



Original Research Paper

Effect of Glyphosate on Reproductive Function and Oxidative Status in Male Rats: Protective Role of Ziziphus lotus Aqueous Leaf Extract

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Key Words	Abstract
Glyphosate; Ziziphus lotus; Reproductive toxicity; Oxidative stress; Sperm quality; Male rats.	The present study was designed to investigate the adverse reproductive effects of glyphosate (G) in male Wistar rats and the possible protective role of Ziziphus lotus (ZL) aqueous leaf extract. Animals were divided into four groups: control (CRL), glyphosate (G; 10 mg/kg/day), ZL (100 mg/kg/day), and G + ZL. Treatments were administered by gavage 5 days/week for 52 consecutive days. Blood, sperm samples, and testicular tissue were collected for hormone measurements, oxidative-status assessment, semen analysis, histopathology, and immunohistochemistry. Glyphosate significantly impaired semen characteristics, reducing spermatid number, sperm count, motility, viability, and testosterone levels while increasing sperm abnormalities, AOPP, and MDA levels. Histopathological and immunohistochemical examinations showed testicular germ cell alterations, connective-tissue enlargement, and p53-positive cells. Co-administration of ZL attenuated several glyphosate-induced changes in semen quality, testosterone, AOPP, MDA, and testicular histology. These findings indicate a protective effect of ZL aqueous leaf extract against glyphosate-induced reproductive toxicity and oxidative stress in male rats...

Introduction

Glyphosate is a non-selective organophosphorus herbicide that has been extensively used worldwide since 1974 because of its broad-spectrum efficacy and its initially perceived low toxicity (Maqueda et al., 2017).

Due to its intensive agricultural application, glyphosate residues have been detected in soil, water, food products, and even in human biological fluids such as blood serum and urine (Conrad et al., 2017; Gillezeau et al., 2022). Consequently, increasing attention has been directed toward its potential toxicological effects on both environmental and human health.

Recent experimental and epidemiological studies have demonstrated that glyphosate exposure may induce toxicity in several organs, including the liver, kidneys, brain, and reproductive system. The principal mechanisms involved include oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis (Martínez et al., 2020; Mesnage et al., 2022). Overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) disturbs cellular redox homeostasis, leading to lipid peroxidation, protein oxidation, and DNA damage (Díaz et al., 2023; Li et al., 2024).

Among the most concerning toxicological

effects, male reproductive dysfunction has attracted growing scientific interest. Several investigations reported that glyphosate and glyphosate-based herbicides (GBHs) impair spermatogenesis and steroidogenesis by altering the expression of essential steroidogenic enzymes such as steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage enzyme (CYP11A1), and 3 β -hydroxysteroid dehydrogenase (3 β -HSD), resulting in reduced testosterone production (Zhao et al., 2021; Wan et al., 2023). In addition, GBHs appear more toxic than glyphosate alone because of the synergistic effects of formulation adjuvants (Romano et al., 2022; Tajai et al., 2023).

Experimental studies in rodents have shown that chronic glyphosate exposure decreases sperm count, motility, and viability while increasing sperm morphological abnormalities and DNA fragmentation (Silva et al., 2024). Histopathological analyses also revealed degeneration of seminiferous tubules, disruption of germinal epithelium, Leydig cell dysfunction, and reduction in seminiferous tubule diameter. These alterations are frequently associated with endocrine imbalance and oxidative damage in testicular tissues.

At the cellular level, glyphosate-induced reproductive toxicity is strongly associated with mitochondrial impairment. Disturbance of mitochondrial membrane potential and ATP depletion promotes activation of intrinsic apoptotic pathways characterized by increased Bax and caspase-3 expression and decreased

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Bcl-2 levels (Shafiee Mehretal., 2024). Furthermore, glyphosate activates inflammatory mediators through NF- κ B and MAPK signaling pathways, contributing to germ cell apoptosis and testicular inflammation (Anifandiset al., 2023; Cattani et al., 2024). Emerging evidence also suggests that glyphosate may alter epigenetic regulation, including DNA methylation and microRNA expression, potentially affecting reproductive capacity and transgenerational fertility.

Because oxidative stress plays a central role in glyphosate toxicity, increasing research efforts have focused on the use of natural antioxidants as protective agents. Plant-derived bioactive compounds such as flavonoids, polyphenols, tannins, and carotenoids possess strong antioxidant and anti-inflammatory activities. These molecules can scavenge free radicals, enhance endogenous antioxidant defenses, and regulate signaling pathways involved in inflammation and apoptosis, particularly through activation of Nrf2 and inhibition of NF- κ B (Georganas et al., 2022; Pandey & Rizvi, 2023).

Ziziphus lotus, a medicinal plant belonging to the Rhamnaceae family, is widely distributed in Mediterranean and arid regions. Different parts of the plant, especially leaves and fruits, are rich in phenolic acids, flavonoids, saponins, alkaloids, and tannins that contribute to its diverse pharmacological properties (Serra et al., 2021; Benhammou et al., 2022). Traditionally, *Z. lotus* has been used in folk medicine for the treatment of inflammatory disorders, metabolic diseases, digestive disturbances, and infections.

Recent pharmacological studies demonstrated that aqueous extracts of *Ziziphus lotus* exert potent antioxidant, anti-inflammatory, anti-apoptotic, and tissue-protective activities. These effects are mainly mediated through enhancement of endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), reduction of lipid peroxidation, and preservation of mitochondrial integrity (Bouriche et al., 2022; Khelifi et al., 2023). In addition, *Z. lotus* extracts have shown the ability to attenuate inflammatory cytokine production and regulate cellular survival pathways.

Although studies investigating its reproductive protective effects remain limited, recent evidence suggests that *Ziziphus lotus* may improve sperm quality, preserve testicular histoarchitecture, and restore hormonal balance under conditions of oxidative stress (Zouaoui et al., 2024; Benali et al., 2025). The beneficial effects of this plant are thought to be related to its high antioxidant capacity and its ability to modulate apoptotic and inflammatory pathways.

Therefore, the present study aims to evaluate the protective effect of aqueous leaf extract of *Ziziphus lotus* against glyphosate-induced reproductive toxicity and oxidative stress in male rats, with particular emphasis on oxidative status, hormonal profile, and

histopathological alterations in testicular tissue.

Material and methods

Glyphosate

Glyphosate is a non-selective herbicide registered for use by the United States Environmental Protection Agency (U.S. EPA) in 1974, CAS number 1071-83-6, chemical formula $C_3H_8NO_5P$. The product used in the experiment was the commercial Fortin Green formulation (isopropylamine salt), with 36% purity and an LD50 greater than 5000 mg/kg (Weckbecker and Cory, 1988), purchased from a local agricultural supplier.

Plant material

Ziziphus lotus (Rhamnaceae) is widely fully grown in North African countries including Algeria, where it is locally named “Sedra” and it has been used in Algerian folk medicine for a long time. In this study, the plant leaves were collected in November 2020 from the M’Sila province region, Algeria.

Preparation of aqueous leaf extract

The leaves were dried at room temperature away from light and then ground manually into a fine powder using a mortar and pestle. The powder was sifted through a sieve to remove large particles, and 50 g of leaf powder was soaked in 500 mL of distilled water and macerated for 24 h, shaken, filtered through Whatman No. 1 filter paper, and lyophilized to obtain the final extract powder used for aqueous leaf extract preparation according to the method of Bougandoura and Bendimerad (2012).

Animals and experimental design

Twenty-four (24) adult male Wistar rats aged from 8 to 12 weeks, weighing 220–250 g were purchased from Algeria’s Kouba Pasteur Institute and acclimatized for two weeks in the animal house before starting the experiment. The animals were divided into four groups and gavaged with glyphosate and *Ziziphus lotus* aqueous leaves extract for 52 days as follows: Group I served as control (CRL) received orally 0.5 mL/kg/day of distilled water, Group II gavaged by Glyphosate (G) at 10 mg/kg/day (Abarikwu et al., 2015), Group III dosed orally by 100 mg/kg/day of *Ziziphus lotus* (ZL) aqueous leaf extract (Goetz and Bureau, 2016) and Group IV received the combination (ZL+ G). All rats were handled in accordance with the guidelines for the care and use of experimental animals and applicable animal-welfare requirements provided by the Algerian Pasteur Institute, Kouba, Algiers, Algeria. The study was approved by the Local Ethical Committee of the Unit of Animal Production, Algerian Pasteur Institute, Kouba (approval no. API/2023).

Semen characteristics

For sperm analysis, the number of testicular spermatids and epididymal spermatozoa was determined according to the method described by Robb et al., 1978. While sperm motility, viability, and morphological abnormalities were measured according to the method described previously (Linder et al., 1995; Llobet et al., 1995).

Hormone measurements

The measurement of total testosterone, FSH, and LH levels in plasma was done by mini VIDAS® automated assay, using the ELFA (Enzyme Linked Fluorescent Assay) technique and specific hormone kits BioMerieux (France). This technique aids in diagnosing disease states caused by an excess or deficiency of these hormones.

Protein content and oxidative stress biomarkers

The content of tissue proteins was quantified according to the colorimetric method of Bradford, 1976, while, the oxidative stress biomarkers were performed as follows: the malondialdehyde (MDA) was measured by Ohkawa et al., 1979, the catalase (CAT) activity was performed according to the method (Aebi, 1984), the advanced products of oxidized proteins (AOPP) are dosed using the Witko-Sarat spectrophotometric method, modified by Cakatay et al., glutathione peroxidase (GPx) activity, was measured by Flohé and Günzler, 1984. the content of reduced glutathione (GSH) was carried out according to the method of Weckbecker and Cory (1988). the nitric oxide (NO) was measured by Montgomery and Dymock.

Histopathological examinations

The testicular fragments were fixed in formaldehyde solution (30%), subjected to a graded alcohol treatment (70%, 80%, 90%), embedded in paraffin, sectioned into 5 µm thick sections, and

stained with hematoxylin and eosin to be examined under a light microscope for the histopathological aspects.

Detection of apoptotic protein p53 immunoreactivity in the testis

The expression of p53 in the germ cell testicular section was detected immunohistochemically using the p53 monoclonal antibody from Leica Biosystems according to the method of Aumpan et al., (2020).

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Bonferroni's multiple-comparison test in GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). A difference at $p < 0.05$ was considered statistically significant.

Results

Effects on sperm quality

The changes in sperm characteristics of rats treated with glyphosate (G), Ziziphus lotus (ZL), and their combination (G + ZL) are summarized in Table I.

Glyphosate treatment significantly reduced spermatid and spermatozoan numbers, sperm motility, and viability, while increasing the proportion of abnormal sperm compared with the control and ZL groups. Co-administration of ZL with G alleviated these adverse effects on semen quality.

Table I. Effect of glyphosate (G), Ziziphus lotus (ZL), and their combination (G+ZL) on spermatid number, sperm count, motility (%), viability (%), and abnormal sperm (%) after 52 consecutive days of treatment.

Animal groups	CRL	G	ZL	G+ZL
Sperm count ($10^6/g$ epididymis)	256.4±38.68	50.08±8.26**	241.1±41.87##	163.0±31.43
Spermatids number ($10^6/g$ testis)	196.2±12.72	65.91±17.57***	163.7±19.88##	166.1±24.27##
Motility (%)	76.83±6.6	30.38±3.51**	61.25±7.35#	77.00±6.88##
Viability (%)	83.83±4.6	35.23±2.2***	79.17±6.27###	66.59±7.11##
Abnormal sperm (%)	17.0±0.7	24.33±0.88***	13.83±0.48*,###	20.83±0.6*,#,+++

Results are expressed as mean ± SD, n = 6. Significant differences ($p < 0.05$) are indicated by * relative to CRL, # relative to the glyphosate (G) group, and + relative to the Ziziphus lotus (ZL) extract group.

Effects on sexual hormones balance

The results of sex-hormone analysis (testosterone, LH, and FSH levels) in rats treated with G, ZL, and their combination (G+ZL) after 52 days

of treatment are represented in Table II. Rat treated with G exhibited a significant decrease ($p < 0.01$) in testosterone levels, accompanied by a significant increase in FSH levels compared to control and ZL groups; such hormonal changes may contribute to impaired reproductive function. Co-administration of ZL with G attenuated the observed changes in testosterone and FSH levels.

Table II. Effect of glyphosate (G), Ziziphus lotus (ZL), and their combination (G+ZL) on hormone levels after 52 consecutive days of treatment.

Animal groups	CRL	G	ZL	G+ZL
Testosterone (ng/ml)	3.89±0.57	0.15±0.06**	3.31±0.71#	2.35±1.07
LH (mUI/mL)	0.42±0.17	0.18±0.04	1.54±1.05	0.46±0.16
FSH (mUI/mL)	0.10±0.00	0.35±0.06***	0.10±0.00###	0.10±0.00###

Results are expressed as mean ± SD, n = 6. Significant differences (p < 0.05) are indicated by * relative to CRL, # relative to the glyphosate (G) group, and + relative to the Ziziphus lotus (ZL) extract group.

Effect on oxidative stress markers

As shown in (Table III), treatment with G caused a significant increase in testicular advanced oxidation protein products (AOPP) and Malondialdehyde (MDA) compared with the control group. The co-administration of ZL along with G can

therefore reverse AOPP levels to near-normal values. Moreover, the antioxidant defense system, both enzymatic components (GPx and CAT activities) and non-enzymatic components (GSH and NO levels), did not exhibit any significant differences among all experimental groups.

Table III. Effect of glyphosate (G), Ziziphus lotus (ZL), and their combination (G+ZL) on protein, MDA, AOPP, GSH, GPx, and NO levels after 52 consecutive days of treatment.

Animal groups	CRL	G	ZL	G+ZL
Protein (ng/ml)	8.59±1.78	6.16±0.09	6.1±0.093	6.18±0.18
AOPP (μM/mg of total protein)	0.41±0.09	2.83±1.07*	0.6±0.13	0.81±0.05
MDA (nmol/mg of protein)	1.47±0.23	2.94±0.33*	2.88±0.25*, ++	1.04±0.26##
Catalase (μM/min/mg of protein)	0.21±0.05	0.98±0.15	0.30±0.08	0.68±0.39
GSH (μM/mg of protein)	0.90±0.18	1.12±0.10	1.07±0.19	1.22±0.14
GPx (μM/min/mg)	0.02±0.01	0.02±0.06	0.05±0.01*	0.04±0.01
NO (μM/mg of protein)	107.3±31.8	203.0±17.57	125.6±33.64	86.93±34.74

Results are expressed as mean ± SD, n = 6. Significant differences (p < 0.05) are indicated by * relative to CRL, # relative to the glyphosate (G) group, and + relative to the Ziziphus lotus (ZL) extract group.

Histopathological and immunohistochemical study of the testis

Histological investigations of testis sections are presented in Figure 1, comparing control rats (a) with treated rats given G (b), ZL (c), and G + ZL (d). Rats treated with G showed degenerative changes in the

seminiferous epithelium, including vacuolization, reduced sperm count in some tubule lumens, and disrupted spermatogenesis with fewer germ cell types. In contrast, co-administering ZL with G significantly reduced and reversed these histological changes, returning the features to normal (Figures 1 and 2).

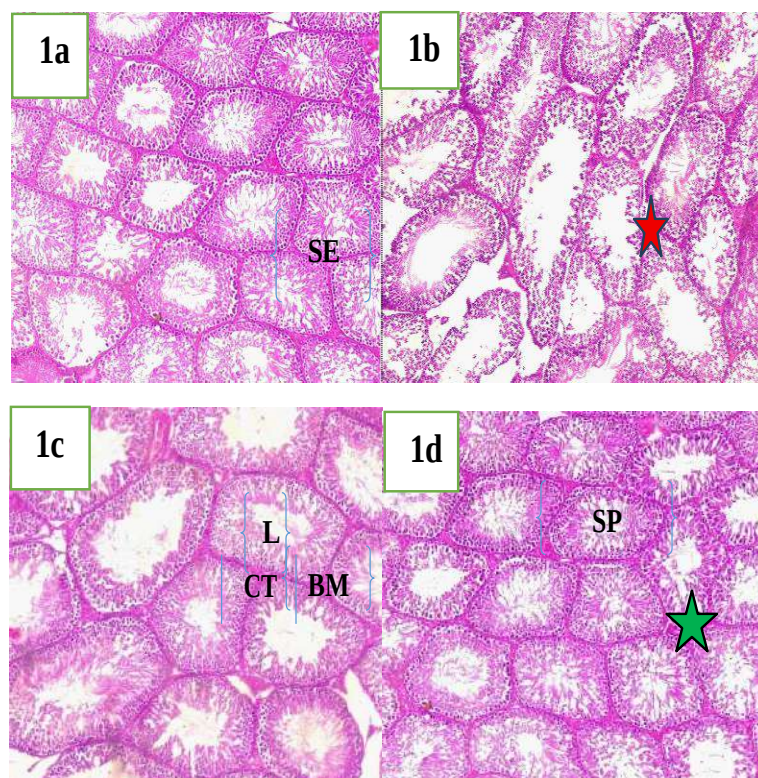


Figure 1. Light microscopic sections of testes from various examined groups: control (1a), G (1b), ZL (1c), and co-administration G+ZL (1d) for 52 days. Sections (5 μ m) were stained with hematoxylin–eosin (G: $\times 100$). L: lumen; CT: connective tissue; SE: seminiferous epithelium; Sp: sperm; BM: basal membrane; red star: deleterious effect; green star: ameliorating effect; G: glyphosate; ZL: Ziziphus lotus.

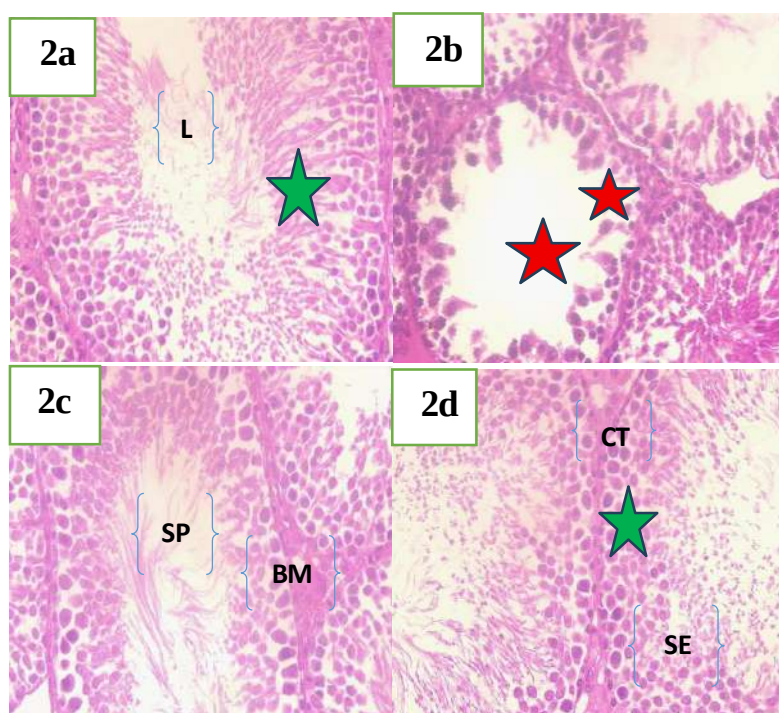


Figure 2. Light microscopic sections of testes from various examined groups: control (2a), G (2b), ZL(2c), and coadministration G+ZL(2d) for 52 days. Sections (5µm) were stained with hematoxylin–eosin (G:×400). L: lumen; CT: connective tissue; SE: seminal epithelium; Sp: sperm; BM: basal membrane; red star: deleterious effect; green star: ameliorating effect; G: glyphosate; ZL: ziziphus lotus.

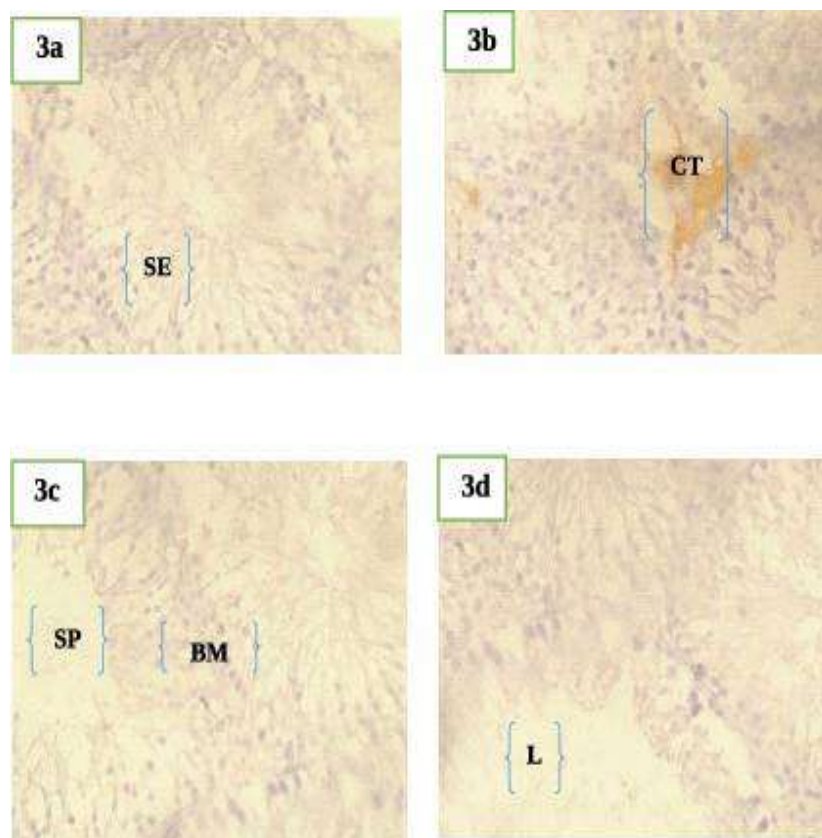


Figure3.

Detection of p53 immunoreactivity in testis tissues. Representative immunohistochemical staining of p53 protein in testis sections from (3a) control, G(3b), ZL(3c), and coadministration G+ZL(3d) experimental groups. Sections were processed using anti-p53 antibodies, followed by appropriate secondary antibodies. The staining intensity and distribution were evaluated under a light microscope. Arrows indicate areas of apoptotic cells exhibiting strong p53 immunoreactivity. Analysis of p53-positive cells is presented in G(B), showing a significant increase in p53 expression in the experimental group compared to the control group.

Discussion

Accumulating evidence indicates that glyphosate exposure exerts harmful effects on male reproductive function. Several experimental and epidemiological studies have demonstrated that chronic exposure to glyphosate and glyphosate-based herbicides (GBHs) negatively affects spermatogenesis, sperm quality, hormonal balance, and testicular structure (Dai *et al.*, 2016; Dallegrave *et al.*, 2007). Recent investigations have further associated glyphosate exposure with hepatotoxicity, nephrotoxicity, neurotoxicity,

endocrine disruption, mitochondrial dysfunction, and chronic oxidative stress (Mesnage *et al.*, 2015; Cattani *et al.*, 2014; Galli *et al.*, 2024). According to Branco *et al.* (2025), the reproductive toxicity of glyphosate is multifactorial and involves dysregulation of redox homeostasis, inflammatory signaling pathways, apoptosis, and altered steroidogenic gene expression.

In the present study, glyphosate exposure significantly impaired reproductive parameters in male rats, as evidenced by reduced spermatid and spermatozoa counts, decreased sperm motility and viability, and increased sperm abnormalities. These findings are consistent with previous reports showing that glyphosate

exposure adversely affects semen quality and male fertility (Séralini *et al.*, 2014; Cassault-Meyer *et al.*, 2014). Similarly, Owagboriaye *et al.* (2017) reported that glyphosate induces abnormal sperm morphology and testicular degeneration, while Ren *et al.* (2026) demonstrated that prolonged exposure may impair reproductive performance through oxidative and endocrine mechanisms.

The decline in sperm quality observed in glyphosate-treated animals may be explained by oxidative stress-mediated cellular damage. Oxidative stress plays a central role in glyphosate-induced reproductive toxicity through excessive production of reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, DNA fragmentation, and mitochondrial dysfunction (Anifandis *et al.*, 2017). Sperm cells are particularly susceptible to oxidative injury because their membranes contain high levels of polyunsaturated fatty acids and possess limited antioxidant defenses. Consequently, ROS overproduction disrupts membrane integrity, reduces sperm motility, and impairs fertilization capacity.

Recent studies conducted between 2022 and 2025 have provided additional insight into the molecular mechanisms of glyphosate toxicity. Mesnage *et al.* (2022) demonstrated that glyphosate exposure alters mitochondrial respiration and ATP production in reproductive tissues, thereby promoting germ cell apoptosis. Likewise, Cattani *et al.* (2024) showed that glyphosate activates inflammatory pathways such as NF- κ B and MAPK, which contribute to testicular inflammation and degeneration. Furthermore, Li *et al.* (2024) reported that chronic glyphosate exposure induces epigenetic modifications and DNA methylation disturbances that may affect reproductive function across generations.

Endocrine disruption also appears to be an important mechanism underlying glyphosate-induced reproductive impairment. Several studies have demonstrated that glyphosate reduces testosterone synthesis by inhibiting steroidogenic enzymes and genes, including *StAR*, *3 β -HSD*, *CYP11A1*, *Cyp17a1*, and *Cyp19a1* (Abarikwu *et al.*, 2015; Zhao *et al.*,

2021; Wang *et al.*, 2023). The decrease in serum testosterone observed in the present study may therefore explain the impairment of spermatogenesis and sperm maturation in glyphosate-treated rats. Additionally, disturbances in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion suggest disruption of the hypothalamic–pituitary–gonadal axis.

Histopathological observations further confirmed the reproductive toxicity of glyphosate. Degeneration of seminiferous tubules, disorganization of the germinal epithelium, reduced sperm density, and enlargement of interstitial spaces were observed in glyphosate-exposed animals. Similar histological alterations were described by Dallegrave *et al.* (2007) and Shafiee Mehr *et al.* (2024). In the present study, p53 immunoreactivity provided evidence of apoptotic involvement. Other apoptotic markers, including Bax, caspase-3, and Bcl-2, have been implicated in glyphosate-induced testicular injury in previous

studies (Hariti *et al.*, 2023), but these markers were not directly measured here.

Natural antioxidants have recently gained considerable attention as potential protective agents against xenobiotic-induced reproductive toxicity. In this context, *Ziziphus lotus* (ZL) represents a promising medicinal plant because of its richness in phenolic compounds, flavonoids, tannins, alkaloids, and saponins. These bioactive metabolites possess strong antioxidant, anti-inflammatory, and cytoprotective properties (Abdoul-Azize, 2016). Benammar *et al.* (2014) demonstrated that aqueous extracts of *Ziziphus lotus* enhance endogenous antioxidant defenses by increasing the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

In the current study, co-administration of *Ziziphus lotus* extract markedly improved sperm count, motility, viability, and morphology in glyphosate-treated rats. These protective effects may be associated with the antioxidant properties of ZL phytochemicals. Previous studies have reported that *Ziziphus lotus* extracts can scavenge ROS, reduce lipid peroxidation, preserve mitochondrial integrity, and improve cellular antioxidant status in oxidative-stress models (Bouriche *et al.*, 2022; Khelifi *et al.*, 2023). Mitochondrial mechanisms were not directly investigated in the present experiment.

Recent studies between 2022 and 2025 have also highlighted the anti-inflammatory and anti-apoptotic properties of *Ziziphus lotus*. Bencheikh *et al.* (2023) demonstrated that ZL extract inhibits pro-inflammatory cytokines and reduces oxidative tissue injury. In addition, Zouaoui *et al.* (2024) reported that *Ziziphus lotus* supplementation improves sperm quality and hormonal balance under oxidative stress conditions. Benali *et al.* (2025) further suggested that ZL polyphenols may protect mitochondrial function and regulate apoptotic pathways in reproductive tissues.

The beneficial effects of *Ziziphus lotus* may also involve modulation of cellular signaling pathways. Polyphenols and flavonoids have been reported to activate the Nrf2 antioxidant pathway and suppress NF- κ B-mediated inflammatory responses, thereby limiting oxidative and inflammatory damage (Georganas *et al.*, 2022; Pandey and Rizvi, 2023). These signaling pathways and mitochondrial-function endpoints were not directly measured in the present study and are therefore considered possible mechanisms rather than demonstrated effects.

Overall, the findings show that *Ziziphus lotus* aqueous leaf extract exerted protective effects against several glyphosate-induced reproductive and oxidative alterations in male rats. These effects may be associated with antioxidant and anti-inflammatory mechanisms reported in the literature; however, inflammatory, anti-apoptotic, endocrine-regulatory, and mitochondrial pathways were not directly investigated in the present study.

Conclusion

Co-administration of *Ziziphus lotus* aqueous leaf extract mitigated several glyphosate-associated changes in semen quality, oxidative-status markers, and testicular histology in male rats, supporting a protective role for this plant preparation. Further studies are needed to clarify the

underlying mechanisms and the broader implications of these findings.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical statement

All animals were handled in accordance with the guidelines for the care and use of experimental animals and applicable animal-welfare requirements provided by the Algerian Pasteur Institute, Kouba, Algiers, Algeria. The study was approved by the Local Ethical Committee of the Unit of Animal Production, Algerian Pasteur Institute, Kouba (approval no. API/2023).

Data Availability Statement

Data are available on request from the corresponding author due to privacy restrictions.

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