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Research Paper

Spirulina Platensis Attenuates Thiamethoxam-Induced Testicular Oxidative Injury and Reproductive Dysfunction in Male Wistar Rats

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ABSTRACT

Background: Thiamethoxam is a neonicotinoid insecticide associated with experimental reproductive toxicity. This study evaluated whether antioxidant-rich *Spirulina platensis* attenuated thiamethoxam-associated biochemical, endocrine, sperm, and histological alterations in male rats.

Methods: We randomized 40 male Wistar rats into control, Spirulina, thiamethoxam, or combined-treatment groups (n = 10). We administered oral Spirulina (800 mg/kg body weight/day) and thiamethoxam (156 mg/kg body weight/day) for three weeks. We assessed testicular oxidative markers, reduced glutathione levels, antioxidant enzyme activities, serum reproductive hormone levels, epididymal sperm characteristics, and testicular histology.

Results: Thiamethoxam increased testicular lipid peroxidation and hydrogen peroxide levels while decreasing glutathione levels and antioxidant enzyme activities. Testosterone levels decreased, whereas follicle-stimulating hormone and luteinizing hormone levels increased. Sperm motility declined from 77.14% in controls to 18.71% following thiamethoxam exposure, and the proportion of abnormal sperm increased from 7.14% to 45.71%. Combined treatment improved motility to 42.57% and reduced abnormalities to 31.43%. Thiamethoxam disrupted seminiferous architecture and spermatogenic organization, with an apparent reduction in Sertoli and germ cells. *Spirulina* coadministration improved biochemical and endocrine profiles and partially preserved spermatogenesis, although residual degenerative changes remained. *Spirulina* alone preserved testicular morphology and caused only minor changes in hormones and sperm.

Conclusion: Spirulina partially attenuated thiamethoxam-associated testicular oxidative injury and reproductive dysfunction, supporting its protective potential under controlled experimental conditions.

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Introduction

Neonicotinoid insecticides are widely used in agricultural pest management because of their systemic activity and efficacy against economically important pests [1]. Thiamethoxam (TMX) acts at insect nicotinic acetylcholine receptors, engaging calcium-dependent neuronal signaling [2]. Receptor selectivity does not preclude mammalian toxicity; a systematic review identified reproductive injury and oxidative stress following non-acute neonicotinoid exposure in rodents [1].

Male reproductive function depends on antioxidant defenses, endocrine regulation, and seminiferous epithelial integrity. Superoxide dismutase, catalase, and glutathione peroxidase scavenge oxidants [3]. Thiobarbituric acid-reactive substances (TBARS) indicate lipid peroxidation [4], while reduced glutathione (GSH) maintains cellular redox homeostasis [5]. Evaluating these markers alongside reproductive hormones, sperm quality, and histology can provide complementary evidence of the functional and structural consequences of toxicant exposure [6, 7].

Oxidative stress and endocrine disruption are associated with TMX-induced reproductive toxicity in experimental mammals. Oral TMX in rats increases testicular lipid peroxidation and hydrogen peroxide levels, decreases GSH and antioxidant defenses, and causes oxidative DNA damage [6, 7]. Increased reactive oxygen species can damage sperm and testicular cells, linking disturbed redox homeostasis to impaired sperm quality and seminiferous degeneration. These changes are also connected with reproductive dysfunction due to the decrease in testosterone levels [6, 7]. Issa et al. [6] observed these changes after 8 weeks, and El-Demerdash et al. [7] after 21 days. Similar endocrine, sperm, and histological changes have been reported during thiacloprid exposure [8].

Cyanobacterial biomass from the phytoplankton species Spirulina platensis (SP), also known as Arthrospira or Limnospira platensis and commonly called blue-green algae, contains phycocyanin, carotenoids, proteins, and other bioactive compounds with reported antioxidant and anti-inflammatory activities [9]. Aqueous SP extract increased hepatic oxidative markers and antioxidant activity in a dexamethasone model and altered stress responses and apoptosis-related gene expression [10]. These findings support investigating *SP*-associated protection against tissue oxidative injury.

Reproductive models also support this rationale. *SP* improved oxidative, endocrine, sperm, and histological outcomes in rats exposed to bromoxynil-octanoate [11] and protected sperm quality and testicular morphology during hydrogen peroxide exposure [12]. Protective effects have also been reported in methotrexate and cisplatin models [13, 14]. Partial attenuation of renal oxidative injury during *SP* and TMX coadministration provides complementary evidence under a TMX exposure regimen [15].

Accordingly, this study evaluated whether oral *SP* at 800 mg/kg body weight attenuated TMX-associated reproductive alterations at 156 mg/kg body weight in male Wistar rats during a three-week intervention. We compared control, SP, TMX, and combined-treatment groups using testicular oxidative markers, antioxidant enzyme activities, reproductive hormones, epididymal sperm characteristics, and histology to assess whether biochemical improvement coincided with endocrine, functional, and structural preservation.

Materials and Methods

Chemicals and *Spirulina* preparation

We obtained Spirulina platensis powder from HerbaForce (United Kingdom). Thiamethoxam (reported purity 99.6%) was obtained from Sigma-Aldrich, United Kingdom. *We administered Spirulina* as an aqueous preparation. *We suspended Spirulina platensis* powder *in distilled water immediately before* administration. We dissolved thiamethoxam in distilled water to achieve the target concentration. We administered both treatments by oral gavage once daily at a standardized volume of 5 mL/kg body weight.

Animals, eligibility, and husbandry

We kept forty male Wistar albino rats aged 8–10 weeks and weighing 180–210 g at baseline for a two-week acclimatization period before allocation. Animals were housed in stainless steel cages in a temperature-controlled room (22 ± 2 °C; relative humidity, 50–60%) under a 12-hour light–dark cycle and were given standard laboratory chow and water ad libitum. The animal care was in accordance with the Guide for the Care and Use of Laboratory Animals, eighth edition. [16].

Inclusion and exclusion criteria

We selected healthy male animals based on normal activity and weight gain during acclimatization. We removed animals that were diseased, physically deformed, or had abnormal baseline behavior before randomization.

Table 1. Experimental treatment groups.

Group	Oral treatment	Dose	Animals
Control	Distilled water	Vehicle control	10
SP	*Spirulina platensis*	800 mg/kg body weight daily	10
TMX	Thiamethoxam	156 mg/kg body weight daily	10
SP + TMX	*Spirulina platensis* and thiamethoxam	800 and 156 mg/kg body weight daily, respectively	10

SP, *Spirulina platensis*; TMX, thiamethoxam.

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Experimental design and treatment

We divided rats into four groups of ten using computer-generated random numbers (Table 1). The control group received distilled water orally; the SP group received 800 mg/kg body weight/day of SP; the TMX group received 156 mg/kg body weight/day of TMX; and the combined group received both treatments at the same dose. The treatment was administered for three weeks. The TMX dose was approximately one tenth of the oral median lethal dose of 1,563 mg/kg reported for rats [6, 7]. In the combined group, *Spirulina platensis* was administered 1 hour before thiamethoxam. Outcome assessors for sperm parameters, biochemical assays, and histopathological evaluation were blinded to treatment-group assignments.

Sample collection and processing

We performed terminal sampling between 08:00 and 10:00, 24 hours after the final dose, to minimize diurnal hormonal variation. Rats were anesthetized with sodium pentobarbital (50 mg/kg body weight, intraperitoneally) and humanely euthanized by cervical dislocation. Blood was collected from the aorta into non-heparinized tubes. Serum was separated by centrifugation at 3,000 rpm for 15 minutes and stored at −18°C until analysis. We excised, weighed, and rinsed the testes with cold 0.9% saline. We assigned the right testes to biochemical analysis and fixed the left testes for histological examination. Tissue for biochemical analysis was homogenized on ice as a 10% weight/volume homogenate in 0.01 M sodium phosphate buffer (pH 7.4) containing 1.15% potassium chloride. We centrifuged homogenates at 10,000 × g for 20 minutes at 4°C and used the supernatants for biochemical assays.

Oxidative stress and antioxidant measurements

We assessed testicular lipid peroxidation using thiobarbituric acid-reactive substances (TBARS) and calibrated with 1,1,3,3-tetramethoxypropane. The assay principle is based on the reaction of lipid-peroxidation products with thiobarbituric acid [4]. For hydrogen peroxide extraction, 100 mg of tissue was homogenized with 5 mL of 0.1% trichloroacetic acid on ice and centrifuged at 12,000 × g for 15 minutes. We measured hydrogen peroxide (H_2O_2) concentration colorimetrically using the horseradish peroxidase-mediated reaction with phenol red at 610 nm, quantified against an H_2O_2 standard curve and expressed as µmol/g tissue.

We determined reduced glutathione (GSH) after protein precipitation with metaphosphoric acid by reacting it with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), producing a colored thiol derivative measured at 412 nm. Contemporary analytical literature describes the DTNB-based detection principle [5]. Concentrations were calculated against a GSH standard curve and expressed as nmol/g tissue. We measured superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) activities using commercial colorimetric kits from Biodiagnostic, Egypt, according to the manufacturer's instructions. Glutathione reductase (GR) activity was measured using a Biodiagnostic kit (Cat. No. GR 24 20). Established assay literature describes the biological functions of these antioxidant enzymes [3]. We determined total tissue protein content by the Bradford assay using bovine serum albumin as a standard, and expressed enzyme activities as U/mg protein.

Reproductive hormones and sperm characteristics

We measured serum testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels using enzyme-linked immunosorbent assay (ELISA) kits from DRG International, United States (Cat. Nos. EIA-1559, EIA-1288, and EIA-1289, respectively), following the manufacturer's instructions. The kits were validated for rat serum assays.

We collected epididymal spermatozoa from the cauda epididymis by mincing the tissue in 2 mL of phosphate-buffered saline (pH 7.4) pre-warmed to 37°C, then incubating for 5 minutes at 37°C to release sperm. We evaluated sperm count, motility, and morphology using an Olympus microscope and a computer-assisted semen analysis (CASA) system (TOX IVOS, Hamilton-Thorne, United States). We assessed and classified 200 spermatozoa per animal as

morphologically normal or abnormal. We classified head, neck, and tail defects according to World Health Organization (WHO) criteria adapted for rodents. We expressed the proportion of abnormal spermatozoa as a percentage, and sperm count as $\times 10^6$ cells/mL.

Histopathological examination

We fixed testicular tissue in 10% formalin, dehydrated it through graded alcohol, cleared it in xylene, and embedded it in paraffin. Sections of 4–6 µm were stained with hematoxylin and eosin. We used an Olympus BH-2 microscope equipped with a Sanyo VVC-6975P digital camera. We descriptively evaluated seminiferous architecture, spermatogenic organization, and degenerative changes.

Statistical analysis

The individual rat was the experimental unit. We used $P < 0.05$ as the threshold for intergroup statistical significance. We conducted statistical analyses using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA). We verified data normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. We used one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for intergroup comparisons. Data are presented as mean ± standard deviation (SD), with n = 10 animals per group contributing to all reported endpoints.

Results

Testicular oxidative stress and antioxidant defenses

TMX administration increased testicular TBARS and hydrogen peroxide levels and decreased GSH levels relative to the control group (Figure 1 and Table 2). *SP* coadministration lowered TBARS and hydrogen peroxide levels and increased GSH levels compared with TMX alone. The combined-treatment profile indicated partial recovery, with oxidative markers remaining above the control profile and GSH levels below it. *SP* alone was associated with lower TBARS and higher GSH levels than the control treatment.

Testicular SOD, CAT, GPx, GR, and GST activities were lower in the TMX group than in controls (Figure 2). Coadministration of *SP* significantly increased these activities relative to TMX alone ($P < 0.05$). *SP* alone showed higher antioxidant enzyme activities than the control treatment.

Serum reproductive hormones

TMX reduced serum testosterone levels and increased FSH and LH levels compared with controls (Figure 3). *SP* alone produced only minor differences in hormone profiles. In the combined group, testosterone levels increased, and FSH and LH levels decreased relative to TMX alone. These changes moved toward the control profile, although the plotted values indicated partial, not complete, hormonal recovery.

Epididymal sperm characteristics

TMX markedly reduced sperm count and motility and increased the proportion of abnormal spermatozoa (Table 3 and Figure 4). Motility was 77.14% in controls and 18.71% following TMX exposure. *SP* coadministration increased motility to 42.57%. The proportion of abnormal spermatozoa increased from 7.14% in controls to 45.71% following TMX exposure and decreased to 31.43% with combined treatment.

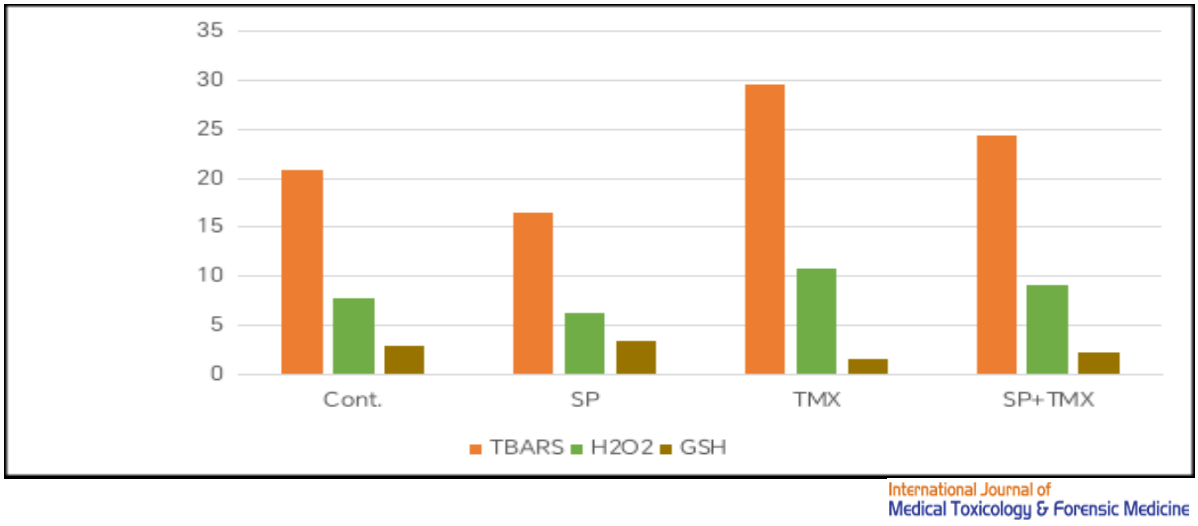


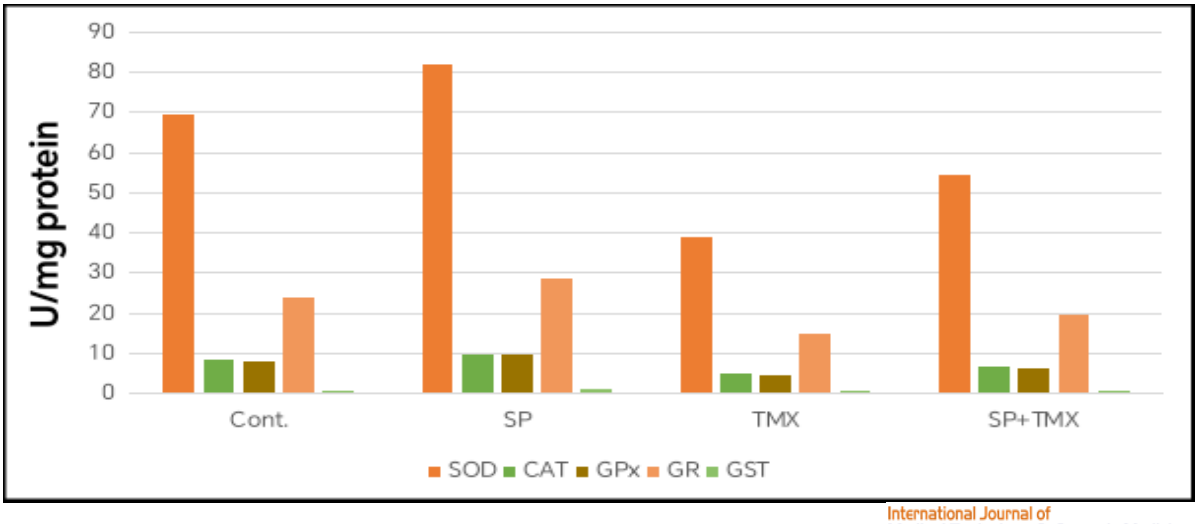
Figure 1. Testicular oxidative stress markers. Treatment profiles for TBARS, hydrogen peroxide levels, and GSH levels in the control, *SP*, TMX, and *SP* + TMX groups.

Table 2. Direction of biochemical and endocrine changes across treatment groups.

Outcome	*SP* versus control	TMX versus control	*SP* + TMX versus TMX
Testicular TBARS	Lower	Higher	Lower
Testicular hydrogen peroxide levels	Lower plotted profile	Higher	Lower
Testicular GSH levels	Higher	Lower	Higher
Testicular SOD, CAT, GPx, GR, and GST	Higher plotted activities	Lower	Higher
Serum testosterone levels	Minor variation	Lower	Higher
Serum FSH and LH levels	Minor variation	Higher	Lower

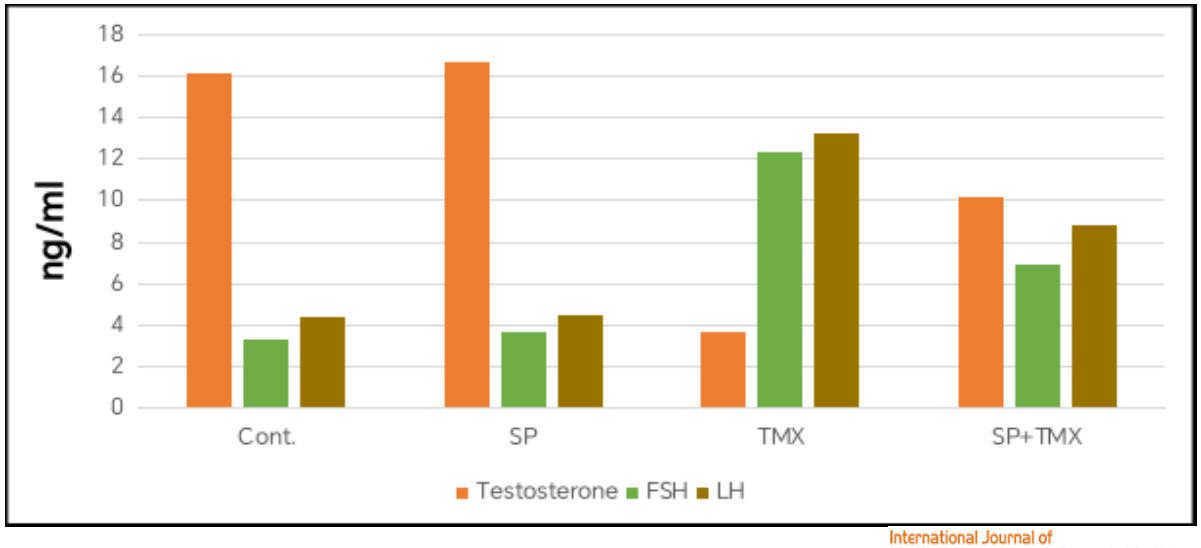
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Directions summarize the reported treatment profiles in Figures 1–3; they are not numerical effect estimates or additional significance tests.



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Figure 2. Testicular antioxidant enzyme activities. Treatment profiles for SOD, CAT, GPx, GR, and GST in the four groups.



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Figure 3. Serum reproductive hormone levels. Testosterone, FSH, and LH levels in the four treatment groups. TMX was associated with reduced testosterone levels and increased FSH and LH levels; combined *SP* treatment shifted these profiles toward the control treatment.

Sperm count increased from 16.00 ×10⁶ cells/mL following TMX exposure to 41.00 ×10⁶ cells/mL with combined treatment. *SP* alone produced comparatively small changes in sperm morphology and higher reported sperm count and motility.

Table 3. Reported epididymal sperm parameters by treatment group.

Parameter	Control Distilled water	*SP* 800 mg/kg/day	TMX 156 mg/kg/day	*SP* + TMX 800 + 156 mg/kg/day
Sperm count (×10⁶/mL)	80.71	87.86	16.00	41.00
Abnormal spermatozoa (%)	7.14	7.43	45.71	31.43
Sperm motility (%)	77.14	83.43	18.71	42.57

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Values are reported for each treatment group (n = 10 animals per group). Doses are expressed as mg/kg body weight/day and were administered orally for 21 days. In the combined group, *SP* and TMX doses were 800 and 156 mg/kg body weight/day, respectively. *SP*, *Spirulina platensis;* TMX, thiamethoxam.

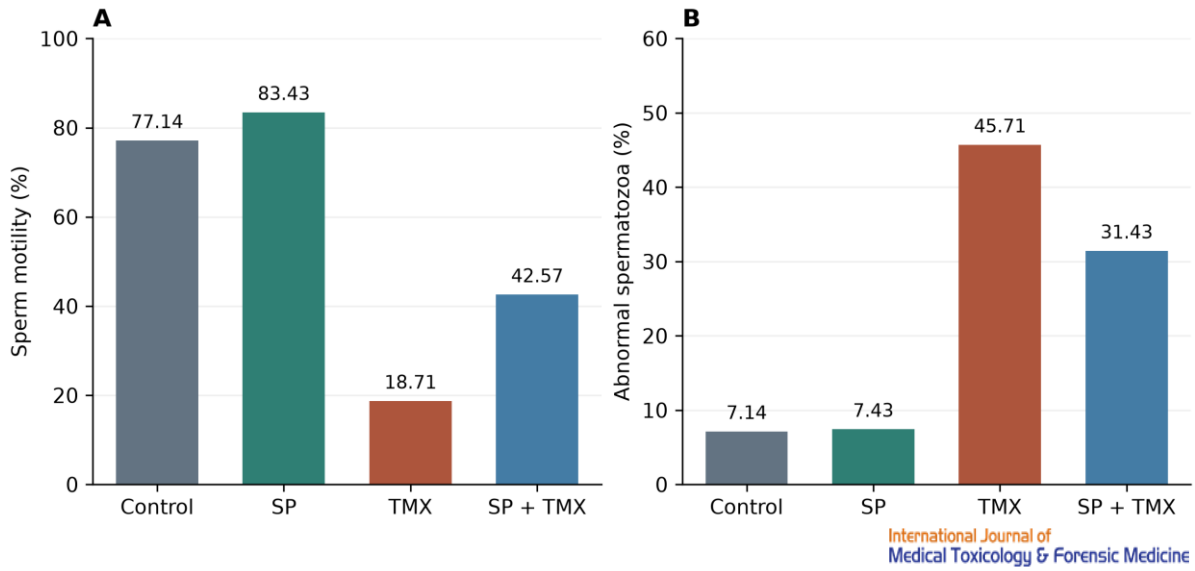


Figure 4. Epididymal sperm motility and abnormalities. Sperm motility (A) and abnormal spermatozoa (B) across treatment groups.

Testicular histopathology

The control and *SP* groups displayed preserved seminiferous epithelial organization and orderly spermatogenic cell layers (Figure 5). TMX exposure was associated with pronounced degeneration, disorganization of the seminiferous epithelium, and an apparent reduction in Sertoli and germ cells, with reduced sperm presence (Figure 6). The combined-treatment group showed partial preservation of spermatogenic organization compared with TMX alone. Residual degeneration and incomplete areas of spermatogenesis remained evident (Figure 7). The histological improvement was consistent with the biochemical and sperm profiles.

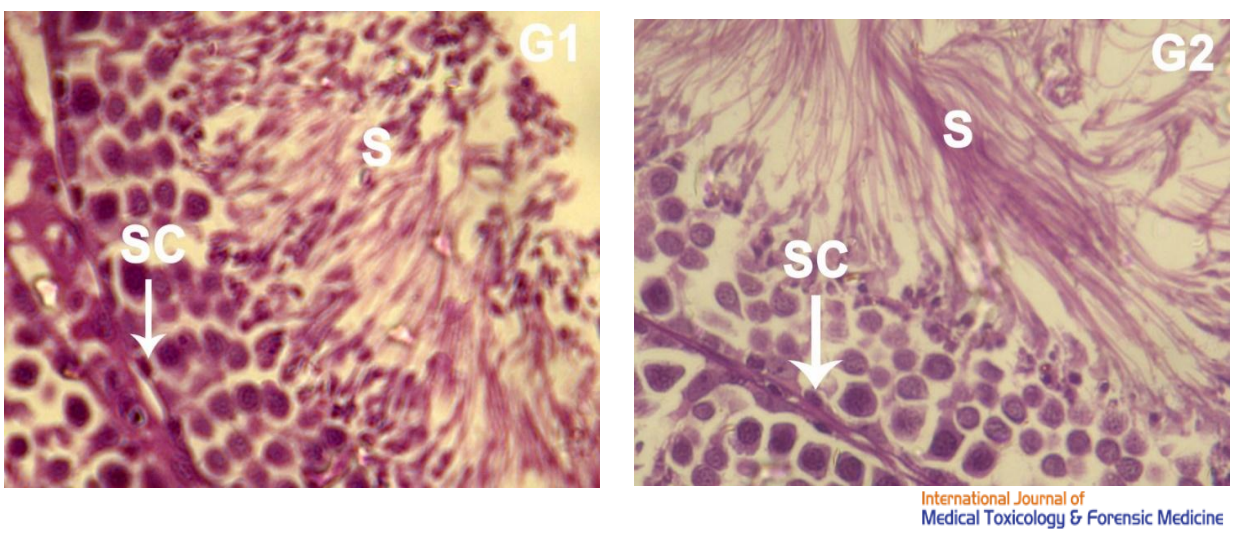


Figure 5. Testicular histology in the control and *SP* groups. Representative sections from control rats (G1) and *SP*-treated rats (G2), showing preserved spermatogenic organization. SC, Sertoli cells; S, spermatozoa. H&E staining; original magnification, ×300.

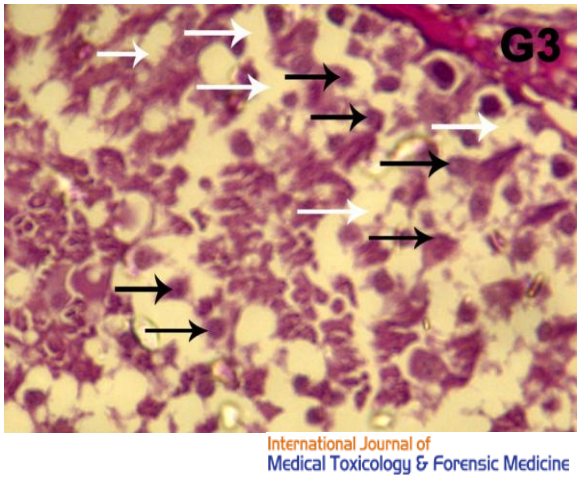


Figure 6. Testicular histology following TMX exposure. The G3 section shows pronounced epithelial degeneration and disorganization. White arrows identify degenerative areas, and black arrows identify disrupted cellular organization. H&E staining; original magnification, ×300.

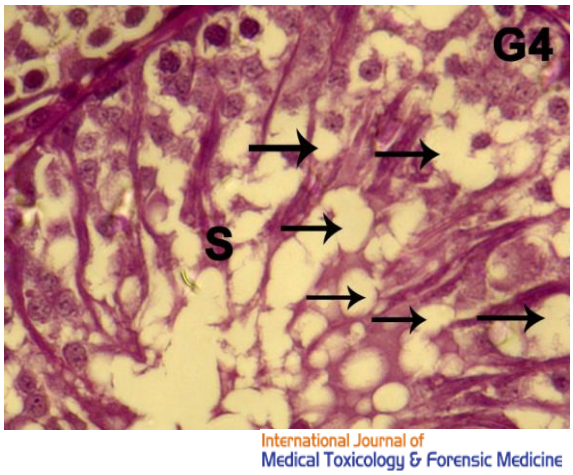


Figure 7. Testicular histology following combined *SP* and TMX treatment. The G4 section shows partial preservation of spermatogenic organization with residual degeneration, indicated by black arrows. S, spermatozoa. H&E staining; original magnification, ×300.

Discussion

This study identified coordinated biochemical, endocrine, sperm, and histological alterations after TMX exposure in male Wistar rats. Increased testicular TBARS and hydrogen peroxide levels accompanied reduced GSH levels and antioxidant enzyme activities, lower testosterone levels, higher FSH and LH levels, impaired sperm quality, and seminiferous degeneration. *SP* coadministration improved these outcomes and partially preserved testicular organization. Agreement among the biochemical, functional, and structural responses supports SP's protective effect under the conditions investigated.

The oxidative changes observed here align with those reported in earlier TMX studies. Issa et al. found that eight weeks of oral exposure to 26, 39, or 78 mg/kg/day lowered CAT and GSH levels, increased malondialdehyde, reduced testosterone, impaired sperm outcomes, and led to progressive testicular degeneration [6]. A similar pattern emerged in the present study after a shorter exposure to 156 mg/kg. Likewise, El-Demerdash et al. reported that 21 days at this dose increased testicular TBARS and hydrogen peroxide, while reducing GSH, antioxidant enzyme activity, testosterone, and sperm quality [7]. These shared oxidative and reproductive effects support the consistency of the current findings. The SP treatment arm also allows assessment of whether these changes can be attenuated.

The simultaneous depletion of GSH and reduced antioxidant enzyme activity provide a plausible context for peroxide accumulation and lipid peroxidation. The TBARS response reflects lipid-peroxidation products [4], while diminished GSH reflects reduced availability of cellular reducing equivalents [5]. SOD removes superoxide, whereas CAT and GPx support peroxide detoxification. GR contributes to glutathione regeneration, and GST participates in glutathione-dependent detoxification [3]. Reduced activity across these complementary defenses is compatible with disturbed testicular redox homeostasis. Higher antioxidant activity and GSH levels, along with lower oxidative markers in the combined group, indicate better maintenance of the biochemical environment associated with spermatogenic function. Reports of oxidative DNA damage during TMX exposure [6,7] further support the parallel improvements in antioxidant defenses, sperm quality, and testicular architecture observed with *SP* coadministration.

Recent evidence from another neonicotinoid provides an informative endocrine comparison. Mahmoud et al. administered thiacloprid at 22.5 or 62.1 mg/kg/day for 56 days and reported reduced testosterone and testicular steroidogenic dehydrogenase activities, increased LH and FSH, lower sperm count and motility, antioxidant depletion, and degenerative histology [8]. Their gonadotropin pattern agrees with the present combination of lower testosterone and higher FSH and LH. This profile is compatible with compensatory gonadotropin responses to impaired gonadal function. El-Demerdash et al. reported lower gonadotropins after TMX [7], indicating variation across experimental settings. *SP*-associated movement of hormone values toward the control profile in the present study suggests partial preservation of endocrine regulation.

The sperm findings establish the functional relevance of the tissue alterations. Motility declined from 77.14% in controls to 18.71% following TMX exposure and increased to 42.57% with combined

treatment. The proportion of abnormal sperm increased from 7.14% to 45.71% and decreased to 31.43% with *SP* coadministration. These improvements were accompanied by higher sperm counts and better spermatogenic organization. Comparable adverse sperm outcomes in the TMX and thiacloprid studies [6–8], together with the broader evidence reviewed by Benchikh et al. [1], place the present findings within an established experimental pattern of neonicotinoid-associated reproductive injury.

SP response is in agreement with recent studies on reproductive protection. El-Nagar and Elsisi found that spraying SP at 300 or 500 mg/kg appeared to help normalize oxidative markers, antioxidant status, testosterone levels, sperm production, and tissue health during a 28-day exposure to bromoxynil octanoate [11]. Their study also described inflammatory and signaling responses, providing supplementary context for *SP*-associated tissue protection. Sedaghatizadeh et al. found that *SP* at 300 mg/kg and phycocyanin at 500 mg/kg improved sperm, antioxidant, and histological outcomes during 60-day hydrogen peroxide exposure [12]. Although the studies examined different toxic substances, both found that better redox balance went hand in hand with preserved reproductive function, a pattern that also appears in the current SP–TMX results.

Khalil et al. Reporting additional evidence from a cisplatin model, a ~68-nm nano-SP preparation given orally at 2,500 mg/kg for eight weeks improved sperm characteristics, oxidative status, testosterone, and key testicular histological and morphometric findings [14]. We show less comet-assay DNA damage and higher StAR and SOD expression in response to joint therapy. The shared reproductive and structural advantages support these findings. However, their nano formulation and cisplatin regimen differ from conventional SP/TMX exposure; our comparison supports only a common protective profile in vivo, rather than a similar phenotype across different formulations or doses. They provide molecular perspectives that reflect the literature without assigning relevance to any endpoints in the current experiment.

Complementary evidence at the same nominal dose and duration came from Neamah et al. Male rats received 800 mg/kg/day SP and 156 mg/kg/day for 3 weeks, with TMX increasing renal TBARS and hydrogen peroxide and reducing GSH and antioxidant enzyme activities, as well as renal dysfunction and tissue injury [15]. *SP* administration improved these kidney outcomes to some degree. The alignment of oxidative-marker directionality and antioxidant recovery across organs is striking. While Neamah et al. evaluated renal protection, this experiment shows corresponding biochemical improvement aligned with testicular endocrine, sperm, and histological stability.

Zedan et al. further characterized *SP*-associated antioxidant responses in dexamethasone-exposed Sprague-Dawley rats. Aqueous *SP* extract at 400 or 800 mg/kg/day for 28 days improved hepatic oxidative markers, CAT and SOD activities, and histological findings, with stronger responses at 800 mg/kg [10]. Hepatic Nrf2, SOD2, Keap1, and apoptosis-related expression responses are therefore plausible candidates for improved tissue stress handling. Spínola et al. more recently reviewed antioxidant activities linked to SP constituents, such as phycocyanin and carotenoids [9]. These sources emphasize the rationale of antioxidant association with protection in contrast to the testicular biochemical outcomes measured in this study.

Endocrinological evidence identified by Salah et al. The paper observed some improvement in testosterone and hepatic oxidative stress with SP in a methotrexate model, though FSH was not recovered [13]. This endpoint-specific response also supports interpreting SP protection as an improvement rather than a return to normality. Sperm and hormone results showed little improvement, with residual seminiferous degeneration in the present experiment. These findings are consistent with a coordinated perturbative response across related outcomes. In summary, the growing body of evidence places SP among the experimental interventions to reduce TMX-associated testicular oxidative injury and reproductive dysfunction.

Conclusion

Oral TMX exposure was associated with testicular oxidative imbalance, reduced antioxidant defenses, altered reproductive hormones, impaired sperm characteristics, and seminiferous degeneration. *SP* coadministration partially improved the biochemical, endocrine, sperm, and histological outcomes. These findings support SP's protective potential against TMX-associated reproductive injury in male Wistar rats under the conditions investigated.

Ethical Approval

Animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, eighth edition [16]. The study received institutional animal-care approval under approval code FMAU.12. Identifying institutional details are provided in the separate title-page file.

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Conflicts of Interest

The authors report that there are no competing interests to declare.

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