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Abatement of the dysfunctional hypothalamic–pituitary–gonadal axis due to ciprofloxacin administration by selenium in male rats

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Abstract

The present study examined the influence of selenium on ciprofloxacin-mediated reproductive dysfunction in rats. The research design consisted of five groups of eight animals each. The rats were administered 135 mg/kg body weight of ciprofloxacin per se or simultaneously with selenium at 0.25 and 0.5 mg/kg for 15 uninterrupted days. Antioxidant and inflammatory indices were assayed using the testes, epididymis, and hypothalamus of the animals after sacrifice. Results revealed that ciprofloxacin treatment per se interfered with the reproductive axis as demonstrated by diminished serum hormonal levels, sperm quality, and enzymatic indices of testicular function, which were, however, abrogated following selenium co-treatment. Besides this, administration of selenium attenuated the depletion of glutathione level, inhibition of catalase, superoxide dismutase, glutathione-*S*-transferase and glutathione peroxidase activities with a concomitant reduction in reactive oxygen and nitrogen species, and lipid peroxidation in ciprofloxacin-treated in rats. Selenium treatment also mitigated ciprofloxacin-mediated elevation in nitric oxide level and of myeloperoxidase activity as well as histological lesions in the animals. Overall, selenium attenuated impairment in the male reproductive axis due to ciprofloxacin treatment through abatement of inflammation and oxidative stress in rats.

KEYWORDS
antioxidant, ciprofloxacin, oxidative stress, reproductive axis, selenium

1 | INTRODUCTION

Ciprofloxacin is a broad-spectrum antibiotic in the family of fluoroquinolones. It is frequently used by fertility specialists and urologists to treat infections of the urinary tracts and reproductive tissues.[1,2] International guiding principles in the treatment of epididymo-orchitis patients actually aimed at apposite antimicrobials that can effectively penetrate epididymal and testicular tissues to exterminate causative microbes without damaging the tissue.[3] Unfortunately, infected patients treated with ciprofloxacin recovered from pathogenic effects but exhibited reduced normal sperm motility and morphology without improvement in sperm agglutination and leukocytospermia.[3,4]

Moreover, previous experimental studies demonstrated that chronic ciprofloxacin administration elicited male reproductive toxicity with significant Leydig cell degeneration.[5,6] Administration of ciprofloxacin reportedly decreased testis weight, testosterone level, and sperm quality in a dose- and time-dependent manner.[7]

The increasing microbial resistance to ciprofloxacin may be associated with its abuse, elevated tissue concentrations, and adverse effects on reproductive tissues. Besides this, the increase in reactive oxygen species (ROS) level during ciprofloxacin antibacterial action may contribute to its deleterious effects on testicular function.[8] Administration of ciprofloxacin decreased fertilization rate and delayed embryonic development through induction of sperm DNA damage and chromatin abnormalities in NMRI mice.[9] As a result, in consideration of the valuable impact of ciprofloxacin therapy on the reproductive health of humans, research into the favorable antidotes for toxicities associated with ciprofloxacin therapy is warranted.

Selenium is an important trace element in human and animal nutrition. Dietary selenium recommendations for humans ranged from 30 to 134 μg/day for adults.[10] Seafood and meat are largely acknowledged main sources of dietary selenium for humans.[11] Inorganic selenium exists as selenates and selenites in water and dietary supplements. Selenium participates in many physiological functions namely immune response, fertility, and thyroid hormone metabolism in animals and humans.[12] Moreover, the three isoforms of iodothyronine deiodinases (DIO1, DIO2, and DIO3) are selenoproteins responsible for the activation and inactivation of thyroid hormones.[13,14] The physiological role of selenium is played almost exclusively by selenoproteins. An appropriate selenium concentration is necessary for the biosynthesis of near 24–25 selenoproteins in rats and selenoenzymes (i.e., thioredoxin reductase and glutathione peroxidase) and maintenance of cellular redox status. Indeed, selenium-biofortified foods reportedly afforded better protection against xenobiotics toxicity and diseases than unfortified foods.[15]

Beneficial effects of selenium on mammalian reproduction include enhancement of sperm production, sperm mobility, and testosterone biosynthesis [16] whereas its deficiency reportedly resulted in a diminution of sperm functional characteristics.[17] The critical role of Gpx4, formerly called phospholipid hydroperoxide glutathione peroxidase, in spermatozoid physiology is associated with the functions of the three different isoforms. The cytosolic form is responsible for the inhibition of interleukin-1-driven nuclear factor κB activation and leukotriene biosynthesis as well as ferroptosis regulation. The nuclear form of Gpx4 is essential for chromatin compaction whereas the mitochondrial GPx4 produces the mitochondrial sheath of spermatozoid and, consequently, secures male fertility.[18–20] The versatility of selenium in biomedical applications has been shown in several studies assessing the antioxidant, antimicrobial and anticancer activities of selenium nanoparticles.[21] Indeed, selenium nanoparticles reportedly improved immune response and stress toughness in fish [22] as well as augmented blood perfusion, reproductive hormones, and hematobiochemical parameters in goats.[23]

The male reproductive axis consists of the hypothalamus, pituitary gland, and testes, which are collectively referred to as the hypothalamic–pituitary–gonadal (HPG) axis.[24] Specifically, the hypothalamus discharges gonadotropin-releasing hormone which excites the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland. Subsequently, LH stimulates testicular Leydig cells to produce testosterone which acts cooperatively with FSH to activate spermatogenesis by stimulating the Sertoli cells.[25,26] The HPG axis is principally responsible for normal reproductive function by regulating endocrine functions and sperm production whereas sperm storage and maturation occur in the epididymis.[27]

The limited reports available on the influence of selenium on ciprofloxacin effectiveness in vitro demonstrated that sodium selenite markedly enhanced the sensitivity of *Clostridium difficile* to ciprofloxacin leading to reduced bacterial growth in samples compared with antibiotic alone.[28] The greater effect of ciprofloxacin loaded selenium-lipid nanoparticle in exterminating bacterial cells than the control has been attributed to its strong antimicrobial and antioxidant activities in the treatment of lung infection or diseases.[29] Moreover, coadministration of selenium and ciprofloxacin elicited a greater preventive effect on chronic bacterial prostatitis than ciprofloxacin alone in rats.[30] Thus far, there is a lack of scientific evidence on the influence of selenium on reproductive toxicity due to ciprofloxacin treatment. It is true that antibiotics, for example, ciprofloxacin, are used in the presence of infection with bacteria. However, the normal cells of the reproductive tissues are not spared of the adverse effects resulting from antibiotic therapy. We, therefore, hypothesized that selenium may render protection to reproductive tissues in patients undergoing ciprofloxacin therapy by possibly modulating biochemical and endocrine changes due to ciprofloxacin treatment.

This study was designed to examine, for the first time, the role of selenium on ciprofloxacin-associated reproductive dysfunction in rats by assessing hormonal concentrations, marker enzymes of testicular function, antioxidant enzyme activities, oxidative stress parameters as well as a histological examination of the epididymis, testes and hypothalamus.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Sodium selenite (Na_2SeO_3), ciprofloxacin, 5,5-dithio-bis-2-nitrobenzoic acid, 2',7'-dichlorodihydrofluorescein diacetate, thiobarbituric acid, 1-chloro-2,4-dinitrobenzene, and all other chemicals were procured from Sigma-Aldrich Chemical Company.

2.2 | Maintenance and handling of animals

Pubertal male Wistar rats (8 weeks old, 145–155 g weight) sourced from the Faculty of Veterinary Medicine, University of Ibadan were used for the current research. The animals were billeted in well-ventilated polycarbonate coops with ample wood shavings quantity as beddings. They were maintained under standard laboratory conditions of a 12 h light:12 h dark photocycle, provided water and

rodent provender ad libitum and allowed to adapt for seven days before commencing the treatment. Animal care and handling during this experimentation were executed in conformity with the authorized rules of the University of Ibadan Ethical Committee and the U.S. National Institute of Health.

2.3 | Design of experiment

Five groups of eight animals each were administered for 15 uninterrupted days as indicated below.

Control: Animals administered 2 mL/kg body weight (bw) of corn oil alone.

Se alone: Animals administered 0.5 mg/kg bw of selenium (Se) alone.

CPFX alone: Animals administered 135 mg/kg of ciprofloxacin alone.

CPFX + Se1: Animals administered 135 mg/kg of ciprofloxacin and 0.25 mg/kg of selenium.

CPFX + Se2: Animals administered 135 mg/kg of ciprofloxacin and 0.5 mg/kg of selenium.

Selenium and ciprofloxacin doses administered in the present study were chosen from previously published articles and pilot studies.[31,32] Moreover, the selected doses of selenium have been established in the literature to effectively protected against oxidative damage, prostatitis, and neurotoxicity induced by various toxicants in rats.[33–35] The animals were weighed 24 h after the final treatment while the blood collected from the animals was processed to get the serum which was subsequently used for hormonal assays. The animals were sacrificed using light ether anesthesia. The epididymis, testes, and hypothalamus were cautiously excised, weighed, and processed for biochemical assays and histological examination.

2.4 | Analysis of sperm characteristics

Motility of sperm cells was assessed as previously described[36] whereas epididymal sperm number was done using the established procedure.[37] Viability and morphological defects of sperm cells were assessed as previously described.[32,38]

2.5 | Assay of hormones from pituitary and testes

To assess the endocrine function integrity in the rats, serum concentrations of FSH, LH, and prolactin from the pituitary gland as well as testosterone from the testes were analyzed using rats' specific ELISA kits from Elabscience Biotechnology Company. Precisely, testosterone (E-EL-R0033), prolactin (E-EL-R0052), FSH (E-EL-R0391), and LH (E-EL-R0026) were analyzed following the manufacturer's guidelines. The sensitivities of testosterone and prolactin were 0.39 and 0.22 ng, while FSH and LH were 0.25 and 0.37 ng, correspondingly. The intra-assay coefficients of variations for testosterone, FSH, prolactin, and LH were 3.1%, 2.6%, 3.3%, and 2.8%, correspondingly.

2.6 | Sample processing for biochemical assays

Epididymis, testes, and hypothalamus excised from the rats were homogenized in 50 mM Tris–HCl buffer of pH 7.4. The supernatants obtained from the centrifugation (12,000*g* for 15 min) of homogenates were used to assays biochemical endpoints. The concentration of protein in the epididymis, testes, and hypothalamus was assayed using Bradford method.[39]

2.7 | Testicular function marker enzymes assay

Analyses of marker enzymes of testicular function were done in the testes supernatants. The activity of glucose-6-phosphate dehydrogenase (G6PD) was analyzed as previously described.[40] Lactate dehydrogenase-X (LDH-X) was analyzed using an established procedure.[41] Acid phosphatase (ACP) activity was assayed in agreement with the established method[27] whereas alkaline phosphatase (ALP) activity was assayed as previously described.[42]

2.8 | Analyses of antioxidant enzyme activities and oxido-inflammatory markers

All other biochemical assays were carried out using a SpectraMax plate reader (Molecular Device) except for activities of superoxide dismutase (SOD) and catalase (CAT) which were done with the aid of 752S UV–VIS Spectrophotometer (Ningbo). SOD and CAT activities were analyzed in agreement with Misra and Fridovich[43] and Claiborne,[44] correspondingly. Glutathione-*S*-transferase (GST) and glutathione peroxidase (GPx) were analyzed in agreement with Habig et al.[45] and Rotruck et al.,[46] correspondingly. The level of glutathione (GSH) was analyzed in agreement with Jollow et al.[47] Additionally, oxidative stress markers such as levels of reactive oxygen and nitrogen species (RONS) and lipid peroxidation (LPO) were analyzed in agreement with Adedara et al.,[48] and Farombi et al.[49] correspondingly. Inflammatory indices such as level of nitric oxide (NO) and myeloperoxidase (MPO) activity were analyzed in agreement with Green et al.[50] and Granell et al.,[51] correspondingly.

2.9 | Microscopic assessment of tissues

Microscopic assessment of the epididymis, testes, and hypothalamus histology was executed in agreement with Bancroft and Gamble.[52] In brief, following fixation of tissues in Bouin's solution, each tissue was separately dehydrated, paraffin-embedded, and then cut using microtome into five micrometers sections. The sections were then stained with hematoxylin and eosin on clean slides before examination

using a light microscope (Leica DM 500) and digital camera (Leica ICC50 E) by pathologists who had no idea about the treatments (i.e., blinded to the treatments). Semiquantitative assessment of lesions identified in the testes were scored as follow: 0, negligible alterations (i.e., less than 5% of tubules affected); 1, slight alterations (i.e., 5%–25% tubules affected); 2, moderate alterations (i.e., 25%–50% tubules affected); 3, marked alterations, (i.e., 50%–75% tubules affected) and 4, severe alterations (i.e., more than 75% tubules affected). The histopathological grading of lesions in caudal epididymis were as follows: 0, no visible effect; 1, slight alterations, normal sperm count with 5–10 necrotic cells in the efferent ductules; 2, moderate alterations, moderate sperm count decrease with 11–50 necrotic cells; 3, marked alterations, marked sperm count decrease with more than 50 necrotic cells; 4, severe alterations with a marked reduction in sperm count in the efferent ducts. The viable and degenerated neurons of the hypothalamus were counted using a graticule and a microscope at different magnifications. Viable neurons possess unique nucleoli, dispersed chromatin without cell death features precisely pyknosis and karyolysis at high power. The number of degenerated neurons was expressed as percentages.

2.10 | Statistical analyses

One-way analysis of variance and Bonferroni's post hoc test using GRAPHPAD PRISM 5 software (Version 4; GraphPad Software) were used to analyze the data of the current investigation. Values of $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Selenium treatment curbed oxidative damage due to ciprofloxacin administration in rats

Figure 1 shows the levels of epididymal, testicular, and hypothalamic RONS and malondialdehyde (MDA) in control, selenium alone, ciprofloxacin alone, and groups co-treated with ciprofloxacin and selenium. Administration of selenium alone did not significantly alter oxidative stress markers compared with the control. Ciprofloxacin alone treatment elevated epididymal, testicular, and hypothalamic RONS and MDA levels in the rats compared with the control. On the other hand, selenium treatment at 0.25 and 0.5 mg/kg significantly diminished the levels of epididymal, testicular, and hypothalamic RONS and MDA in ciprofloxacin-exposed rats.

3.2 | Selenium treatment augments cellular redox status in ciprofloxacin-exposed rats

Figures 2–4 show epididymal, testicular and hypothalamic antioxidant enzyme activities and GSH level in control, selenium alone, ciprofloxacin alone, and groups co-treated with selenium and ciprofloxacin. Administration of selenium alone did not significantly modify cellular redox status compared with the control. However, ciprofloxacin treatment alone significantly reduced the activities of SOD, GPx, CAT, and GST cum GSH level in the epididymal, testicular, and hypothalamic tissues compared with the control. Selenium treatment at 0.25 and

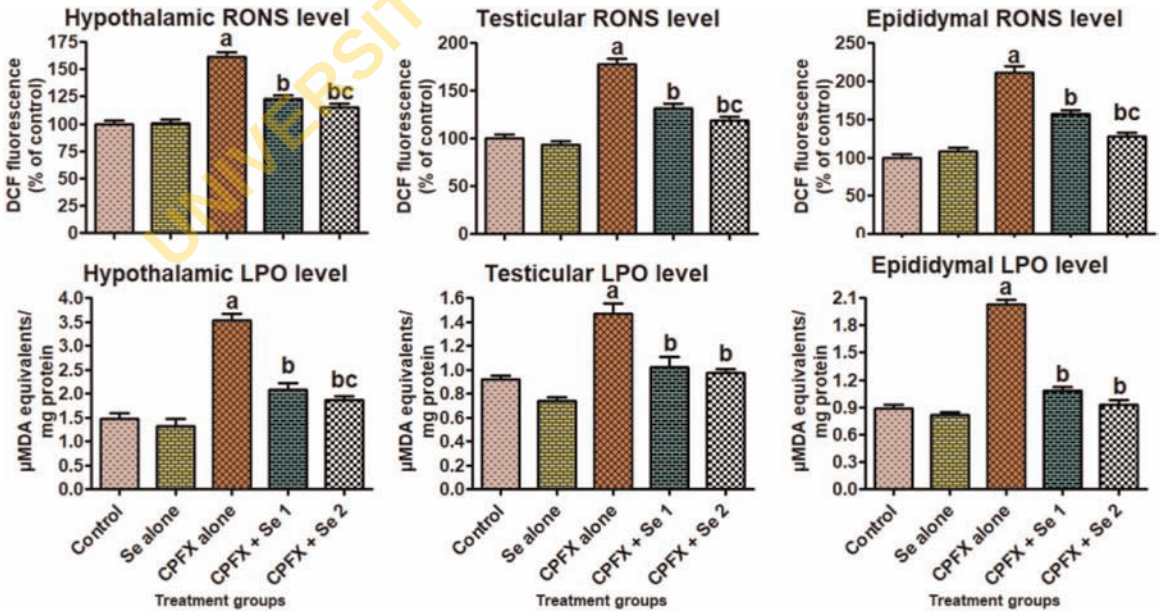

FIGURE 1 Role of selenium on testicular, epididymal, and hypothalamic levels of reactive oxygen and nitrogen species (RONS) and lipid peroxidation (LPO) in ciprofloxacin-treated rats. CPFX stands for ciprofloxacin at 135 mg/kg; Se stands for selenium alone (0.5 mg/kg); Se1 stands for selenium at 0.25 mg/kg; Se2 stands for selenium at 0.50 mg/kg. Results are presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

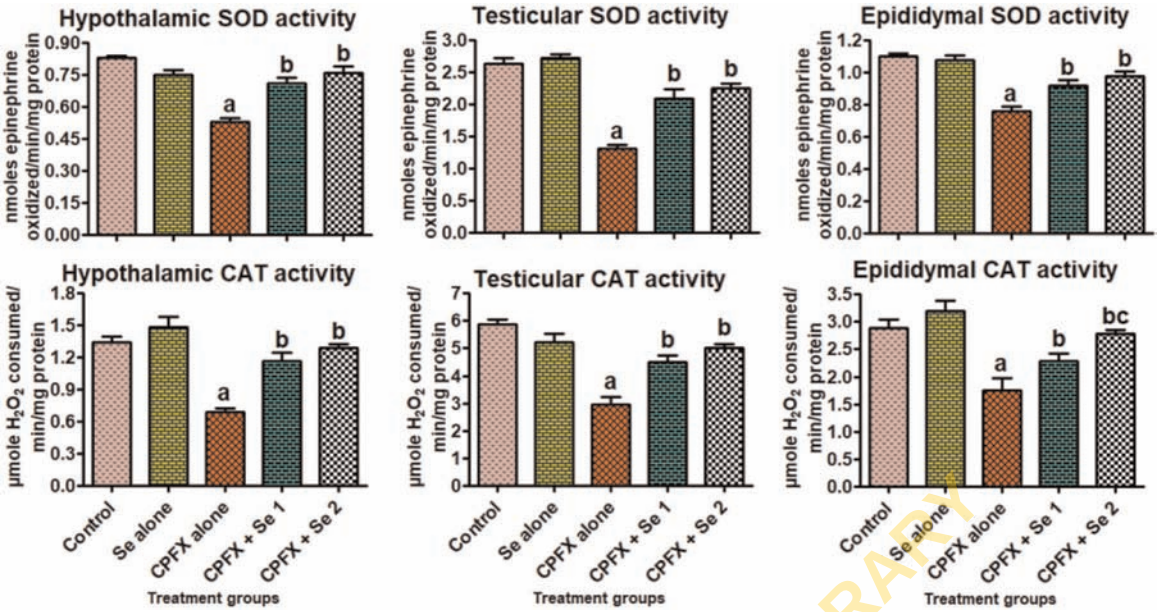

FIGURE 2 Role of selenium on testicular, epididymal and hypothalamic superoxide dismutase (SOD) and catalase (CAT) activities in ciprofloxacin-treated rats. CPFX stands for ciprofloxacin at 135 mg/kg; Se stands for selenium alone (0.5 mg/kg); Se1 stands for selenium at 0.25 mg/kg; Se2 stands for selenium at 0.50 mg/kg. Results are presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

0.5 mg/kg significantly augmented cellular redox status in the epididymal, testicular, and hypothalamic tissues to normalcy in ciprofloxacin-treated animals compared with the control.

3.3 | Selenium modulated inflammatory biomarkers in ciprofloxacin-treated rats

Figure 5 shows NO level and MPO activity in the epididymal, testicular, and hypothalamic tissues of rats in the control, selenium alone, ciprofloxacin alone, and groups co-treated with selenium and ciprofloxacin. Selenium alone did not significantly alter inflammatory markers namely NO level and MPO activity compared with the control. However, ciprofloxacin treatment alone significantly elevated epididymal, testicular and hypothalamic NO level and MPO activity in the rats compared with the control. Selenium treatment at 0.25 and 0.5 mg/kg significantly diminished the levels of epididymal, testicular, and hypothalamic NO level and MPO activity in ciprofloxacin-treated rats.

3.4 | Selenium assuaged deficits in endocrine and sperm quality in ciprofloxacin-treated rats

Figure 6 shows the serum concentrations of hormones as well as sperm functional indices in control, selenium alone, ciprofloxacin alone, and groups co-treated with ciprofloxacin and selenium. Administration of selenium alone did not significantly affect endocrine and sperm quality when compared with the control. Ciprofloxacin alone treatment caused a significant decrease in the serum of testosterone, LH, FSH, prolactin concentrations in comparison with control. Also, rats administered ciprofloxacin singly exhibited a significant decrease in sperm count and forward mobility along with a marked increase in sperm morphological defects compared with control. Aberrations in the sperm morphology of rats administered ciprofloxacin alone were largely curved mid-pieces and bent tails. However, the endocrine and sperm quality of rats treated with ciprofloxacin and selenium at 0.25 and 0.5 mg/kg were significantly improved compared with rats administered ciprofloxacin alone.

3.5 | Selenium treatment enhanced enzymatic indicators of testicular function in ciprofloxacin-exposed rats

Figure 7 shows enzymatic indicators of testicular function in control, selenium alone, ciprofloxacin alone, and groups co-treated with selenium and ciprofloxacin. Administration of selenium alone did not significantly increase testicular activities of LDH, G6PD, ALP, and ACP activities compared with the control. Ciprofloxacin per se induced testicular toxicity by considerably diminishing testicular activities of LDH, G6PD, ALP, and ACP compared with the control whereas their activities were significantly improved in rats administered ciprofloxacin and selenium at 0.25 and 0.5 mg/kg compared with ciprofloxacin alone.

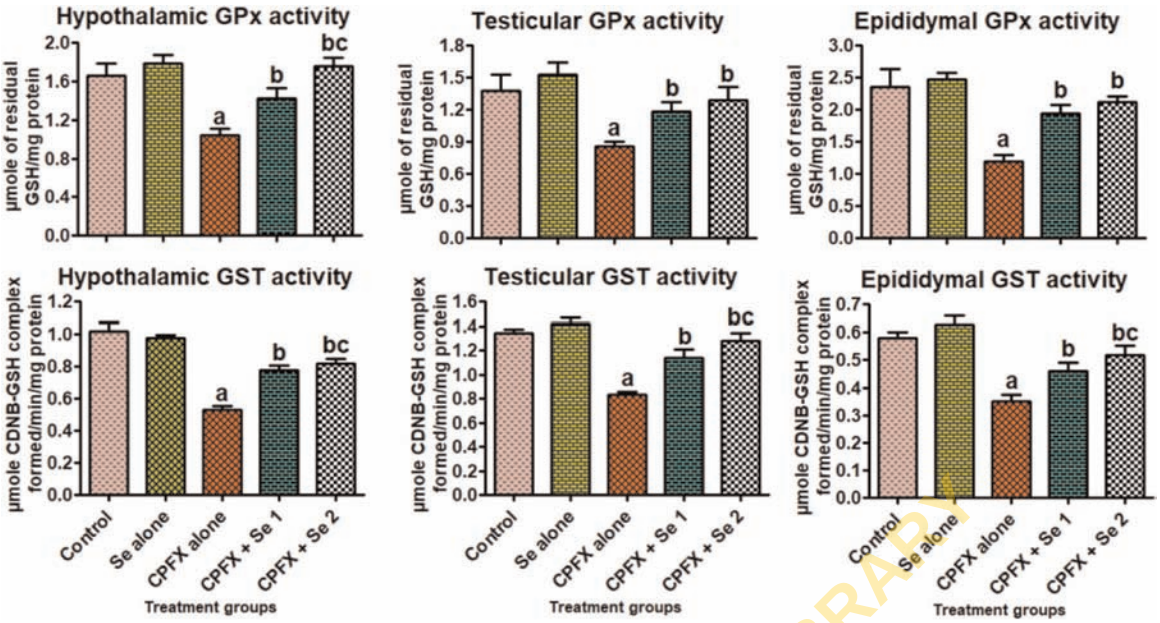

FIGURE 3 Role of selenium on testicular, epididymal, and hypothalamic glutathione-*S*-transferase (GST) and glutathione peroxidase (GPx) activities in ciprofloxacin-treated rats. CPFX stands for ciprofloxacin at 135 mg/kg; Se stands for selenium alone (0.5 mg/kg); Se1 stands for selenium at 0.25 mg/kg; Se2 stands for selenium at 0.50 mg/kg. Results are presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

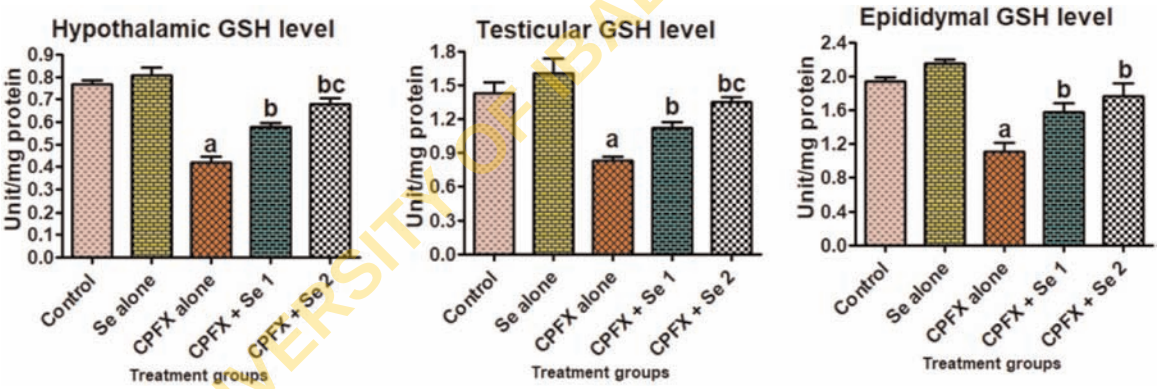

FIGURE 4 Role of selenium on testicular, epididymal, and hypothalamic glutathione (GSH) level in ciprofloxacin-treated rats. CPFX stands for ciprofloxacin at 135 mg/kg; Se stands for selenium alone (0.5 mg/kg); Se1 stands for selenium at 0.25 mg/kg; Se2 stands for selenium at 0.50 mg/kg. Results are presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

3.6 | Selenium treatment modulated histological injury in ciprofloxacin-treated rats

Figures 8–10 show representative histological features and semi-quantitative data of epididymal, testicular, and hypothalamic tissues from rats in the control, selenium alone, ciprofloxacin alone, and groups co-treated with ciprofloxacin and selenium. Histological features of the epididymis, testes, and hypothalamus from control and selenium alone groups were normal. Animals administered ciprofloxacin singly exhibited marked neuronal degeneration and extensive area of vacuolation of the neuropil in the hypothalamus, tubular atrophy with inadequate sperm cells in the lumen of the seminiferous tubules whereas epididymal tubules contained reduced sperm cells with marked degeneration. Histological features of epididymal, testicular, and hypothalamic tissues of rats co-treated with ciprofloxacin and selenium at 0.25 and 0.50 mg/kg were comparable with the control group.

4 | DISCUSSION

The novel findings from the current investigation revealed that selenium at 0.25 and 0.5 mg/kg efficiently abrogated endocrine deficit, sperm toxicity, oxidative damage, inflammatory response, and

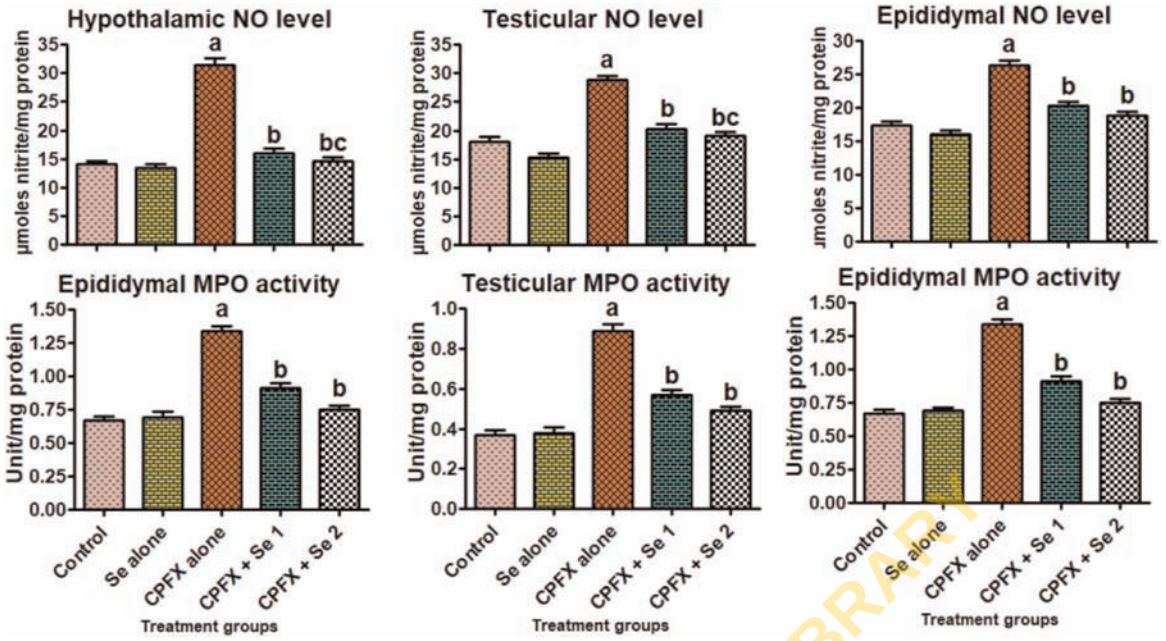

FIGURE 5 Role of selenium on testicular, epididymal, and hypothalamic nitric oxide (NO) and myeloperoxidase (MPO) activity in ciprofloxacin-treated rats. CPFX stands for ciprofloxacin at 135 mg/kg; Se stands for selenium alone (0.5 mg/kg); Se1 stands for selenium at 0.25 mg/kg; Se2 stands for selenium at 0.50 mg/kg. Results are presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

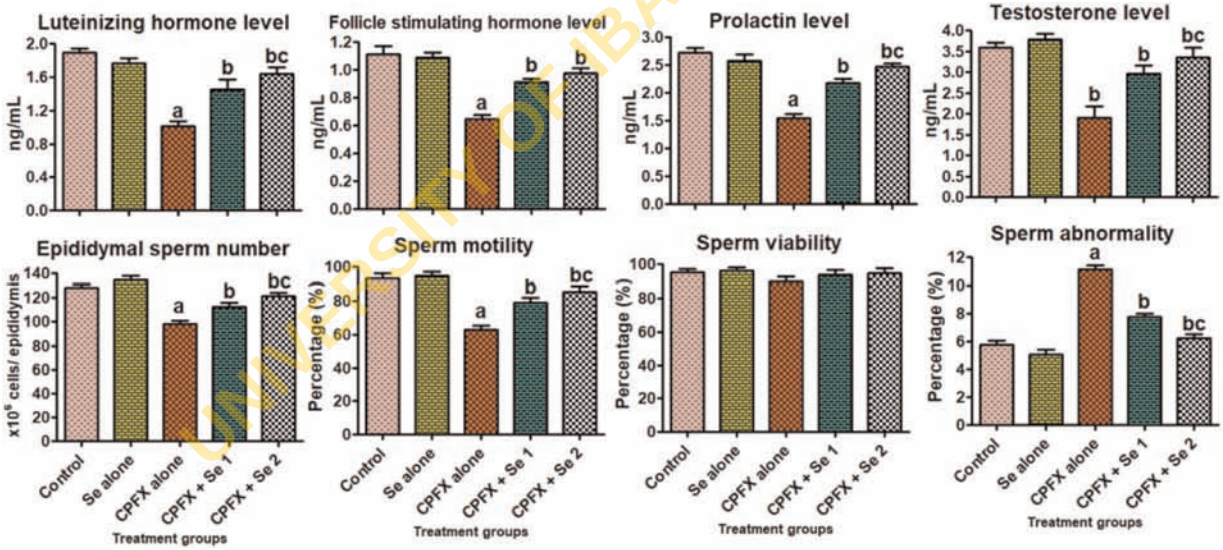

FIGURE 6 Role of selenium on the endocrine and spermiogram in ciprofloxacin-treated rats. CPFX stands for ciprofloxacin at 135 mg/kg; Se stands for selenium alone (0.5 mg/kg); Se1 stands for selenium at 0.25 mg/kg; Se2 stands for selenium at 0.50 mg/kg. Results are presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

histological lesions associated with ciprofloxacin treatment in rats. Enzymatic and nonenzymatic antioxidants are responsible for the continuous inactivation of ROS to retain small concentration required to sustain normal cellular activities. However, disproportionate production of ROS causes oxidative destruction of the membrane phospholipids to finally produce 4-hydroxylnonenal and malodialdehyde, which further propagates cellular injury.[53] Administration of ciprofloxacin per se elevated the levels of RONS and MDA in the hypothalamic, epididymal, and testicular tissues in the current research, thus indicating the involvement of oxidative stress in ciprofloxacin-mediated reproductive toxicity in rats. Earlier studies have demonstrated involvement of oxidative stress in ciprofloxacin-induced testicular damage in mice.[8] The abatement of RONS and MDA levels subsequent to selenium treatment might be

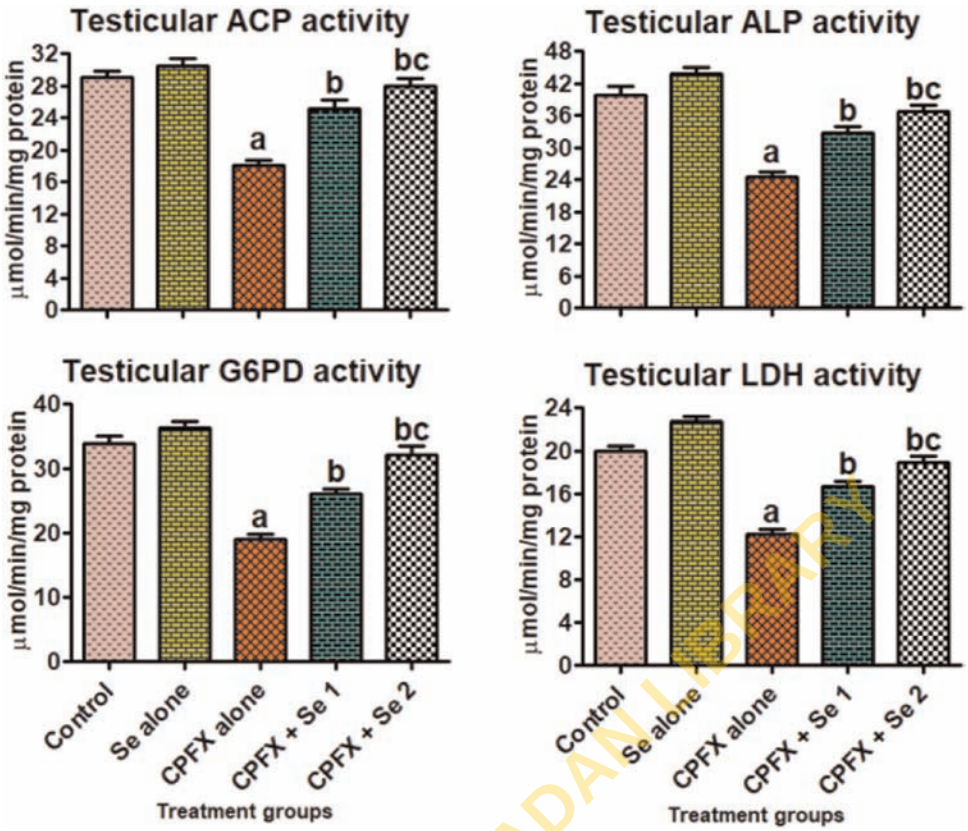

FIGURE 7 Role of selenium on enzymatic indices of testicular function in ciprofloxacin-treated rats. CPFX stands for ciprofloxacin at 135 mg/kg; Se stands for selenium alone (0.5 mg/kg); Se1 stands for selenium at 0.25 mg/kg; Se2 stands for selenium at 0.50 mg/kg. Results are presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

linked to its anti-peroxidative and antioxidant activities of selenium previously reported.[54]

Additionally, the diminution in the hypothalamic, epididymal, and testicular activities of SOD and CAT following treatment with ciprofloxacin indicates inhibition of their antioxidant role which may consequently escalate the level of oxidants namely hydrogen peroxide, superoxide radicals, and hydroxyl radical generation through Haber-Weiss reaction. The preservation of cellular redox status, GSH-dependent enzyme functions and detoxification of xenobiotics within the aqueous portion of the cell is largely achieved by GSH.[55] Thus, significant diminution in the hypothalamic, epididymal and testicular GSH level as well as GPx and GST activities in rats administered ciprofloxacin per se in the present investigation indicates over utilization of GSH and inhibition of these GSH-dependent enzymes which consequently can cause oxidative damage. Overproduction of hydrogen and lipid peroxides reportedly results in GSH depletion and consequently, inhibition of GSH-dependent enzymes due to reduced substrate availability.[56] However, the significant restoration of these cellular antioxidants in hypothalamus, epididymis, and testes of animals co-treated with selenium indicates its antioxidant effect in the animals.

Inducible nitric oxide synthase is normally activated to synthesize NO and cytokines during cellular inflammatory response.[57] In fact, moderate tissue NO level plays a key role in cellular and molecular signaling during normal physiological conditions whereas excessive NO production following exposure to xenobiotics is well known to cause nitration of tyrosine residues in proteins leading to protein dysfunction and interference with signal transduction pathways.[58] Furthermore, MPO in addition to having cytokine-related activity is well known to activate neutrophils leading to induction of inflammation and reactive oxygen species production.[59] Thus, a marked increase in MPO activity and NO level in the hypothalamic, epididymal, and testicular tissues of animals treated with ciprofloxacin per se indicate induction of oxido-inflammatory stress in the animals. However, the significant reduction in the NO level and MPO activity in the hypothalamic, testicular, and epididymal tissues subsequent to selenium treatment indicates its protective influence on inflammatory response induced by ciprofloxacin in the animals.

The marked wane in the serum LH, FSH, and prolactin levels in rats administered ciprofloxacin per se in the current investigation indicates the interference of the drug with the pituitary function. Besides this, prolactin plays a pivotal role in testosterone and sperm production by enhancing the sensitivity of LH receptors on the Leydig cells to LH.[60,61] The decrease in serum prolactin concentration following administration of ciprofloxacin per se denotes

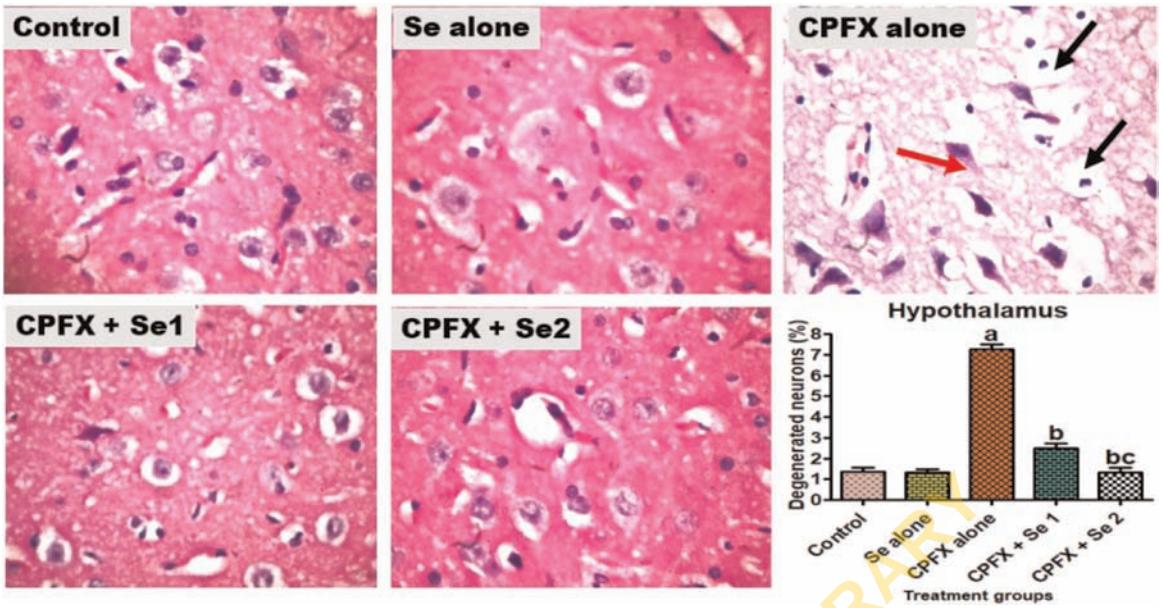

FIGURE 8 Representative hypothalamus sections. The hypothalamus of control and Se alone-treated rats showed normal morphology. The hypothalamus of animals treated with ciprofloxacin per se showing marked neuronal degeneration (red arrow) and extensive area of vacuolation of the neuropil (black arrow). Hypothalamus morphology of rats co-treated with ciprofloxacin and Se at 0.25 mg/kg (Se 1) and 0.5 mg/kg (Se 2) appear normal similar to control. Magnification, ×400. The result of the semiquantitative analysis is presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

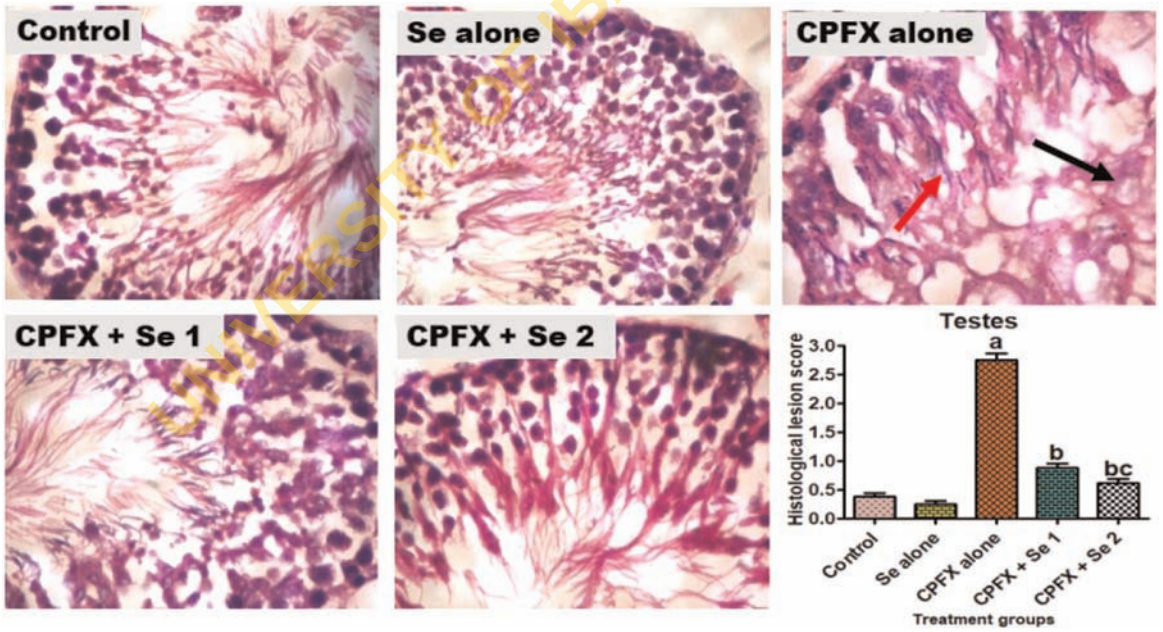

FIGURE 9 Representative testes sections. Control and selenium alone-treated animals showed normal morphology of the testes. The testes of animals treated with ciprofloxacin per se showing tubular atrophy (black arrows) with inadequate sperm cells in the lumen (red arrow) of the seminiferous tubules. Testicular morphology of animals co-treated with ciprofloxacin and selenium at 0.25 mg/kg (Se 2) and 0.5 mg/kg (Se 2) appear normal with adequate sperm cells. Magnification, ×400. The result of the semiquantitative analysis is presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

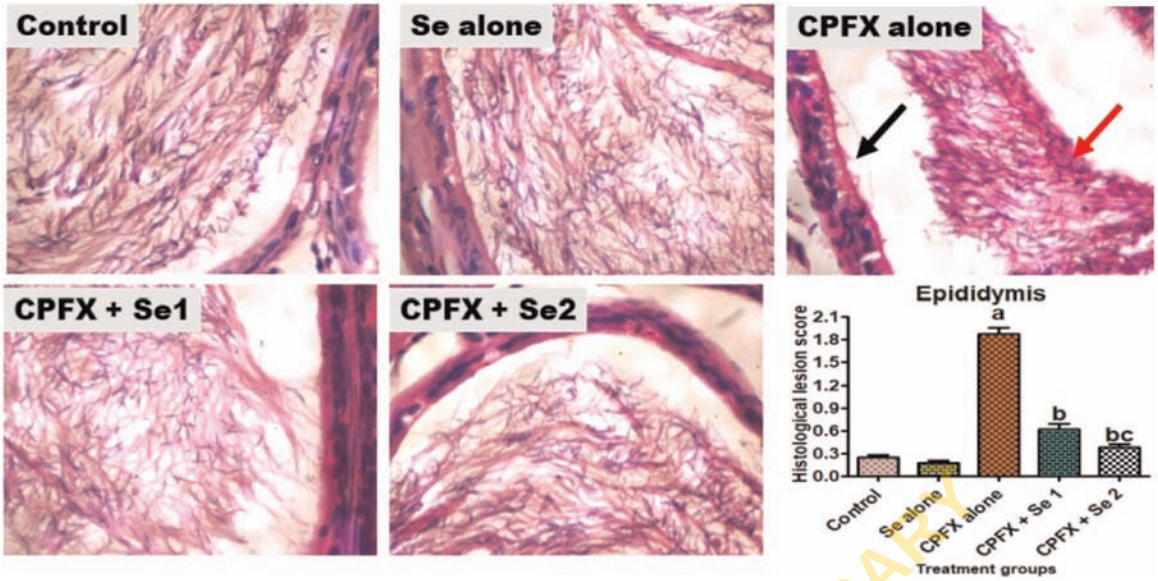

FIGURE 10 Representative epididymal sections. The epididymis of control and selenium alone-treated rats showed normal morphology. The epididymis of animals treated with ciprofloxacin per se had tubules containing reduced sperm cells (red arrow) with marked degeneration (black arrow). Epididymis of rats co-treated with ciprofloxacin and selenium at 0.25 mg/kg (Se 1) and 0.5 mg/kg (Se 2) appear normal similar to control. Magnification, ×400. The result of the semiquantitative analysis is presented as mean ± *SD* for eight rats per group. [a]Values differ significantly from control ($p < .05$). [b]Values differ significantly from CPFX alone ($p < .05$). [c]Values differ significantly from CPFX + Se1 ($p < .05$)

the direct inhibitory effect of ciprofloxacin on the pituitary to produce prolactin, which consequently may contribute to reduced testosterone production by testicular Leydig cells in the rats. This observation bared the likely interference of ciprofloxacin with the pituitary–testicular axis in the control of spermatogenesis in animals. The observed diminution in serum reproductive hormones in ciprofloxacin-treated rats is in agreement with previous studies in mice.[6] However, the restoration of the testosterone, FSH, prolactin, and LH levels subsequent to selenium treatment revealed the protective effects of selenium on reproductive endocrine shortage due to ciprofloxacin in the exposed animals.

Moreover, the reduction of sperm characteristics detected in rats administered ciprofloxacin alone in the current research may result from the low hormonal levels, thus indicating an indirect consequence of ciprofloxacin on testicular function. Sperm count is an acknowledged sensitive index for appraising spermatogenesis in both animal and humans because it represents all phases of spermiogenesis, meiosis, and maturation.[36] The reduction in mobility and concentration of sperm along with elevated sperm defects following ciprofloxacin treatment in the current research evidenced an adverse impact of oxidative stress on the reproductive function in the treated rats. Oxidative stress is an acknowledged contributing factor to defective sperm function. The three major mechanisms associated with oxidative stress-induced infertility in males reportedly include reduced sperm motility, impairment of fertilization, and oxidative DNA damage.[62] Administration of ciprofloxacin adversely impacted the mid-piece and tail segments of the rats in the present investigation. Elevated abnormalities in sperm morphology due to ciprofloxacin treatment may be associated with induction of oxidative stress in the animals. Oxidative damage to the sperm tail which shields the structural machineries directly responsible for sperm mobility, and the mid-piece where the mitochondria that produce cellular energy for sperm reside may consequently diminish the fertilization of the ovum by the sperm.[63] Our findings on ciprofloxacin-induced sperm toxicity corroborate previous studies.[64] However, the re-establishment of sperm functional parameters subsequent selenium co-treatment reveals the protective effect of selenium on spermatotoxicity induced by ciprofloxacin in rats.

Testicular activities of metabolic enzymes namely G6PD, LDH, ACP, and ALP are crucial in the preservation of normal germ cell development and sperm production. These enzymes are acknowledged indices of testicular function commonly assessed in reproductive toxicology.[40] Testicular ALP hydrolyses 6-phosphoglucose, an intermediate in the glycolytic pathway, to free glucose which is essential for the proliferation and differentiation of spermatogenic cells. Testicular ACP activity is largely linked to functionality and survival of spermatogonial stem cell.[27] Hence, the reduction in ALP and ACP activities in rats treated with ciprofloxacin per se indicates an inhibition of their roles in the testicular reutilization of glucose and spermatogenic cell division in the animals. Additionally, the significant reduction in LDH activity in rats treated with ciprofloxacin per se may impede its crucial roles in lactate metabolism and spermatogenic cells maturation. The reduction in the testicular G6PD activity in animals treated with ciprofloxacin per se may interfere with NADPH synthesis by the pentose phosphate pathway which consequently, may account GSH depletion in the testes of the treated animals. The remarkable upturn in these marker enzymes of testicular function in rats following treatment with selenium

demonstrates the protective influence of selenium on gonadal toxicity associated with ciprofloxacin treatment in rats. Furthermore, the ameliorative effects of selenium on the histological lesions induced by ciprofloxacin treatment in the hypothalamus, testes, and epididymis substantiates the biochemical data in the current investigation.

The current data accentuate the reproductive health effects of selenium in ciprofloxacin-treated rats. Selenium through anti-inflammatory and antioxidant response mechanisms modulated ciprofloxacin-induced reproductive dysfunction. Thus, selenium may be a favorable trace element to improve male reproductive health during ciprofloxacin therapy.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The original data and materials of the current study are available with the corresponding author and would be made available on justifiable request.

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