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RESEARCH ARTICLE

Quantitative Prediction of Azoxystrobin-Induced Genetic Damage, Histopathology, Brain and Testicular Disparities in Male Albino Rats

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ABSTRACT

Azoxystrobin is a widely used broad-spectrum fungicide in agriculture and public health, raising concerns about its potential toxicological effects. This study evaluated the neurotoxic and testicular effects of azoxystrobin in male albino rats by assessing oxidative stress, antioxidant defense mechanism, histopathological alterations and DNA damage at sub-chronic doses. Twenty-eight rats (active, healthy and free of infections) were randomly divided into four groups (n = 7 each). The control group (A0) received a standard diet, while groups A1, A2 and A3 were administered azoxystrobin at doses of 30, 60, and 90mg/kg/day, respectively, for 60 days. No mortality was observed; however, dose-dependent clinical signs such as diarrhea, lethargy, and depression were recorded in rats treated with higher doses (60 and 90 mg/kg/day) after days 30 of experiment. Biochemical analysis revealed a significant increase in oxidative stress markers, including thiobarbituric acid reactive substances (TBARS) and reactive oxygen species (ROS), along by a marked reduction within antioxidant enzymes (SOD, CAT, GSH, and POD) in brain and testicular tissues. Histopathological examination showed edema, spermatid necrosis, Leydig cell degeneration, vacuolation, and disruption of the seminiferous epithelium in testes of rats given higher doses of fungicides. Histopathological analyses of brain exhibited microgliosis, neuronal degeneration, necrosis, and inflammatory changes, especially at higher doses. Results on genotoxicity determined by comet assay indicated a significant increase in DNA damage in both brain and testicular cells. Overall, azoxystrobin exposure induced oxidative stress, histopathological damage, and genotoxic effects highlighting its potential neurotoxic and reproductive risks.

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INTRODUCTION

Modern agriculture relies heavily on pesticides, including fungicides, to control plant diseases and enhance crop productivity, thereby supporting global food security (Wu *et al.*, 2021; Naseem *et al.*, 2022). Fungicides account for a substantial proportion of agrochemical use and are also applied in public health, horticulture, and wood preservation (Rathore and Pandey, 2025). Among these, azoxystrobin (AZX), a broad-spectrum strobilurin fungicide, is widely used on cereals, rice, fruits, and vegetables due to its high efficacy in controlling fungal infections (Wan *et al.*, 2025).

However, the extensive application of azoxystrobin has raised environmental and toxicological concerns. It is not fully degraded after application and can persist in soil, leach into groundwater, and contaminate aquatic ecosystems, contributing to diffuse environmental pollution (Burandt *et al.*, 2024; Lu *et al.*, 2024). Such persistence increases the risk of exposure to non-target organisms, including mammals, through contaminated food and water sources (Wang *et al.*, 2026).

Recent studies have highlighted the potential toxicity of azoxystrobin to terrestrial organisms, particularly its adverse effects on the nervous and reproductive systems (El-Hak *et al.*, 2022). These systems are especially vulnerable due to their high metabolic activity and structural sensitivity. Therefore, evaluating its toxicological impact is essential. The albino rat (*Rattus norvegicus*) is a well-established experimental model widely used to investigate such effects and to provide insights into potential human health risks.

Studies on the reproductive toxicity of azoxystrobin in male albino rats have demonstrated dose- and time-dependent testicular damage (Fotouh *et al.*, 2025). The testes, as the primary male reproductive organs, are particularly vulnerable targets. Histopathological analyses have revealed degenerative alterations, including sloughing of the germinal epithelium, vacuolation within seminiferous tubules, and structural damage to Leydig and Sertoli cells (Szabó *et al.*, 2023). These changes are associated with functional impairment, manifested as reduced sperm count, decreased motility, and increased morphological abnormalities such as abnormal head and tail structures (Wu *et al.*, 2021; Ahmed *et al.*, 2025).

Mechanistically, these effects are linked to disruption of the hypothalamic–pituitary–testicular (HPT) axis, resulting in decreased levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone. Oxidative stress has been identified as a key underlying factor (Wang *et al.*, 2026), as azoxystrobin exposure leads to extreme ROS production, tremendous endogenous antioxidant fortifications such as catalase and glutathione systems (Ouyang *et al.*, 2021; Fotouh *et al.*, 2025). Due to high metabolic activity and relatively limited antioxidant capacity, testicular tissue is particularly susceptible to oxidative damage, leading to cellular dysfunction and apoptosis (Rathore and Pandey, 2025).

Azoxystrobin also exhibits significant neurotoxic potential, targeting the mammalian brain. Due to its high oxygen consumption, lipid-rich composition, and relatively limited antioxidant capacity, the brain is particularly susceptible to oxidative stress–induced damage (Gupta *et al.*, 2025). Azoxystrobin disrupts mitochondrial function and oxidative phosphorylation, leading to excessive free radicals within neuronal cells (Guo *et al.*, 2024). This oxidative imbalance results in lipid peroxidation, protein oxidation, and DNA damage, ultimately impairing neuronal function and promoting cell death. Such alterations may contribute to neurobehavioral deficits, although the precise histopathological and functional outcomes following exposure remain insufficiently characterized.

Despite growing evidence of its toxicity, a comprehensive evaluation of the combined effects of azoxystrobin on both the nervous and reproductive systems within a single experimental model is limited (El-Hak *et al.*, 2022). Therefore, the present study was designed to provide an integrated assessment of azoxystrobin-induced neurotoxicity and testicular toxicity in sexually mature male albino rats.

MATERIALS AND METHODS

Experimental rats and treatments: This research experiment was accomplished within the Faculty of Veterinary Medicine, Aswan University, Egypt, over the period of November 2024 to March 2025, following a completely randomized design (CRD) with appropriate experimental groups to evaluate the toxicological effects of the azoxystrobin in albino rats. Experimental rats (28) were procured from a local laboratory animal house and were acclimatized to standard laboratory conditions for a period of seven days before experimentation in different groups, and each group contained 7 rats. All testing was performed succeeding the Ethical Committee's Guidelines for the Care and Utilization of Laboratory Animals set forth via the Aswan University Ethics Committee, Faculty of Veterinary Medicine (Protocol No.: ASWU/VM 21-10-2024). All the rats were housed in polypropylene cages with stainless steel grid tops under controlled environmental conditions, maintaining temperature at 25±2°C, relative humidity at 60±2%, and a 12-hour light-dark photoperiod cycle. Standard pelleted feed and water were provided ad libitum throughout the experimental period. Azoxystrobin mixed in the diet was fed to rats in groups (A0, A1, A2 and A3) @0.00, 30, 60 and 90mg/kg/day, respectively, for a period of 60 days.

Oxidative and antioxidative stress biomarkers

Tissue preparation: For the assessment of oxidative and anti-oxidative stress bio-parameters, the brain and testes were immediately removed at the time of necropsy of each rat. After separation, the testes and brain were rinsed and placed in a clean Petri dish. After that, the testes and brain tissues were collected from each treated and control rat, minced and homogenate was obtained, and finally centrifugation was carried out at 5000rpm for 5 min to remove cellular debris for the measuring the stress parameters such as oxidative and antioxidative. After centrifugation, the supernatant was extracted and placed at -4°C for further analysis (Wang *et al.*, 2026).

Biochemical analysis: Oxidative stress biomarkers, including thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and reactive oxygen species (ROS), were quantified using a UV–visible spectrophotometer. Antioxidant enzyme activities, namely superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), were determined following previously established protocols (Khairy *et al.*, 2023; Ali *et al.*, 2025; Fotouh *et al.*, 2025). Measurements were conducted in brain and testicular tissues at specific wavelengths: 505 nm (ROS), 532nm (TBARS), 240nm (CAT), 560nm (SOD), and 470nm (POD).

Determination of genetic potential of azoxystrobin by Comet assay: Upon completion of the experimental period, animals were subjected to overnight fasting with free access to water and subsequently euthanized using chloroform. For estimation of genetic damage in isolated cells of the brain and testes, the visceral organs were collected from each rat and were immediately homogenized using chilled deionized water, then centrifuged at 3000rpm for 15 minutes at 4°C for isolation of cells. The separated cells were confirmed by staining with Giemsa solution. Finally, the isolated cells were subjected to the comet assay, a reliable technique under alkaline conditions according to an earlier protocol (Singh *et al.*, 1988). For estimation of genetic damage, approximately 0.95% normal melting point and 1.0% low melting point agarose were dissolved in deionized water at 60-65°C . After that, thin-layer normal melting point agarose (80µL) was prepared on frosted glass and placed on ice for solidification. After solidification of the first layer, a second layer containing isolated cells (5µL) mixed in low-melting-point agarose (100µL) was prepared using a cover slip, and again the slides were kept on ice for solidification. Then a third layer (70µL) of 0.5% low melting point agarose was prepared. All the glass slides arranged and then immersed in newly made and chilled lysing solution at 40°C for 4h for lysing purposes. After lysing the slides, electrophoresis was carried out for 25 min at 25V. Following electrophoresis, the slides were then neutralized using Tris- HCl buffer, pH 7.5, and were subjected to ethidium bromide staining. A total of 250 cells from each rat of each group were examined for DNA damage in terms of DNA material incandescing nearby the nucleus and or makes a tail stained with ethidium bromide in isolated cells of brain and testicular tissues.

Histopathological analysis: Brain and testicular tissues were immediately excised from control and treated rats at necropsy. The collected organs were gently rinsed with normal saline to remove blood and debris, then fixed in 10% neutral buffered formalin. After fixation, tissues were processed using standard histological procedures. Thin sections (4–5µm) were prepared using a rotary microtome, mounted on clean glass slides, and deparaffinized according to established protocols (Fotouh *et al.*, 2025; Rasheed *et al.*, 2025). Subsequently, the sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histopathological evaluation.

Histopathological alterations were assessed using a semi-quantitative scoring system based on lesion severity. The grading criteria were defined as follows: (–) no detectable lesion, (+) mild changes, (++) moderate changes, (+++) severe changes, and (++++) very severe changes. Scoring was performed in a blinded manner to minimize observer bias, and at least five random microscopic fields were evaluated per tissue section.

Statistical analysis: Data was analyzed by ANOVA using the SPSS (SPSS Inc., Illinois, USA) program. Mean ± SE values for antioxidants enzymes, oxidative stress profile, frequency of DNA damage and seminiferous tubules of rats of control and treated groups were compared by Tukey's test. P<0.05 was considered statistically significant.

RESULTS

Clinical observation: No mortality was recorded in rats across all the treatments. Mild to moderate physical disturbances like depression, lethargy and watery fecal contents were examined in rats fed higher doses of insecticides.

Effects at oxidative stress and anti-oxidative enzymes within brain and testes of male albino rats: The results demonstrated significant modulation of oxidative stress biomarkers with elevated lipid peroxidation and ROS generation, indicating compromised cellular oxidative homeostasis. Antioxidant enzyme activities exhibited substantial variations reflecting adaptive or overwhelmed defense mechanisms. Results on oxidative stress analysis revealed significant perturbations in brain of rats in terms of elevated concentrations of ROS and lipid metabolism by-product like TBARS, while displayed marked decrease in contents of antioxidant enzymes indices including SOD, POD, CAT and GSH in rats fed azoxystrobin at high doses 60 and 90mg/kg/day (Fig. 1).

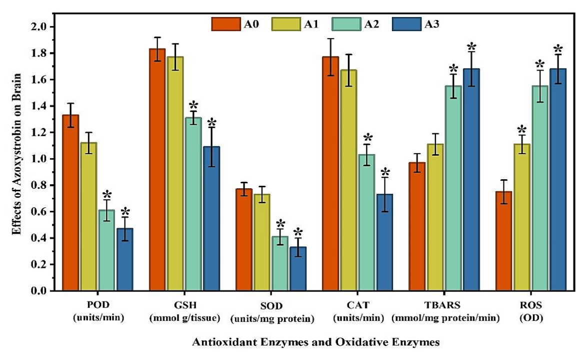

Fig. 1: Photograph exhibiting comparison of oxidative stress and antioxidant profile in the brain of rats treated with different doses of azoxystrobin and normal rats. *P<0.05 vs control (A0).

The results on oxidative and antioxidative profile in rats given different doses of azoxystrobin revealed significantly higher contents of free radicals and lipid peroxidation product TBARS in testicular tissues when compared to normal rats at higher doses 60 and 90mg/kg/day (Fig. 2). The results on antioxidant enzymes displayed marked decrease in contents of antioxidant enzymes like SOD, POD, CAT and GSH in rats testing fed azoxystrobin at elevated dosage 60 and 90mg/kg/day (Fig. 2).

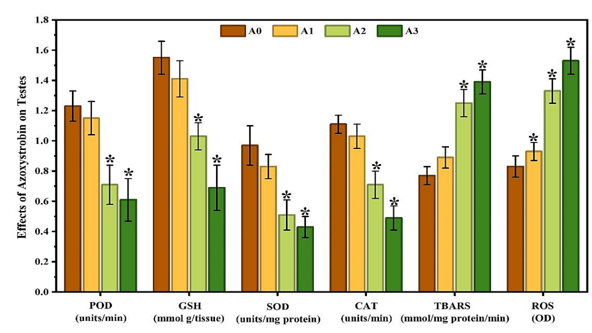

Fig. 2: Photograph exhibiting contrast of oxidative stress and anti-oxidant profile within testes of control and rats treated with different doses of azoxystrobin. *P<0.05 vs control (A0).

Microscopic evaluation revealed distinctive mild to moderate histopathological features (Table 1) in different sections of brain including degeneration and atrophy of neuron, congestion, eccentric and necrosis of neurons, inflammatory exudate and microgliosis in rats fed azoxystrobin at high dose 60mg/kg/day (Fig. 3). Severe histopathological alterations including atrophy of neuron, enlarged cytoplasm of neurons, eccentric nuclei of neuron congestion, inflammatory exudate, necrosis of neurons, and microgliosis in rats fed azoxystrobin at high dose 90 mg/kg/day (Fig. 4). The microscopic results on the intensity/severity of histoarchitectural changes in testes of rats fed various levels of azoxystrobin are recorded in table 1. Minor to modest histological changes in rats fed with azoxystrobin @60mg/kg/day revealed prominent features like necrosis of germinal epithelium of seminiferous tubules, halt of procedure of spermatogenesis, presence of necrotic cells and tissues debris in seminiferous tubules midpoint, mortification of spermatids and detachment of germinal cells (Fig. 5) In contrast, rats treatment by high dosage of azoxystrobin @90mg/kg/day exhibited severe microscopic changes (presence of lumen necrotic cells of seminiferous tubules, germinal epithelium necrosis of seminiferous tubules, vacuolation within epithelium of seminiferous tubules multinucleated giant cells, necrosis of spermatids and halting of development of spermatogenesis (Fig. 6).

Effect on diameter of seminiferous tubule, seminiferous tubules with normal spermatogenesis and genetic Damage: The results indicated significantly lower seminiferous tubule diameter and percentage of seminiferous tubules with normal procedure of spermatogenesis in rat's testicular tissues fed with higher levels of azoxystrobin compared to normal rats (Table 2). The results on genetic damage showed significantly increased percentage of isolated cells of testes (Fig. 7), and brain of rats given higher doses of azoxystrobin compared to normal rats (Fig. 8).

Table 1: Intensity and severity of various testicular and brain lesions examined in male albino rats fed azoxystrobin.

Lesions	Groups/Treatments			
	A0	A1	A2	A3
Testes				
Necrosis of spermatids	-	++	+++	++++
Necrosis of germinal epithelium		++	+++	++++
Increased weight of testes	-	++	++	+++
Decreased size of testes	-	+	+++	++
Presence of necrotic cells of seminiferous tubules lumen	-	++	+++	++++
Inflammatory processes	-	+	++++	++++
Thinning of germinal epithelium	-	++	++++	++++
Decrease within diameter of seminiferous tubules	-	+++	++++	++++
Vacuolation in epithelium of seminiferous tubules	-	++	++++	++++
Decrease frequency of seminiferous tubules with normal cells	-	++	++++	++++
Brain				
Atrophy of neuron	-	+	+++	++++
Enlarged cytoplasm of neurons	-	++	++++	++++
Eccentric nuclei of neuron	-	++	+++	++++
Congestion	-	+	++	++++
Inflammatory exudate	-	+	+++	++++
Microgliosis	-	+	+++	++++
Necrosis of neurons	-	+	++++	++++

Table 2: Seminiferous tubule diameter, percentile rate of seminiferous tubules exhibiting normal spermatozoa and frequency of DNA damage in testes of treated and untreated male albino rats

Parameters	GROUPS/TREATMENT			
	A0	A1	A2	A3
Seminiferous tubule diameter (µm)	191.3±2.6	181.3 ±2.4	146.3±1.3*	131.3±3.3*
Frequency of seminiferous tubules normal spermatogenesis (%)	96.7±1.3	89.5±1.7	65.3±2.7*	55.5±3.3*
Genetic damage (%) in isolated cells of testes	2.79±0.15	3.17±0.14	9.7 ±1.21*	11.5±1.11*
Genetic damage (%) in isolated cells of brain	1.77±0.13	1.85±0.16	6.3 ±0.21*	9.7±0.31*

Values are presented as mean±SE (*n*=7 per group). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. *P<0.05 was considered statistically significant compared to the control group (A0).

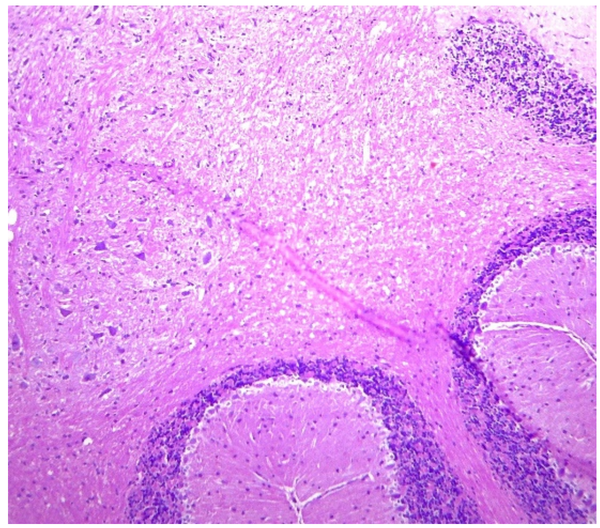

Fig. 3: Microscopic sections of brain of rats treated with azoxystrobin (60mg/kg/day) showing moderate pathological lesions like necrosis of neurons, microgliosis degeneration of neurons and eccentric nuclei of neuron. H&E stain; 400X.

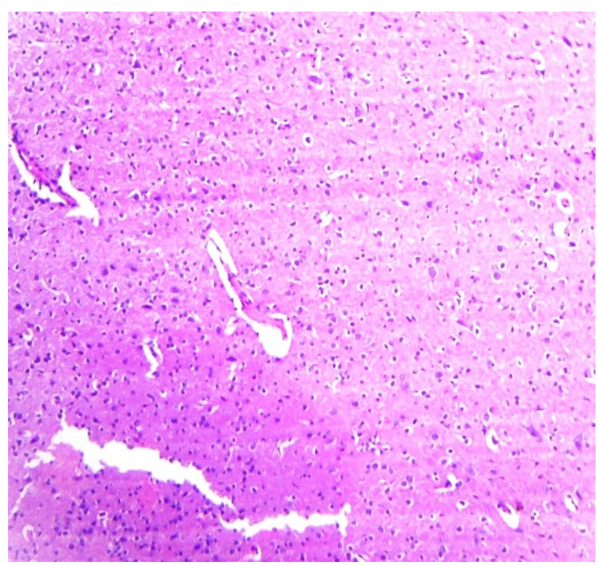

Fig. 4: Microscopic sections of brain of rats treated with higher doses of fungicide (90mg/kg/day) showing severe pathological lesions including neuron erosion and necrosis, hemorrhages, neuron inflamed cytoplasm along with eccentric nuclei of neuron. H&E stain; 400X.

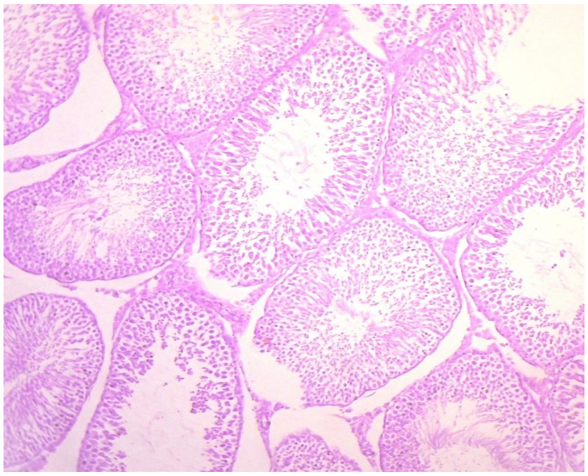

Fig. 5: Microscopic section of testes of rats given azoxystrobin (60mg/kg/day) showing moderate pathological lesions comprising halting the progression of spermatogenesis, vacuolation, necrosis of spermatids and mixing of necrotic spermatids of seminiferous tubules lumen. H&E stain; 400X.

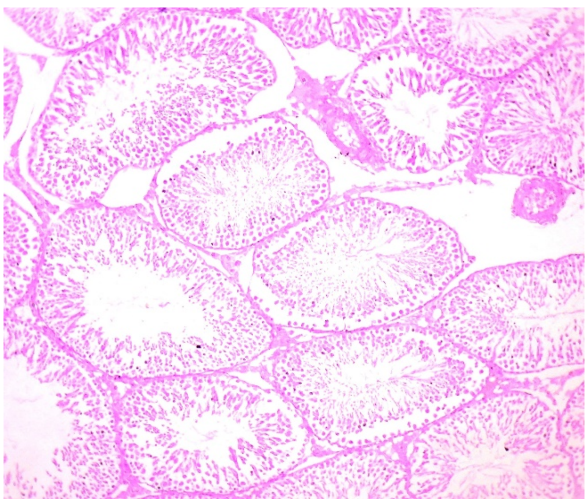

Fig. 6: Microscopic sections of testes of rats given azoxystrobin (90mg/kg/day) showing severe pathological lesions containing halt of procedure of spermatogenesis, sloughed cells, vacuolation, necrosis of spermatids and mixing of necrotic spermatids of seminiferous tubules lumen. H&E stain; 400X.

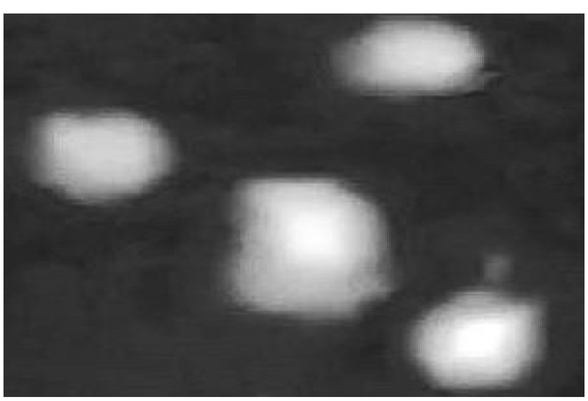

Fig.7: Photomicrograph indicating genetic damage (DNA fluorescing nearby nucleus/ making a tail) in isolated cells of the testicular organ of rats fed high doses (90mg/kg/day) of azoxystrobin 1000X; Ethidium bromide stain.

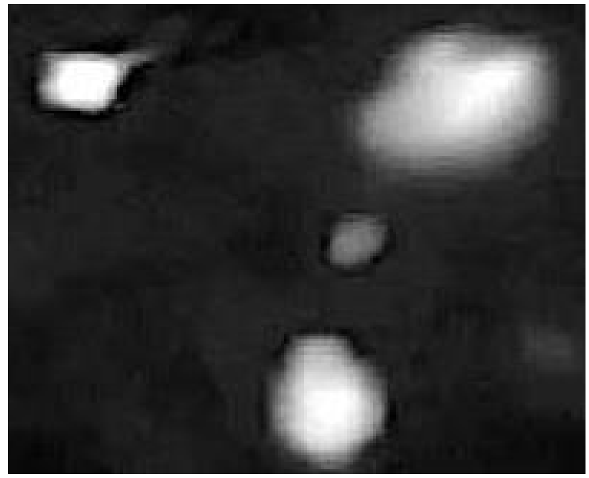

Fig. 8: Photomicrograph indicating genetic damage (DNA flaming surrounding nucleus/ making a tail) in isolated cells of the brain of rats fed high doses (90mg/kg/day) of azoxystrobin 1000X; Ethidium bromide stain.

DISCUSSION

The clinical observations such as depression, lethargy, and gastrointestinal disturbances in azoxystrobin treated rats may reflect systemic toxicity and disruption of normal physiological functions. These behavioral and physical manifestations could result from neurochemical imbalances secondary to oxidative stress-induced neuronal damage, or from general malaise associated with hepatic and renal dysfunction (Yang *et al.*, 2021). However, organ-specific toxicity except the brain and testes was not evaluated in the current investigation (Ahmed *et al.*, 2025). The present investigation demonstrated that azoxystrobin administration at elevated doses (60 and 90mg/kg/day) induces pronounced oxidative stress and cellular damage in brain and testicular tissues of male albino rats manifesting through multiple interconnected pathological mechanisms. The observed elevation in ROS and TBARS coupled with concurrent depletion of enzymatic antioxidants including SOD, POD, CAT, and reduced GSH indicates a severe disruption of cellular redox homeostasis (Ali *et al.*, 2025) This oxidative imbalance appears to overwhelm the endogenous antioxidant defense systems, leading to lipid peroxidation of cellular membranes and subsequent cytotoxicity in both neural and reproductive tissues (Rehman *et al.*, 2021; Al-Saeed *et al.*, 2023).

The mechanism underlying azoxystrobin-induced oxidative stress can be attributed to its interference with mitochondrial respiration, particularly through inhibition of complex III in the electron transport chain (Naseem *et al.*, 2022). This disruption results in excessive electron leakage and subsequent generation of superoxide anions, which are converted to various ROS, including hydrogen peroxide and hydroxyl radicals. These findings align with recent investigations (Zhang *et al.*, 2021), which reported similar oxidative perturbations in hepatic tissues due to insecticide exposure, demonstrating significant elevations in MDA levels and concurrent reductions in antioxidant enzyme activities. Furthermore, Wang *et al.* (2026) documented that strobilurin fungicides, including azoxystrobin, induce mitochondrial dysfunction through dissipation of mitochondrial membrane potential, subsequently triggering apoptotic cascades mediated by cytochrome c release and caspase activation. The profound histopathological alterations observed in brain tissue, including neuronal atrophy, cytoplasmic enlargement, nuclear eccentricity, microgliosis, and inflammatory exudation, reflect the neurotoxic potential of azoxystrobin. These morphological changes are consistent with oxidative stress-mediated neurodegeneration, where excessive ROS production leads to protein oxidation, DNA strand breaks, and lipid peroxidation of neuronal membranes (Qiao *et al.*, 2021). The presence of microgliosis suggests an inflammatory response to neuronal injury, as activated microglial cells release pro-inflammatory cytokines that can exacerbate oxidative damage. Recent work corroborates these findings, demonstrating that azoxystrobin exposure induces neurobehavioral deficits in zebrafish models through oxidative stress-dependent mechanisms, with significant alterations in locomotor activity and acetylcholinesterase inhibition (Liu *et al.*, 2023).

The testicular pathology observed in the current study is particularly noteworthy, characterized by necrosis of germinal epithelium, arrest of spermatogenesis, decreased seminiferous tubule diameter, vacuolation, and reduced frequency of tubules exhibiting normal spermatogenesis. These reproductive toxicological effects appear to be mediated through oxidative stress-induced damage to the blood-testis barrier and direct cytotoxic effects on germ cells, particularly spermatids and spermatocytes, which are highly vulnerable to ROS-mediated injury due to their high content of polyunsaturated fatty acids (Rajeh, 2026a; Riaz *et al.*, 2025). The observed reduction in seminiferous tubule diameter from 191.3µm in control animals to 131.3 µm in the highest dose group, coupled with a dramatic decrease in normal spermatogenesis from 96.7 to 55.5%, indicates severe impairment of spermatogenic function. These observations are consistent with recent findings reported that azoxystrobin exposure in male mice resulted in significant reductions in sperm count, motility, and morphological abnormalities, accompanied by elevated oxidative stress markers in testicular tissue and decreased testosterone levels (Chen *et al.*, 2020; Rajeh, 2026b).

The genotoxic effects demonstrated in the present study, with DNA damage frequencies increasing from baseline levels of 2.79 to 11.5% in testicular cells and from 1.77 to 9.7% in brain cells at the highest dose, represent a critical toxicological endpoint. This genetic damage likely results from multiple mechanisms, including direct ROS-mediated DNA strand breaks, oxidative modification of nucleotide bases (particularly 8-hydroxy-2'-deoxyguanosine formation), and impairment of DNA repair mechanisms (Naseer *et al.*, 2025). The vulnerability of both neural and reproductive tissues to genotoxic insult reflects their high metabolic activity and limited regenerative capacity. Recent investigations (Kumar *et al.*, 2022) demonstrated similar genotoxic effects of azoxystrobin in human peripheral blood lymphocytes using the comet assay, revealing dose-dependent increases in DNA damage, micronucleus formation, and chromosomal aberrations, suggesting potential mutagenic and carcinogenic risks associated with prolonged exposure.

The dose-dependent relationship between azoxystrobin administration and the severity of pathological manifestations underscores the importance of exposure assessment in risk evaluation. The progression from mild alterations at lower doses to severe degenerative changes at higher doses suggests a threshold effect, beyond which the cellular antioxidant and repair mechanisms become overwhelmed. This pattern is consistent with recent published research by Pereira *et al.* (2021), who investigated the toxicological profile of azoxystrobin in aquatic organisms and reported similar dose-dependent oxidative stress responses, with significant correlations between exposure concentrations and biomarker alterations, including lipid peroxidation, protein carbonylation, and antioxidant enzyme modulation.

The implications of these findings extend beyond individual organ toxicity to suggest potential systemic effects of azoxystrobin exposure. The concurrent impairment of neural and reproductive functions raises concerns about the fungicide's impact on neuroendocrine regulation, as the hypothalamic-pituitary-gonadal axis plays a crucial role in reproductive physiology. Recent evidence (Tang *et al.*, 2023) supports this hypothesis, demonstrating that azoxystrobin exposure disrupts hormone homeostasis in zebrafish, altering expression of genes involved in steroidogenesis and causing significant reductions in plasma testosterone and estradiol levels. From a mechanistic perspective, the observed toxicological effects appear to involve a complex interplay between oxidative stress, mitochondrial dysfunction, inflammatory responses, and impaired cellular repair mechanisms.

The depletion of GSH, a critical intracellular antioxidant and cofactor for glutathione peroxidase, represents a particularly significant finding as it compromises the cell's ability to detoxify both ROS and xenobiotics through Phase II conjugation reactions. This is supported by recent studies by Zhao *et al.* (2022) and Rashid *et al.* (2025), who identified significant perturbations in glutathione metabolism, amino acid metabolism, and energy metabolism pathways in rats exposed to azoxystrobin and fish treated with heavy metals, suggesting widespread metabolic dysregulation. It has been recorded that the investigation of histopathological analysis is a critical diagnostic technique for examining tissue at a cellular level to identify the mechanisms of toxicity of harmful substances (Afzal *et al.*, 2022). The histopathological alterations in rats observed in testicular tissues could be linked to the oxidative stress initiation via the rapid generation of free radicals by azoxystrobin in rats. Light microscopic investigation of diverse sections of brain and testes of rats given higher dosage of insecticides including necrosis of spermatids, arrest of process of spermatogenesis, detachment of germinal epithelium in testes. Previously similar histopathological lesions due to pesticides in testes of bird have also been observed (Hussain *et al.*, 2017), while necrosis of neuron, microgliosis and inflammatory exudate in brain provided substantial evidence of systemic physiological alterations encompassing oxidative damage, metabolic dysfunction, and structural tissue damage collectively indicating significant modulation of cellular homeostasis and tissue integrity with profound implications for overall organismal health and physiological adaptation mechanisms (Wu *et al.*, 2021). The microscopic alterations observed in rats in this experimental research might also be corelated to oxidative stress production induced damages in testes and brain.

Conclusions: This study provides comprehensive evidence that azoxystrobin exposure induces significant oxidative stress, cellular damage, and genotoxicity in neural and reproductive tissues of male rats through mechanisms involving ROS generation, lipid peroxidation, antioxidant depletion, and impairment of cellular integrity. These findings, corroborated by recent literature, underscore the need for careful risk assessment of azoxystrobin exposure in occupational and environmental settings and warrant further investigation into potential protective strategies, including antioxidant supplementation and regulatory measures to limit human and environmental exposure to this widely used fungicide.

Authors contribution: NA, AA, AAA: Writing –original draft, Writing – review & editing, Investigation, Data curation, Conceptualization, Funding acquisition. AEA, YH, NKE: Methodology, Investigation, Supervision. FA, ME: Data curation, Resources. AMAM, KA: Writing – review & editing, Conceptualization, Project administration, Resources. All authors read and approved the manuscript.

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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