducing membrane damage and restoring of spermatogenesis, which agrees with previous studies (Lapyuneyong et al. 2022; Murugesan et al. 2022).

Conclusions

This study has confirmed the endocrine disorders and reproductive toxicity generated by chlorpromazine (synthetic antipsychotic drugs). Chlorpromazine causes hormonal disruption and alterations in the signaling pathways of *PRM I* and *II* genes responsible for spermatogenesis. The administration of *Mucuna pruriens* seed improved male reproductive function associated with chlorpromazine by elevating testosterone, FSH, and LH serum levels and controlling the *PRM I* and *II* signaling pathways in animal models.

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