



Testicular Gene Expression Response to *Icacina Trichantha* Root Ethanol Extract Therapy in Paroxetine-Induced Reproductive Dysfunction in Male Wistar Rats

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Abstract. Male infertility is increasingly linked to pharmacological agents, particularly selective serotonin reuptake inhibitors (SSRIs) such as paroxetine, which may disrupt spermatogenesis through endocrine and molecular mechanisms. Paroxetine is therefore used as an agent in studying some intervention strategy in male infertility in experimental animals. This study investigated the effects of paroxetine on the expression of key spermatogenic genes, cyclic AMP response element modulator (CREM), protamine I (PRM I), and protamine II (PRM II) in male Wistar rats and evaluated the remedial potential of *Icacina trichantha* root ethanol extract (ITREE) in comparison with tadalafil and clomiphene citrate. Twenty-five rats were randomly assigned to five groups: control (Group A), paroxetine only (Group B), paroxetine + ITREE (Group C), paroxetine + tadalafil (Group D), and paroxetine + clomiphene (Group E). The oral treatment regimen spans through 56 days. Paroxetine significantly increased CREM (92.64 ± 2.35 vs. 28.80 ± 2.94), PRM I (58.17 ± 1.73 vs. 21.64 ± 1.29), and PRM II (66.08 ± 3.25 vs. 42.74 ± 1.91) compared with control, and elevated the PRM I/PRM II ratio from 0.51 in control to 0.88. Co-administration of ITREE partially restored expression levels (CREM 68.87 ± 1.46 , PRM I 42.15 ± 1.63 , PRM II 55.71 ± 3.28) and attenuated the PRM I/PRM II ratio to 0.76. Tadalafil and clomiphene produced varying degrees of modulation, with CREM expression of 66.96 ± 2.07 and 105.12 ± 3.85 , respectively. These findings indicate that paroxetine disrupted spermatid differentiation and chromatin remodeling, while ITREE effectively mitigates these molecular alterations. The study highlights the therapeutic potential of *I. trichantha* roots in preventing SSRI-induced male reproductive toxicity and provides a molecular basis for plant-based interventions.

Keywords: Paroxetine, Spermatogenesis, CREM, Protamines, *Icacina trichantha*

1. Introduction

Male infertility is a growing global health concern, accounting for approximately 40–50% of infertility cases worldwide (Kumar & Singh, 2015). A wide range of factors including genetic abnormalities, endocrine disruption, environmental exposures, and pharmacological agents have been implicated in the impairment of male reproductive function (Agarwal *et al.*, 2021). Among pharmacological agents, antidepressants particularly selective serotonin reuptake inhibitors (SSRIs) have attracted increasing attention because of their potential adverse effects on reproductive physiology and sperm function. One of the most widely prescribed SSRIs is paroxetine, which is commonly used in the treatment of depression, anxiety disorders, obsessive–compulsive disorder, panic disorder, and post-traumatic stress disorder (Nevels *et al.*, 2016).

Paroxetine exerts its therapeutic action primarily through inhibition of the serotonin reuptake transporter (SERT), resulting in increased synaptic concentrations of serotonin in the central nervous system. While this mechanism is beneficial in the management of psychiatric disorders, excessive serotonergic activity has been associated with

disturbances in the hypothalamic–pituitary–gonadal (HPG) axis, leading to impaired reproductive hormone regulation (Mosbah *et al.*, 2019). Elevated serotonin levels may suppress gonadotropin-releasing hormone (GnRH) secretion and subsequently reduce follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone production, thereby disrupting spermatogenesis and sperm maturation (Schenker *et al.*, 1992; Mosbah *et al.*, 2019). Experimental and clinical studies have demonstrated that paroxetine administration can lead to reduced sperm count, impaired sperm motility, abnormal sperm morphology, and increased DNA fragmentation (Mosbah *et al.*, 2021; Yardimci *et al.*, 2019). Histopathological alterations including degeneration of seminiferous tubules, cellular disorganization, vacuolization, and reduced numbers of spermatozoa within the epididymal lumen have also been reported in animal models following paroxetine exposure (Elkelany & Kashef, 2023).

Beyond endocrine disruption and structural testicular damage, growing evidence suggests that male infertility may also result from alterations in the expression of genes essential for spermatogenesis. Spermatogenesis is a highly coordinated process involving mitotic proliferation of spermatogonia, meiotic division of spermatocytes, and differentiation of haploid spermatids into mature spermatozoa (Sánchez-Jasso *et al.*, 2023). This process is tightly regulated by transcription factors and signaling pathways that control stage-specific gene expression. Among these regulatory molecules, the cyclic adenosine monophosphate (cAMP) response element modulator (CREM) plays a central role in post-meiotic germ cell differentiation.

CREM is a cAMP-responsive transcription factor belonging to the CREB/ATF family of transcription regulators and is considered a key regulator of spermiogenesis. Through alternative promoter usage and extensive alternative splicing, the CREM gene produces multiple isoforms that function either as transcriptional activators or repressors (Behr & Weinbauer, 2001). During normal spermatogenesis, a developmental “switch” occurs in which CREM repressor isoforms expressed in pre-meiotic germ cells are replaced by activator isoforms, particularly CREM τ , in post-meiotic haploid spermatids (Peri & Serio, 2000). This switch is essential for the transcription of several spermatid-specific genes involved in sperm structural development and chromatin remodeling. Studies in CREM-deficient mice have demonstrated complete infertility characterized by arrest of germ cell maturation at the round spermatid stage, underscoring the indispensable role of CREM in spermatid differentiation (Blendy *et al.*, 1996; Nantel *et al.*, 1996). Similarly, abnormal CREM expression patterns have been observed in men with spermatogenic failure and round spermatid maturation arrest, indicating that disruption of CREM signaling contributes to male infertility (Steger, 1999; Sánchez-Jasso *et al.*, 2023).

Among the downstream targets regulated by CREM are the protamine genes, which encode sperm-specific nuclear proteins essential for chromatin condensation during the final stages of spermiogenesis. Protamines are small arginine-rich proteins that replace histones in elongating spermatids, leading to the formation of highly compacted sperm chromatin. In mammals, two major protamines, protamine 1 (PRM1) and protamine 2 (PRM2) are expressed and function cooperatively to achieve the extreme condensation of paternal DNA within the sperm head (Ren *et al.*, 2021). This histone-to-protamine transition is critical for maintaining genomic integrity, protecting sperm DNA from oxidative damage, and ensuring proper transmission of paternal genetic information during fertilization (Chioccarelli *et al.*, 2020; Kobayash *et al.*, 2021).

The expression of PRM1 and PRM2 is tightly regulated at transcriptional, translational, and post-translational levels during spermatogenesis. Any imbalance in the expression or ratio of PRM1 to PRM2 can disrupt chromatin packaging, resulting in abnormal sperm morphology, reduced motility, and increased DNA fragmentation (Pardede *et al.*, 2020; Ren *et al.*, 2021). Altered protamine expression has therefore been widely recognized as a biomarker of impaired spermatogenesis and male infertility. Environmental toxicants, pharmacological agents, and oxidative stress have all been reported to interfere with protamine expression and chromatin remodeling processes in germ cells (Bashiri *et al.*, 2021).

Given the increasing use of antidepressant medications and their potential reproductive toxicity, there is growing interest in identifying therapeutic agents capable of mitigating drug-induced reproductive dysfunction. Natural products and plant-derived bioactive compounds have been widely investigated for their protective effects on reproductive health due to their antioxidant, anti-inflammatory, and endocrine-modulating properties (Agarwal *et al.*,

2021). In this context, medicinal plants used in traditional medicine represent promising candidates for the management of male reproductive disorders.

Despite the documented reproductive toxicity of paroxetine, limited studies have explored its impact on the molecular regulators of spermatogenesis, particularly transcription factors such as CREM and downstream structural proteins such as protamines. Furthermore, the potential of phytotherapeutic agents to restore paroxetine-induced alterations in spermatogenic gene expression remains largely unexplored. Therefore, this study was designed to investigate the effects of paroxetine on the expression of CREM and protamine genes in the testes of male rats and to evaluate the remedial potential of *Icacina trichantha* root ethanolic extract in comparison with established therapeutic agents. Understanding the molecular mechanisms underlying these effects may provide valuable insight into novel strategies for the management of drug-induced male infertility.

Given the increasing incidence of antidepressant use and the growing concern about their potential reproductive side effects, there is a need to explore therapeutic strategies capable of mitigating drug-induced reproductive dysfunction. Plant-derived bioactive compounds have gained considerable attention in recent years due to their diverse pharmacological properties, including antioxidant, anti-inflammatory, and endocrine-modulating effects that may protect against reproductive toxicity (Agarwal *et al.*, 2021; Bashiri *et al.*, 2021). Medicinal plants used in traditional medicine therefore represent promising candidates for the management of male infertility associated with xenobiotic exposure.

Icacina trichantha, a medicinal plant widely used in African ethnomedicine, has been reported to possess several biological activities including antioxidant, anti-inflammatory, and cytoprotective properties (Nwankwo *et al.*, 2022). These pharmacological properties suggest that extracts derived from *I. trichantha* may have the potential to protect or restore reproductive function following chemical or drug-induced testicular damage. However, despite the increasing interest in medicinal plants as therapeutic agents, there is limited information regarding the potential role of *I. trichantha* in modulating molecular pathways involved in spermatogenesis.

In addition to phytotherapeutic agents, pharmacological interventions such as clomiphene citrate and tadalafil are commonly used in the management of male reproductive disorders. Clomiphene citrate is a selective estrogen receptor modulator that enhances endogenous testosterone production by stimulating gonadotropin secretion through blockade of estrogen-mediated negative feedback on the hypothalamic–pituitary axis (Krzastek *et al.*, 2019). Tadalafil, a phosphodiesterase type-5 inhibitor, is widely used in the treatment of erectile dysfunction and has also been shown to improve testicular blood flow, sperm parameters, and overall reproductive function (Corona *et al.*, 2020). These agents therefore serve as useful pharmacological comparators when evaluating the potential reproductive benefits of novel therapeutic compounds.

Although the adverse effects of paroxetine on sperm quality and testicular histology have been documented, there is limited information on its influence on key molecular regulators of spermatogenesis such as the cyclic AMP response element modulator (CREM) and protamine proteins. Since CREM acts as a critical transcription factor regulating post-meiotic germ cell differentiation and protamines are essential for chromatin condensation during spermiogenesis, alterations in the expression of these genes may contribute significantly to impaired sperm development and male infertility.

Therefore, the present study was designed to investigate the effects of paroxetine-induced reproductive toxicity on the expression of CREM and protamine genes in the testes of male Wistar rats and to evaluate the potential remedial effects of *Icacina trichantha* root ethanolic extract (ITREE). In addition, the therapeutic efficacy of ITREE was compared with established pharmacological agents, tadalafil and clomiphene citrate. It was hypothesized that paroxetine exposure would disrupt the expression of genes critical for spermatogenesis, particularly CREM and protamine, and that treatment with ITREE may ameliorate these molecular alterations and restore normal spermatogenic function.

2. Materials and Methods

2.1 Preparation of *Icacina trichantha* Root Ethanol Extract (ITREE):

Roots of *Icacina trichantha* were collected and washed thoroughly to remove soil and debris. They were then air-dried at room temperature and subsequently pulverized into coarse powder using a mechanical grinder. A measured quantity of the powdered root material was macerated in 40% ethanol at a ratio of 1:5 (w/v) and agitated on a mechanical shaker at room temperature for 72 hours, as previously described by Olumese *et al.* (2015). The mixture was filtered using Whatman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator. The concentrated extract was further lyophilized using a freeze dryer to obtain a dry ethanol extract, which was stored in a desiccator until use.

2.2 Experimental Animals

25 adult male Wistar rats weighing 180–220 g were used for this study. The animals were obtained from the Animal House Facility of the Faculty of Life Sciences, University of Benin, Nigeria. The rats were housed in standard plastic cages under controlled environmental conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity 50–60%, and a 12 h light/dark cycle). Animals had free access to standard laboratory chow and clean drinking water *ad libitum*. The animals were acclimatized for two weeks prior to the commencement of the experiment.

2.3 Experimental Design

The experimental animals were randomly assigned into five groups (n = 5 per group). Paroxetine was administered to induce reproductive toxicity in group B which served as negative control, while *Icacina trichantha* root ethanolic extract (ITREE) was evaluated for its potential remedial effects by co-administration with paroxetine in group C. Tadalafil and clomiphene citrate served as standard reference drugs and were co-administered with paroxetine in groups D and E respectively. All treatments were administered orally using an oral gavage once daily for 56 days. The experimental design is presented in Table 1.

Table 1: Experimental design for evaluation of the effects of ITREE on paroxetine-induced alterations in gene expression.

Group	Number of rats	Treatment	Duration
A (Control)	5	Distilled water	56 days
B (Negative control)	5	Paroxetine (10 mg/kg body weight)	56 days
C	5	Paroxetine (10 mg/kg bw) + ITREE (200 mg/kg bw)	56 days
D	5	Paroxetine (10 mg/kg bw) + Tadalafil (5 mg/kg bw)	56 days
E	5	Paroxetine (10 mg/kg bw) + Clomiphene citrate (10 mg/kg bw)	56 days

2.4 Sample Collection

At the end of the experimental period (56 days), the rats were fasted overnight and sacrificed by cervical dislocation in accordance with established procedures for laboratory animals (Aguwa *et al.*, 2020). The animals were dissected and the testes were carefully excised.

2.5 Gene Expression Analysis

Gene expression of CREM, PRM I, PRM II and PDE5 in the testis of male Wistar rats was determined via Reverse Transcriptase Polymerase Chain Reaction Analysis (Elekofehinti *et al.* 2020; Olumegbon *et al.*, 2020) in the following steps;

2.6 RNA Isolation

Total RNA was extracted from testicular tissue using the Quick-RNA MiniPrep™ Kit (Zymo Research, USA) according to the manufacturer's instructions. To remove genomic DNA contamination, RNA samples were treated

with DNase I (New England Biolabs, USA). The concentration and purity of RNA were determined spectrophotometrically by measuring absorbance at 260 nm and the A260/A280 ratio.

2.7 cDNA Synthesis

Complementary DNA (cDNA) was synthesized from 1 µg of DNase-treated RNA using the ProtoScript® II First-Strand cDNA Synthesis Kit (New England Biolabs, USA). Reverse transcription was performed under the following conditions: 65°C for 5 min, 42°C for 60 min, and 80°C for 5 min to terminate the reaction.

2.8 Polymerase Chain Reaction (PCR) Amplification

PCR amplification of the target genes was carried out using OneTaq® 2X Master Mix (New England Biolabs, USA) and gene-specific primers synthesized by Inqaba Biotec (Pretoria, South Africa). The genes analyzed included cyclic AMP response element modulator (*Crem*), protamine 1 (*Prm1*), protamine 2 (*Prm2*), and phosphodiesterase 5A (*Pde5a*). Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used as the housekeeping gene. PCR reactions were performed in a final volume of 25 µL containing cDNA template, forward and reverse primers, nuclease-free water, and PCR master mix. Amplification was carried out under the following conditions: Initial denaturation: 95°C for 5 min

30 cycles of amplification: Denaturation at 95°C for 30 s, annealing for 30 s and extension at 72°C for 60 s, Final extension: 72°C for 10 min.

2.9 Agarose Gel Electrophoresis

The PCR products were separated on a 1.0% agarose gel containing ethidium bromide and visualized under ultraviolet illumination. Band intensities were quantified using ImageJ software (National Institutes of Health, USA). Relative gene expression levels were normalized to the housekeeping gene, *Gapdh* (Elekofehinti *et al.*, (Olumegbon *et al.*, 2020).

2.10 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25. One-way analysis of variance (ANOVA) was used to determine differences among groups, followed by the Least Significant Difference (LSD) post hoc test for multiple comparisons. Statistical significance was considered at $p < 0.05$.

2.11 Ethical Approval

Ethical approval for the study was obtained from the Research Ethics Committee of the Faculty of Life Sciences, University of Benin, Nigeria. All experimental procedures involving animals were conducted in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals (National Research Council, 2011).

3. Results

The effects of the various treatments on the expression of key genes involved in spermatogenesis and sperm chromatin remodeling in the testes of male Wistar rats are presented in Figures 1 to 4. The genes evaluated included cyclic AMP response element modulator (CREM), protamine I (PRM I), protamine II (PRM II), and the PRM I/PRM II ratio.

The negative control group (Group B), which received paroxetine alone, showed a significant ($p < 0.05$) increase in the expression of CREM (92.64 ± 2.35), PRM I (58.17 ± 1.73), and PRM II (66.08 ± 3.25) compared with the control group (Group A) which recorded values of 28.80 ± 2.94 , 21.64 ± 1.29 , and 42.74 ± 1.91 , respectively. This indicates that administration of paroxetine markedly altered the expression of spermatogenesis-related genes in the testes of the experimental animals.

Treatment with the plant extract and standard drugs produced varying degrees of modulation of gene expression relative to the negative control group. Co-administration of paroxetine with ITREE (Group C) resulted in significant reduction in gene expression levels among the treatment groups, with CREM, PRM I, and PRM II values of 68.87 ± 1.46 , 42.15 ± 1.63 , and 55.71 ± 3.28 , respectively. These values were significantly different ($p < 0.05$) from both the control and negative control groups. Similarly, Groups D co-administered Tadalafil and ITREE demonstrated partial normalization of gene expression levels compared with the negative control group, although the magnitude of reduction was less pronounced than that observed in Group C particularly for PRM I. In contrast, Group E co-administered Clomiphene and ITREE showed relatively unperturbed expression of PRM I and II compared to negative control but a particularly notable increase in CREM (105.12 ± 3.85) expression.

The PRM I/PRM II ratio, an important indicator of proper sperm chromatin packaging and spermatid maturation, was also altered across the experimental groups. The negative control group exhibited an increased ratio (0.88) compared with the control group (0.51), suggesting disruption of normal chromatin condensation dynamics. Among the treatment groups, the paroxetine + ITREE group (Group C) demonstrated the greatest restoration toward the control ratio (0.76). Groups E also showed a very partial improvement with ratio of 0.84, while Group E administered clomiphene exhibited an elevated ratio (1.23), indicating a greater imbalance in protamine expression.

Overall, the findings as presented in Figures 1 to 4 demonstrate that paroxetine administration significantly altered the expression of genes involved in spermatogenesis and chromatin remodeling, while co-administration with plant extracts and standard therapeutic agents resulted in varying degrees of modulation of these molecular markers.

Figure 1: Effect of administration of ITREE on CREM gene expression

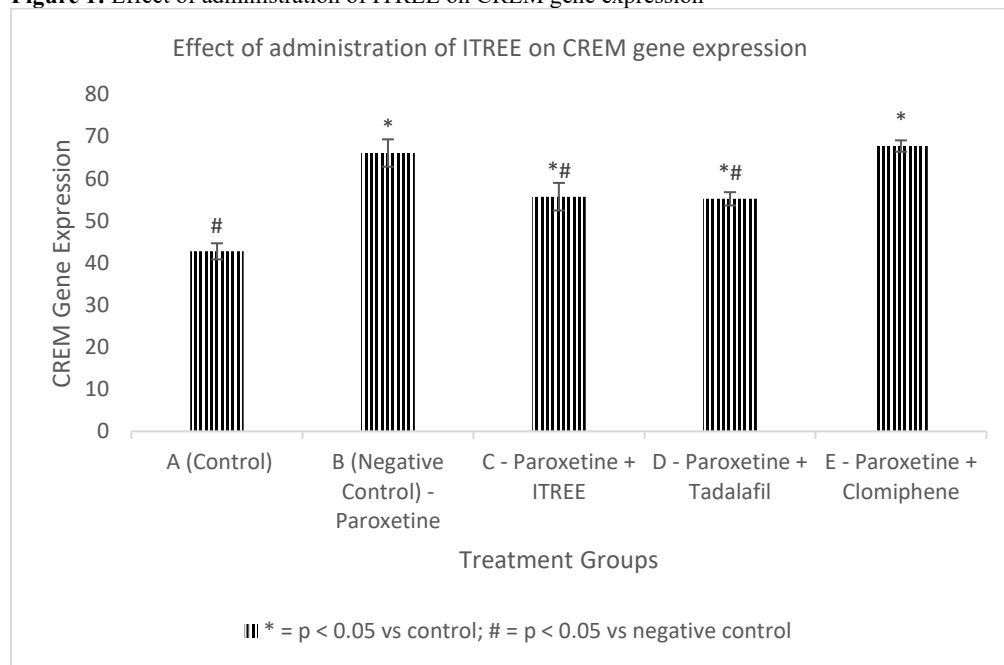


Figure 2: Effect of administration of ITREE on PRM I gene expression

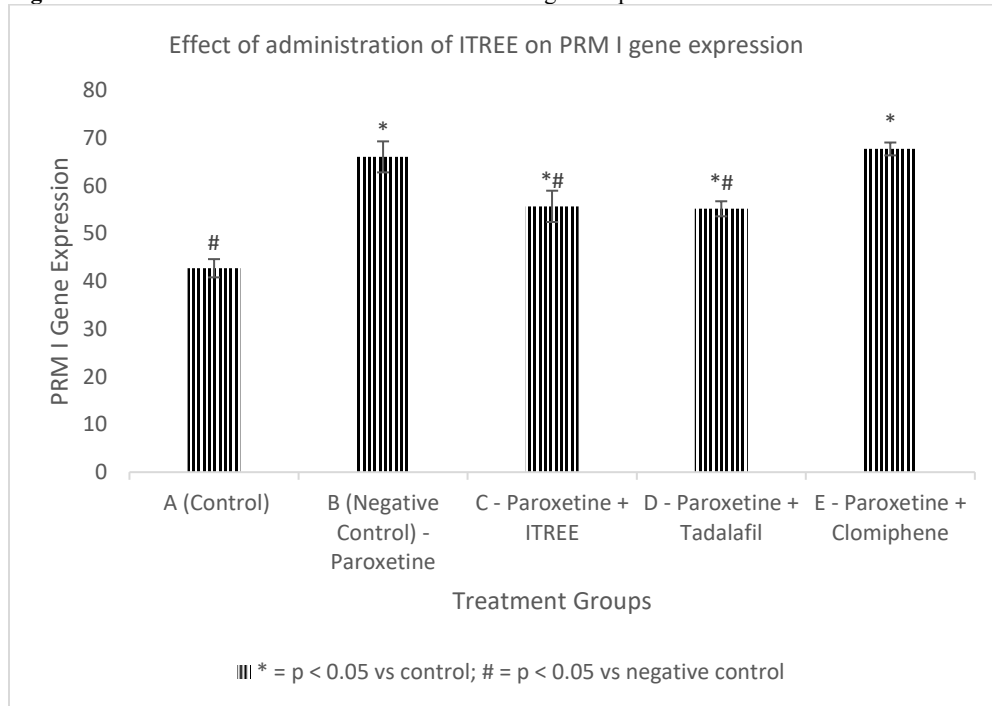


Figure 3: Effect of administration of ITREE on PRM II gene expression

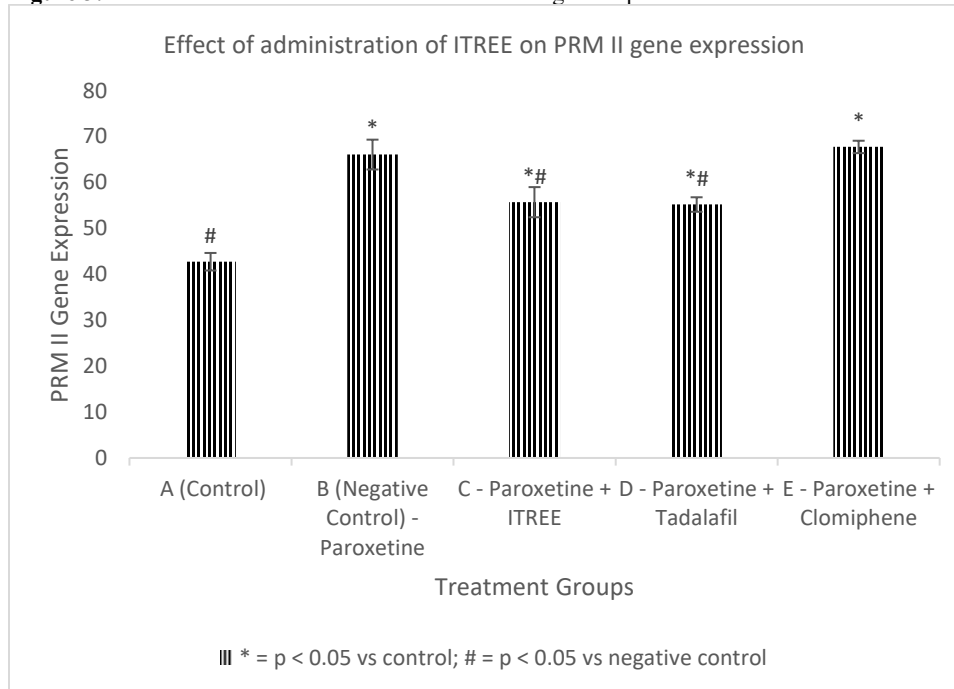
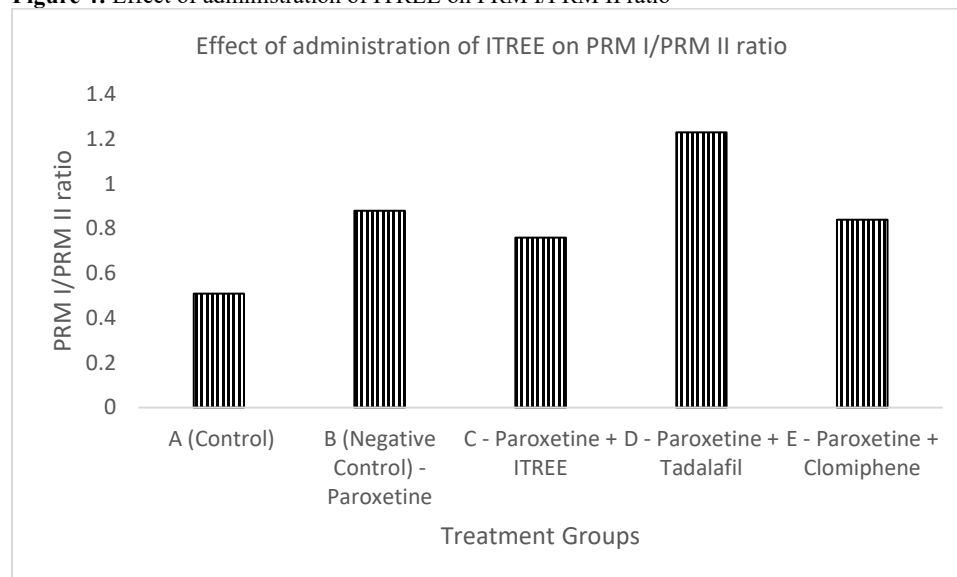


Figure 4: Effect of administration of ITREE on PRM I/PRM II ratio



4. Discussion

The present study investigated the effect of paroxetine on the testicular expression of key genes involved in spermatogenesis, cyclic AMP response element modulator (CREM), protamine I (PRM I), and protamine II (PRM II) and evaluated the potential ameliorative role of ITREE in comparison with established therapeutic agents, tadalafil and clomiphene. These genes are central regulators of post-meiotic germ cell differentiation and chromatin remodeling during spermiogenesis; therefore, alterations in their expression may provide important molecular insights into mechanisms underlying drug-induced male reproductive dysfunction.

The findings of this study demonstrated that administration of paroxetine resulted in a marked dysregulation of spermatogenesis-related gene expression in the testes of male Wistar rats. Specifically, the negative control group (Group B) exhibited a significant elevation in the expression of CREM, PRM I, and PRM II compared with the control group (Group A). This observation suggests that paroxetine exposure disrupts the molecular processes governing spermatid differentiation and sperm chromatin packaging. Increasing evidence indicates that selective serotonin reuptake inhibitors (SSRIs), including paroxetine, may negatively affect male reproductive physiology through oxidative stress, endocrine disturbances, and disruption of testicular gene expression (Tanrikut & Schlegel, 2007). The significant overexpression of the investigated genes observed in the present study therefore supports the hypothesis that paroxetine can interfere with the transcriptional regulatory networks essential for normal spermatogenesis.

CREM is a pivotal transcription factor that mediates the expression of numerous genes required for the transition of round spermatids into elongated spermatids during spermiogenesis. It functions within the cyclic AMP signaling pathway and regulates genes involved in chromatin condensation, acrosome formation, and flagellar development. Previous studies have confirmed that CREM plays a critical role in both human and murine spermatogenesis (Peri & Serio, 2000; Behr & Weinbauer, 2001). Experimental evidence from knockout models has demonstrated that mice lacking the CREM gene are infertile due to an arrest of germ cell maturation at the round spermatid stage (Blendy *et al.*, 1996; Nantel *et al.*, 1996). In such models, the absence of CREM leads to the loss of expression of several post-meiotic genes essential for spermiogenesis, ultimately resulting in impaired sperm production (Sánchez-Jasso *et al.*, 2023).

Regulation of CREM expression is highly complex and occurs at multiple levels, including transcriptional control through the use of multiple promoters (Molina *et al.*, 1993; Stehle *et al.*, 1993; Daniel *et al.*, 2000), alternative transcript splicing (Foulkes *et al.*, 1991; Foulkes *et al.*, 1992), alternative polyadenylation sites (Foulkes *et al.*, 1991),

and translational regulation (Delmas *et al.*, 1992; Gellersen *et al.*, 1997). These regulatory mechanisms give rise to various CREM isoforms that may function either as transcriptional activators or repressors depending on the physiological context. Consequently, tight regulation of CREM expression is required to maintain the proper balance of gene activity during spermatogenesis.

In the present study, paroxetine administration resulted in a marked overexpression of CREM compared with the control group. Dysregulated or excessive expression of CREM may disturb the tightly controlled transcriptional network involved in germ cell differentiation. Recent evidence suggests that aberrant CREM expression may contribute to abnormal spermatid development, defective chromatin remodeling, and impaired sperm maturation (Sánchez-Jasso *et al.*, 2023). Furthermore, altered CREM expression has been associated with abnormal sperm morphology, reduced motility, and idiopathic male infertility (Karami Hezarcheshmeh *et al.*, 2024). Environmental and xenobiotic exposures have also been implicated in the disruption of CREM-regulated pathways (Sánchez-Jasso *et al.*, 2023). Therefore, the overexpression of CREM observed following paroxetine exposure in this study may represent a compensatory or pathological response to testicular stress that ultimately interferes with normal spermatogenic progression.

Co-administration of ITREE with paroxetine resulted in a substantial reduction in CREM expression compared with the paroxetine-only group, although the levels remained higher than those of the control group. This finding suggests that ITREE possesses the capacity to partially restore the normal regulatory balance of spermatogenic transcription factors. The improvement observed may be attributed to the presence of bioactive phytochemicals in *I. trichantha*, which have been reported to exhibit antioxidant and cytoprotective activities capable of mitigating cellular damage induced by xenobiotics. Oxidative stress is widely recognized as a major mechanism through which pharmacological agents impair male reproductive function; therefore, the protective effect of ITREE on CREM expression may be related to its ability to reduce oxidative damage within the testicular microenvironment. The observation that ITREE improved CREM expression toward normal levels suggests a potential regulatory influence on the molecular pathways controlling spermiogenesis.

In comparison, treatment with tadalafil also resulted in a reduction in CREM expression relative to the paroxetine group, indicating partial restoration of transcriptional regulation. Tadalafil, a phosphodiesterase-5 inhibitor commonly used in the management of erectile dysfunction, has been reported to improve testicular perfusion and enhance reproductive function through improved blood flow and modulation of nitric oxide signaling pathways. Improved vascular supply to the testes may enhance the microenvironment required for germ cell development, thereby contributing to normalization of gene expression patterns.

Interestingly, administration of clomiphene in combination with paroxetine resulted in an even greater increase in CREM expression compared with the paroxetine-only group. Clomiphene is a selective estrogen receptor modulator that stimulates endogenous testosterone production by blocking estrogen-mediated negative feedback on the hypothalamic–pituitary–gonadal axis. While this mechanism may increase androgen levels and improve certain aspects of male reproductive function, the elevated CREM expression observed in this group suggests that clomiphene may not effectively correct the specific transcriptional imbalance induced by paroxetine. Instead, the increased gonadotropin stimulation may have further enhanced testicular transcriptional activity, thereby amplifying CREM expression.

Protamine proteins represent another essential component of spermatogenesis and are directly involved in the final stages of sperm chromatin condensation. During spermiogenesis, histones in the spermatid nucleus are progressively replaced by transition proteins and ultimately by protamines, leading to highly compacted chromatin that protects paternal DNA during fertilization. PRM I and PRM II are the two major protamines present in mammalian sperm, and their precise expression ratio is critical for maintaining proper chromatin structure and sperm function (Oliva, 2006; Jodar & Oliva, 2014).

In the present study, paroxetine administration resulted in significant increases in the expression of both PRM I and PRM II relative to the control group. This finding indicates that paroxetine may disrupt the regulatory pathways governing chromatin remodeling during spermiogenesis. Although protamines are essential for DNA condensation,

their expression must be tightly coordinated. Excessive or dysregulated expression can lead to abnormal chromatin packaging, which may compromise sperm morphology, motility, and fertilizing ability.

The PRM I/PRM II ratio is widely regarded as a sensitive biomarker of sperm chromatin integrity. In healthy individuals, this ratio remains relatively stable; however, alterations in the ratio have been associated with defective spermatogenesis and male infertility (Steger & Balhorn, 2018; Pardede *et al.*, 2020). In the current study, paroxetine increased the PRM I/PRM II ratio from 0.51 in the control group to 0.88 in the negative control group, indicating disruption of the normal balance between the two protamines. Such imbalance may impair chromatin condensation and increase susceptibility of sperm DNA to damage. Treatment with ITREE partially corrected this imbalance by reducing the PRM I/PRM II ratio to 0.76, suggesting an improvement in chromatin packaging dynamics. This observation supports the potential protective role of ITREE in maintaining the molecular processes required for proper sperm maturation. Similarly, clomiphene administration resulted in a moderate reduction of the ratio to 0.84, indicating partial normalization. In contrast, tadalafil treatment produced a pronounced increase in the PRM I/PRM II ratio (1.23), which represents a greater deviation from the normal value observed in the control group. This finding suggests that while tadalafil may improve certain physiological aspects of reproductive function, its influence on the molecular regulation of protamine expression may be limited or indirect.

Overall, the findings of this study demonstrate that paroxetine induces significant alterations in the expression of key spermatogenic genes, including CREM, PRM I, and PRM II, thereby disrupting the molecular mechanisms responsible for sperm chromatin remodeling. Co-administration of ITREE was able to partially restore normal gene expression patterns and improve the balance between protamine proteins, indicating a protective effect on spermatogenic processes. When compared with the conventional therapeutic agents evaluated in this study, ITREE demonstrated a comparable or potentially superior ability to modulate the molecular markers associated with sperm maturation.

These results suggest that *Icacina trichantha* root ethanolic extract may represent a promising natural therapeutic candidate for mitigating drug-induced reproductive toxicity. Nevertheless, further investigations involving larger experimental models, protein-level analyses, and fertility outcome studies are necessary to elucidate the precise molecular mechanisms through which ITREE exerts its protective effects on male reproductive function.

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Declaration of Conflict of Interest

The authors declare no conflicts of interest

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