

Impact of Varying Doses and Timing of Pesticide ‘Atrazine’ on the Testes of Male Wistar Rats

Enyioma-Alozie, S.*

Department of Anatomy, Faculty of Basic Medical Sciences, Baze University, Abuja

*Corresponding Author: swesme.alozie@bazeuniversity.edu.ng

ABSTRACT

Background: The testes are vital for spermatogenesis and testosterone synthesis, both essential for male fertility. Environmental contaminants, particularly pesticides such as Atrazine, have been implicated in testicular dysfunction and endocrine disruption. Atrazine, a widely used herbicide, persists in the environment and may affect non-target organisms, including mammals.

Aim: This study evaluated the impact of varying doses and exposure durations of Atrazine on body weight, reproductive hormones, sperm quality, and testicular histoarchitecture in male Wistar rats. **Methodology:** Twenty-five adult male Wistar rats were randomly divided into five groups (n=5). Group 1 received distilled water (control), while Groups 2–5 received 38.5, 77, 154, and 200 mg/kg body weight/day of Atrazine orally for 21 days. Body weight, serum testosterone, luteinizing hormone (LH), sperm parameters, and testicular histology were assessed using standard biochemical and histological techniques. **Results:** Atrazine exposure led to a dose-dependent reduction in body weight gain, from 56.6% in controls to 28.1% at 200 mg/kg. Serum testosterone significantly decreased from 3.36 ± 0.61 ng/mL (control) to 1.48 ± 0.30 ng/mL at 77 mg/kg ($p \leq 0.05$), with partial recovery at 200 mg/kg (2.99 ± 0.64 ng/mL). Sperm count declined progressively from $54.95 \pm 18.23 \times 10^6$ (control) to $37.05 \pm 14.14 \times 10^6$ at 200 mg/kg, with corresponding decreases in motility and progressivity. Despite these functional impairments, testicular histology remained normal across all groups. **Conclusion:** Atrazine exerts dose-dependent endocrine and spermatogenic disruptions in male rats, with reduced testosterone and sperm quality preceding histological damage. These findings underscore the reproductive risks associated with prolonged Atrazine exposure.

Keywords: Atrazine, Reproductive toxicity, Testosterone, Sperm parameters, Wistar rats

INTRODUCTION

The testes are critical organs of the male reproductive system, responsible for the dual functions of spermatogenesis and the synthesis of testosterone, the principal male sex hormone. These processes are fundamental to male fertility and the development of secondary sexual characteristics (Peate, 2025). The efficiency of spermatogenesis is highly sensitive to environmental and physiological factors such as temperature fluctuations, hormonal balance, and exposure to toxic substances. Elevated testicular

temperature, for instance, disrupts normal sperm production, which explains the anatomical positioning of the testes within the scrotum, maintaining an optimal temperature approximately 2°C below core body temperature (Shahat, 2020).

In recent decades, environmental contaminants, particularly pesticides, have been increasingly implicated in male reproductive dysfunction due to their ability to interfere with normal testicular physiology and sperm quality (Environmental Impacts on Reproductive Health, 2022).

Pesticides play an indispensable role in modern agriculture by controlling weeds, insects, and pathogens that threaten crop yield (Khan *et al.*, 2023). However, their extensive use has raised public health concerns, particularly regarding their potential as endocrine-disrupting chemicals (EDCs). These compounds can mimic or block endogenous hormones, thereby disturbing the delicate endocrine regulation of reproduction (Zlatnik, 2016). The reproductive consequences of chronic pesticide exposure include reduced fertility, alterations in sperm morphology and motility, impaired steroidogenesis, and testicular atrophy (Mostafalou *et al.*, 2013). Moreover, epidemiological and toxicological studies have demonstrated associations between pesticide exposure and disorders such as infertility, testicular dysgenesis, and hormone-dependent cancers. For instance, a 2023 study at Stanford University identified 22 pesticides associated with increased prostate cancer risk, emphasizing the long-term health hazards linked to pesticide exposure (Soerensen *et al.*, 2024). Similarly, recent evidence suggests that neonicotinoids, one of the most commonly applied pesticide classes, may disrupt neurodevelopment and delay sexual maturation in males (Donley *et al.*, 2024).

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine; ATZ) is a widely used chloro-s-triazine herbicide commonly applied to control broadleaf and grassy weeds in corn, sorghum, sugarcane, cotton, and pineapple cultivation (Seiler, 1992). Due to its efficiency and low cost, it remains one of the most extensively used herbicides globally and ranks as the second most applied pesticide in the United States (Fatka, 2025). According to Syngenta (2025), Atrazine supports over 85,000 jobs in the agricultural industry and contributes to a yield increase of approximately 900 million bushels of corn annually, translating to an economic benefit of \$34–\$48 per acre. However, the extensive and repeated application of Atrazine has resulted in its persistence in the environment, contaminating soil, groundwater, and surface water, with potential consequences for both wildlife and humans (Rohr, 2021, Matthiessen *et al.*, 2018). Studies have reported that Atrazine and its metabolites persist in the environment, particularly during farming seasons, and may affect non-target species such as amphibians by inducing developmental and reproductive abnormalities (Soare *et al.*, 2019).

Although early assessments suggested that Atrazine posed minimal health risks, accumulating evidence now indicates the need for re-evaluation of its safety, especially in relation to endocrine and reproductive health (Mountaki, 2025). Given the central role of the testes in male fertility and the widespread use of Atrazine in agriculture, it is essential to investigate its potential reproductive toxicity.

Despite extensive research on Atrazine's environmental fate and toxicity, data remain limited regarding the specific effects of varying doses and timing of exposure on testicular structure and function. Considering that both dose and duration are critical determinants of toxicological outcomes, elucidating their relationship with testicular damage is necessary for accurate risk assessment. Therefore, this study seeks to evaluate the effects of varying doses and exposure durations of Atrazine on the testes of male Wistar rats. Findings from this research will provide valuable insight into the reproductive hazards associated with Atrazine exposure, contributing to improved understanding of its health implications and informing policy on safer pesticide usage and environmental protection.

MATERIALS AND METHODS

Procurement and Housing of Experimental Animals

Twenty-five (25) adult male Wistar rats with an average weight of 120g were purchased from the Animal House of the College of Medicine and Health Sciences, Baze University, Abuja. The animals were housed in five well-ventilated plastic cage measuring 20cm x 30cm. They were fed with standard rat feed and water *ad libitum*, and kept at room temperature (25 ± 1 °C) with 12 hours light and 12 hours dark cycle throughout the period of the research, which included a 14 days acclimatization period, and a 21 days treatment period. The weights of the Wistar rats were measured before and after acclimatization, and also before and after the administration using an electric weighing balance.

Preparation of Atrazine Solution

Laboratory-prepared technical grade Atrazine pesticide (>98% purity) was purchased from a commercial chemical store in Abuja with the dosing solution (water). The prepared Atrazine and its dosing solution were stored at room temperature in the animal house throughout the period of this experiment.

Animal Feed

The rat feed (vital feed) was purchased from a feed store in Abuja, and stored at room temperature in the animal house.

Laboratory Materials

Other laboratory materials include: gloves, sterile bottles, syringes, needle, dissecting board, kit, fixative (10% formal saline), H&E stain, cover slips, glass slides, microscope, microtome, centrifuge, distilled water, oral gavage, feeding plates and water bottles.

Experimental Design

The twenty-five (25) adult male Wistar rats were randomly divided into five (5) groups of five rats each, and treated Atrazine orally (Olayinka *et al.*, 2022) as follows:

Group 1 (Control Group): 5ml/kg distilled water with vital feed only for the 21 days period of the experiment

Group 2: 38.5 mg/kg bw/day oral administration of Atrazine using oral gavage for 21days

Group 3: 77 mg/kg bw/day oral administration of Atrazine using oral gavage for 21 days

Group 4: 154 mg/kg bw/day oral administration of Atrazine using oral gavage for 21 days

Group 5: 200 mg/kg bw/day oral administration of Atrazine using oral gavage for 21 days

Animal Sacrifice

At the end of the 21day treatment period, all the 25 rats were fasted overnight and then sacrificed by chloroform inhalation as anaesthesia. The blood samples were taken in sterile bottles for biochemical analysis, and the organ of interest (testis and epididymis) was harvested and fixed in 10% formal saline for histological analysis and tissue processing.

Assay of Reproductive Hormones

The concentrations of reproductive hormones, specifically testosterone and luteinizing hormone (LH), were determined using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. For testosterone, a competitive ELISA method was employed according to Ghazal *et al.*, (2022). In this assay, testosterone molecules present in the serum samples competed with enzyme-labeled testosterone conjugates for binding to specific antibodies pre-coated on the wells of the microplate. The reaction was carried out by adding standards, controls, and samples into antibody-coated wells, followed by the addition of the enzyme conjugate and incubation at 37°C for 60 minutes. After washing to remove unbound reagents, a tetramethylbenzidine (TMB) substrate was added to develop color, which was subsequently stopped using sulfuric acid. The optical density was read at 450 nm using a microplate reader.

The intensity of the color produced was inversely proportional to the testosterone concentration in the samples, and values were derived from a standard calibration curve and expressed in nanograms per milliliter (ng/mL).

Luteinizing hormone (LH) concentration was also determined using the ELISA technique, based on the competitive binding principle. In this procedure, standards, controls, and serum samples were added to microplate wells pre-coated with LH-specific antibodies and incubated at 37°C for 60 minutes to facilitate antigen-antibody binding. Following incubation, unbound substances were washed off, and an enzyme-linked secondary antibody was introduced. After a second incubation and washing step, TMB substrate was added to initiate color development, which was allowed for 10–15 minutes before the reaction was terminated with sulfuric acid. The absorbance was then measured at 450 nm, and LH concentrations were determined by comparison with a standard curve (Elabscience, 2020). The results were expressed in milli-international units per milliliter (mIU/mL).

Sperm Analysis

Sperm parameters were evaluated following a modified method of Yokoi and Mayi (2004). The cauda epididymis was minced in 5 mL of physiological saline, gently agitated for 10 minutes, and incubated at room temperature for 2 minutes. The supernatant was diluted (1:100) in a solution containing 5 g sodium bicarbonate and 1 mL of 35% formalin. Total sperm count was determined using an improved Neubauer

hemocytometer. A 10 μL aliquot of the diluted suspension was loaded into each chamber, allowed to settle, and examined under a light microscope. Spermatozoa were counted in five 16-celled squares, and the concentration was expressed as $(X \times 10^6)/\text{mL}$, where X represents the mean sperm count.

Sperm motility was assessed microscopically at $\times 40$ magnification using samples from the original dilution. For morphological evaluation, spermatozoa were fixed in 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada) at a 1:20 dilution. A total of 500 spermatozoa per sample were examined under phase-contrast microscopy, and morphological abnormalities were recorded. The percentage of morphologically normal spermatozoa was calculated, and representative micrographs were taken for reference.

Hematoxylin & Eosin Tissue Processing and Microscopic Examination

Testicular tissues were fixed in 10% buffered formalin and processed through graded alcohols (70%, 80%, 90%, 95%, and absolute ethanol), cleared in xylene, and embedded in molten paraffin wax. The paraffin blocks were solidified at 5°C for 15 minutes and sectioned at 3 μm thickness using a rotary microtome. Sections were floated on a 55°C water bath, mounted on frosted-end glass slides, and air-dried on a hot plate for 40 minutes to ensure proper adhesion. Sections were stained using the standard Hematoxylin and Eosin (H&E) technique. Slides were dewaxed in xylene, hydrated through descending grades of alcohol, stained in Harris hematoxylin for 5 minutes, differentiated in 1% acid alcohol, blued in running tap water, and counterstained with 1% eosin. The sections were then dehydrated through ascending grades of alcohol, cleared in xylene, and mounted with dibutylphthalate propylene xylene (DPX) (Ladics and Ryan, 2019). The stained slides were examined under a light microscope for evaluation of testicular histoarchitecture, and representative photomicrographs were taken.

Ethical Clearance

All the procedures in this research were conducted in accordance with the standard procedures as set by the Research Ethical Committee of the Department of Anatomy, Faculty of Basic Medical Sciences, Baze University, Abuja. The research protocol was reviewed and cleared by the committee with a reference number BU/URES/ANA/0009.

Statistical Analysis

Statistical data obtained in the research was analyzed using the statistical package for the Social Sciences (IBM SPSS) version 23. Mean and Standard Deviation (SD) was calculated for all the values. Comparison between the control and treated groups was done using a one-way analysis of variance (ANOVA) with LSD multiple range tests. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Body Weight Differences across Groups

The mean body weight changes across groups in this study were compared on one-way ANOVA, as presented on Table 1. For the initial body weight of the rats, final body weight, and body weight differences, no group showed a statistically significant difference at $p \leq 0.05$ when compared to the control group. Intergroup comparison also showed no statistically significant difference in mean. The control group showed the

highest body weight increase (56.6%), indicating normal growth without Atrazine exposure. Group 2 (38.5 mg/kg bw/day) and Group 3 (77 mg/kg bw/day) showed a decrease in body weight gain compared to the control. Group 2 had a 41.5% weight increase, while Group 3 had a 43.5% increase, both lower than the control. A further decline in body weight gain was observed in Group 4 (154 mg/kg bw/day) and Group 5 (200 mg/kg bw/day). Group 4 had a 29.9% increase, and Group 5 had the lowest weight gain at 28.1%. The results suggest that Atrazine exposure led to a dose-dependent reduction in body weight gain of the rats.

Table 1: Showing the Mean Body Weight Changes across Groups Compared on One-Way ANOVA

GROUP (N)	INITIAL BODY WEIGHT (g)	FINAL BODY WEIGHT (g)	BODY WEIGHT DIFFERENCES (%)
GROUP 1 (Control)	116.8±8.8	187.5±22.9	56.6±36.1
GROUP 2 (38.5 mg/kg ATZ)	123.8±27.3	175.6±20.1	41.5±24.9
GROUP 3 (77 mg/kg ATZ)	136.0±29.8	190.3±43.9	43.5±32.9
GROUP 4 (154 mg/kg ATZ)	122.3±11.4	159.6±12.7	29.9±25.1
GROUP 5 (200 mg/kg ATZ)	128.4±8.1	156.5±17.1	28.1±24.6

Values are expressed as MEAN±SD; N = 5; ATZ = Atrazine; * $P < 0.05$ Compared to the Control

Reproductive Hormone Levels: Testosterone and Luteinizing Hormone (LH)

Table 2 shows the mean reproductive hormone levels across groups compared on one-way ANOVA. Compared to the control group (3.36±0.61), Atrazine exposure resulted in a dose-dependent decrease in testosterone levels: Group 2 (38.5 mg/kg) showed a significant reduction (1.76±0.83, $p \leq 0.05$) indicating early suppression. Group 3 (77 mg/kg) showed further decrease (1.48±0.30, $p \leq 0.05$), suggesting a worsening effect. Group 4 (154 mg/kg) showed a slight recovery (1.81±0.53, $p \leq 0.05$), but still significantly lower than the control. Group 5 (200 mg/kg) also showed partial restoration (2.99±0.64), indicating potential adaptation or compensatory mechanisms at the highest dose. Atrazine exposure suppresses testosterone production at lower doses (38.5–154 mg/kg), likely due to disruption of the hypothalamic-pituitary-gonadal (HPG) axis. However, at higher doses (200 mg/kg), testosterone levels recover, which may indicate an adaptive physiological response or detoxification effects.

For Luteinizing Hormone (LH) levels, compared to the control group (2.01±0.19), LH levels fluctuated with Atrazine exposure. In Group 2 (38.5 mg/kg bw/day), LH levels decreased (1.43±0.45), suggesting suppression. Group 3 (77 mg/kg bw/day) showed a persistent low LH level (1.88±0.17). Group 4 (154 mg/kg bw/day) had LH level similar to the control (2.01±0.28). Group 5 (200 mg/kg bw/day) had an LH level slightly lower than the control (1.89±0.38).

Table 2: Showing the Mean Reproductive Hormone Levels across Groups Compared on One-Way ANOVA

GROUP (N)	TESTOSTERONE (ng/mL)	LUTEINIZING HORMONE (mIU/mL)
GROUP 1 (Control)	3.36±0.61	2.01±0.19
GROUP 2 (38.5 mg/kg ATZ)	1.76±0.83*	1.43±0.45
GROUP 3 (77 mg/kg ATZ)	1.48±0.30*	1.88±0.17
GROUP 4 (154 mg/kg ATZ)	1.81±0.53*	2.01±0.28
GROUP 5 (200 mg/kg ATZ)	2.99±0.64	1.89±0.38

Values are expressed as MEAN±SD; N = 5; ATZ = Atrazine; * $P < 0.05$ Compared to the Control

Sperm Analysis: Sperm Count, Motility, Morphology and Progressivity

Table 3 shows the mean sperm parameters of rats across groups compared on one – way ANOVA. For Sperm Count, the control group (Group 1, 0 mg/kg bw/day) had a sperm count of $54.95 \pm 18.23 \times 10^6$. At the lowest dose (38.5 mg/kg bw/day, Group 2), sperm count slightly increased to $60.45 \pm 4.22 \times 10^6$, suggesting no adverse effect at this level. However, at 77 mg/kg bw/day (Group 3), sperm count declined to $47.30 \pm 10.26 \times 10^6$, and further decreased in Groups 4 (154 mg/kg bw/day) and 5 (200 mg/kg bw/day) to $40.00 \pm 18.73 \times 10^6$ and $37.05 \pm 14.14 \times 10^6$, respectively. This downward trend indicates that Atrazine exposure reduces sperm count at higher doses, potentially due to testicular toxicity, hormonal disruption, or impaired spermatogenesis.

The control group (Group 1) receiving 0 mg/kg bw/day, exhibited the highest sperm motility (65.48 ± 14.92). At a low dose (38.5 mg/kg bw/day, Group 2), sperm motility remained similar (65.75 ± 10.04), suggesting no significant effect at this level. However, as the Atrazine dose increased, sperm motility progressively declined. Group 3 (77 mg/kg bw/day) showed a noticeable reduction in motility (59.60 ± 15.26), while Groups 4 (154 mg/kg bw/day) and 5 (200 mg/kg bw/day) exhibited a substantial decline to 43.88 ± 16.44 and 42.33 ± 23.41 , respectively. Similarly, the control group had a sperm morphology of 72.34 ± 8.89 .

At the lowest dose (38.5 mg/kg bw/day, Group 2), sperm morphology slightly increased to 76.85 ± 9.22 , and at 77 mg/kg bw/day (Group 3), it remained relatively stable at 75.20 ± 16.48 . However, at higher doses (154 mg/kg bw/day, Group 4), morphology declined to 69.97 ± 13.69 , and at 200 mg/kg bw/day (Group 5), it returned to a value similar to the control (72.47 ± 4.88). For Sperm Progressivity, the control group exhibited a sperm progressivity of 1.75 ± 0.71 , while Group 2 (38.5 mg/kg bw/day) had a slightly improved progressivity of 2.00 ± 0.00 , which may be an incidental finding. From Group 3 onwards, sperm progressivity steadily declined: 1.25 ± 0.71 in Group 3, and 1.00 ± 0.00 in both Groups 4 and 5. The progressive reduction in sperm movement suggests that Atrazine affects sperm motility and directional movement, which may impair fertilization potential.

Table 3: Showing the Mean Sperm Parameters across Groups Compared on One-Way ANOVA

GROUP (N)	COUNT (x10⁶/mL)	MOTILITY (%)	MORPHOLOG Y (%)	PROGRESSI VITY (Scale 1 - 4)
GROUP 1 (Control)	54.95±18.2 3	65.48±14.92	72.34±8.89	1.75±0.71
GROUP 2 (38.5 mg/kg ATZ)	60.45±4.22	65.75±10.04	76.85±9.22	2.00±0.00
GROUP 3 (77 mg/kg ATZ)	47.30±10.2 6	59.60±15.26	75.20±16.48	1.25±0.71
GROUP 4 (154 mg/kg ATZ)	40.00±18.7 3	43.88±16.44	69.97±13.69	1.00±0.00
GROUP 5 (200 mg/kg ATZ)	37.05±14.1 4	42.33±23.41	72.47±4.88	1.00±0.00

Values are expressed as MEAN±SD; N = 5; ATZ = Atrazine; **P*<0.05 Compared to the Control

Histological Evaluation

The examination of testicular tissues from all five groups of Wistar rats, stained with Hematoxylin and Eosin (H&E), demonstrated normal testicular architecture without any indications of toxicity or structural abnormalities.

Seminiferous Tubules: The seminiferous tubules in all groups were well-organized, featuring intact basement membranes and a normal luminal structure. There were no signs of degeneration, atrophy, or distortion within the tubules.

Germ Cell Population and Spermatogenesis: A complete spermatogenic cycle was observed, encompassing spermatogonia, primary and secondary spermatocytes, spermatids, and mature spermatozoa. There was no evidence of germ cell apoptosis, necrosis, or vacuolation.

Leydig Cells and Interstitial Tissue: The Leydig cells were abundant and morphologically normal, suggesting undisturbed testosterone production. The interstitial spaces showed no signs of edema, fibrosis, or inflammatory infiltration.

Sertoli Cells and Supporting Structures: Sertoli cells maintained their structural integrity and were appropriately aligned within the seminiferous epithelium, ensuring proper support for spermatogenesis. There were no signs of dysfunction or detachment.

Vascular and Connective Tissue Integrity: The testicular blood vessels remained intact, with no indications of congestion, hemorrhage, or infarction. The connective tissue framework was well-preserved, free from fibrosis or degenerative changes.

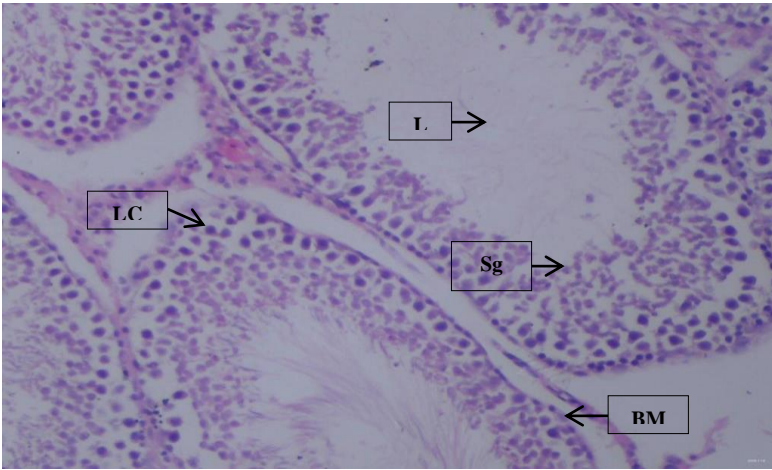


Plate 1: Photomicrograph of the Testis from Group 1 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

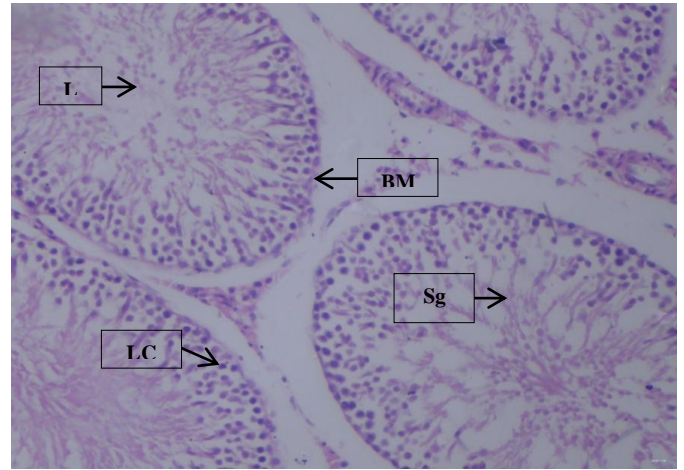


Plate 2: Photomicrograph of the Testis from Group 2 showing Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

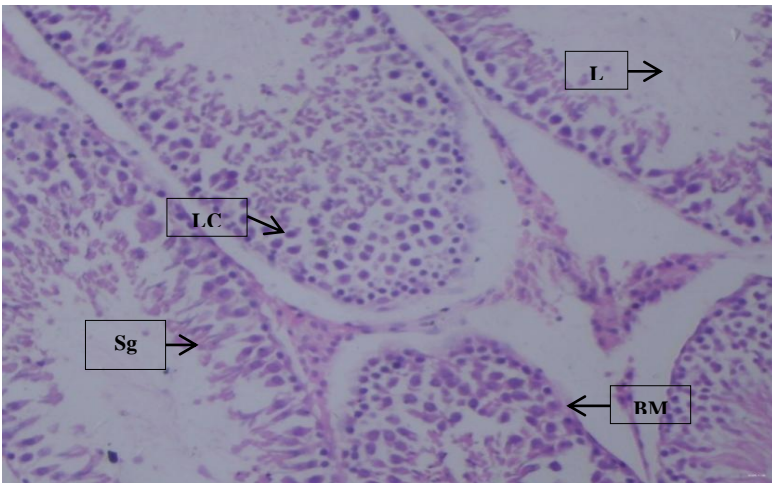


Plate 3: Photomicrograph of the Testis from Group 3 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

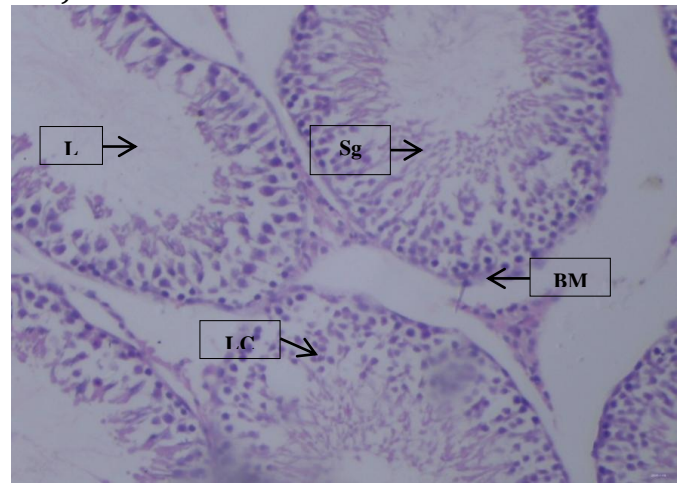


Plate 4: Photomicrograph of the Testis from Group 4 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

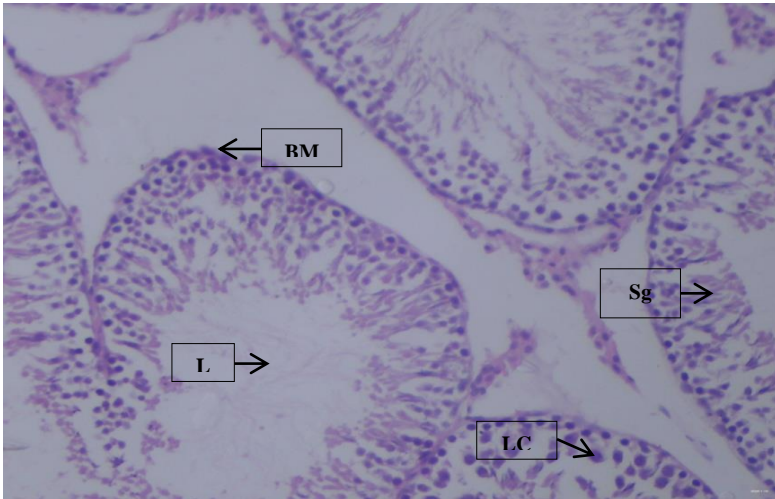


Plate 5: Photomicrograph of the Testis from Group 5 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

DISCUSSION

The reduction in body weight observed among Atrazine-treated rats indicates systemic toxicity associated with the herbicide. Although no statistically significant differences were detected across groups, the progressive decline in body weight gain with increasing doses suggests dose-related metabolic stress or appetite suppression. Similar findings were reported by Okafor *et al.* (2022), who demonstrated that chronic Atrazine exposure at 100 mg/kg reduced body weight in Wistar rats, possibly due to oxidative stress and mitochondrial dysfunction. Likewise, Abarikwu *et al.*, (2010) reported reduction in body weights of in rats exposed to high doses of Atrazine, that may have altered metabolic enzyme activities, impairing energy metabolism and nutrient absorption.

The dose-dependent reduction in serum testosterone levels in this study supports earlier reports that Atrazine disrupts the hypothalamic-pituitary-gonadal (HPG) axis. Atrazine is known to inhibit luteinizing hormone (LH)-stimulated testosterone synthesis in Leydig cells, thereby suppressing androgen production (Pedrozo *et al.*, 2024). The observed partial recovery of testosterone at the highest dose (200 mg/kg) may reflect adaptive detoxification mechanisms or induction of hepatic cytochrome P450 enzymes involved in steroid metabolism (Siboto *et al.*, 2023). The fluctuating LH levels seen across treatment groups also align with the feedback disruption of the HPG axis reported in similar studies ((Pedrozo *et al.*, 2024). These hormonal alterations suggest that Atrazine acts as an endocrine disruptor capable of impairing reproductive hormonal balance even at sub-lethal doses.

Atrazine exposure produced a consistent dose-related decline in sperm count, motility, and progressivity, indicating impaired spermatogenesis and sperm function. Reduced sperm motility and progressivity are hallmarks of testicular oxidative stress (Rotimi *et al.*, 2023), which compromises sperm membrane fluidity and mitochondrial energy production. Comparable results were reported by Rotimi *et al.*,(2023) and Abarikwu *et al.* (2023), who observed a significant reduction in sperm quality indices following

Atrazine exposure in male rodents. The slight improvement in sperm morphology at low doses in this study could be incidental or related to compensatory antioxidant defense mechanisms. However, the overall decline at higher doses indicates that Atrazine disrupts spermatogenic efficiency, likely via oxidative or hormonal pathways.

Despite the biochemical and sperm alterations, histological examination revealed normal testicular architecture across all experimental groups. This finding suggests that short-term Atrazine exposure (21 days) at the tested doses may impair functional parameters without inducing overt structural damage. Abarikwu *et al.* (2010) and Owagboriaye *et al.*, (2022) similarly reported that low- to moderate-dose Atrazine exposure caused reversible functional impairments in testicular physiology without visible histological degeneration. However, prolonged exposure or co-exposure with other environmental toxicants may potentiate degenerative effects, as shown by Olayemi *et al.* (2020), who observed seminiferous tubule degeneration following 60-day Atrazine administration.

Collectively, these findings indicate that Atrazine exerts subtle but significant reproductive toxicity in male Wistar rats through endocrine disruption and impaired spermatogenesis, while overt tissue damage may require prolonged or higher cumulative exposure.

CONCLUSION

This study demonstrates that oral administration of Atrazine produces dose-dependent effects on body weight, serum testosterone, and sperm quality in male Wistar rats. Atrazine exposure resulted in reduced testosterone levels and impaired sperm motility and count, consistent with endocrine disruption of the hypothalamic-pituitary-gonadal axis. However, testicular histology remained largely normal, indicating that functional impairments may precede structural degeneration.

CONFLICTS OF INTEREST: The author declares no conflicts of interest

REFERENCES

- Abarikwu, S. O., Adesiyun, A. C., Oyeloja, T. O., Oyeyemi, M. O., & Farombi, E. O. (2010). Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, atrazine. *Archives of environmental contamination and toxicology*, 58(3), 874-882.
- Abarikwu, S. O., Ezim, O. E., Ikeji, C. N., & Farombi, E. O. (2023). Atrazine: cytotoxicity, oxidative stress, apoptosis, testicular effects and chemopreventive Interventions. *Frontiers in Toxicology*, 5, 1246708.
- Abarikwu, S. O., Oleribe, A. L., Mgbudom-Okah, C. J., Onuah, C. L., Chikwendu, C. S., & Onyeike, E. N. (2022). The protective effect of fluted pumpkin seeds against atrazine-induced testicular injury. *Drug and Chemical Toxicology*, 45(2), 799–809. <https://doi.org/10.1080/01480545.2020.1776723>

- Donley, N., Peterson, R., & Zhang, Y. (2024). Emerging concerns on neonicotinoid exposure and delayed sexual maturation in mammals. *Reproductive Toxicology*, *119*, 108–117.
- Elabscience. (2020). *Human LH (Luteinizing Hormone) ELISA Kit – Manual*. Elabscience
- Environmental Impacts on Reproductive Health. (2022). *Global report on environmental contaminants and male reproductive dysfunction*. World Health Organization.
- Fatka, M. (2025). Atrazine: The enduring herbicide supporting modern agriculture. *AgriBusiness Today*, *47*(2), 22–27.
- Ghazal, K., Brabant, S., Prie, D., & Piketty, M. L. (2022). Hormone immunoassay interference: a 2021 update. *Annals of laboratory medicine*, *42*(1), 3-23.
- Khan, B. A., Nadeem, M. A., Nawaz, H., Amin, M. M., Abbasi, G. H., Nadeem, M., ... & Ayub, M. A. (2023). Pesticides: impacts on agriculture productivity, environment, and management strategies. In *Emerging contaminants and plants: Interactions, adaptations and remediation technologies* (pp. 109-134). Cham: Springer International Publishing.
- Ladics, G. S., & Ryan, K. L. (2019). Histopathological techniques in toxicologic pathology. In J. D. Elmore (Ed.), *Animal histopathology* (pp. 45-66). Springer.
- Matthiessen, P., Wheeler, J. R., & Weltje, L. (2018). A review of the evidence for endocrine disrupting effects of current-use chemicals on wildlife populations. *Critical reviews in toxicology*, *48*(3), 195-216.
- Mountaki, E. (2025). *The Impact Of Endocrine Disrupting Chemicals On Male Fertility: a narrative* (Doctoral dissertation, ARISTOTLE UNIVERSITY OF THESSALONIKI).
- Mostafalou, S., Baeri, M., & Abdollahi, M. (2013). Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives. *Toxicology and Applied Pharmacology*, *268*(2), 157–177.
- Okafor, E. C., Odo, U. E., & Obiora, C. F. (2022). Chronic low-dose Atrazine exposure induces oxidative stress and growth retardation in rats. *Journal of Applied Toxicology*, *42*(6), 951–960.
- Olayemi, F. O., Bolarinwa, A. F., & Fashina, C. D. (2020). Prolonged Atrazine exposure impairs testicular histology and sperm function in rats. *Environmental Toxicology and Pharmacology*, *79*, 103411.

- Olayinka, E. T., Ore, A., Adewole, K. E., & Oyerinde, O. (2022). Evaluation of the toxicological effects of atrazine-metolachlor in male rats: In vivo and in silico studies. *Environmental Analysis, Health and Toxicology*, 37(3), e2022021.
- Owagboriaye, F., Oladunjoye, R., Adekunle, O., Adeleke, M., Salisu, T., Adenekan, A. Sulaimon, A., Dedeke, G., & Lawal, O. (2022). First report on atrazine monitoring in drinking water from Ijebu-North, South-West Nigeria: human health risk evaluation and reproductive toxicity studies. *Frontiers in Toxicology*, 4, 975636.
- Peate, I. (2025). *Anatomy and physiology for nurses and healthcare professionals* (6th ed.). Wiley-Blackwell.
- Pedrozo, A. L., Oda, H. M., Dogan, N., da Silva Matusiaki, W. F., & Inoue, M. H. (2024). Atrazine as an Endocrine Disruptor and its Effects on Male Reproductive Health. *Aracê*, 6(2), 2131-2148.
- Rohr, J. R. (2021). The atrazine saga and its importance to the future of toxicology, science, and environmental and human health. *Environmental Toxicology and Chemistry*, 40(6), 1544-1558.
- Rotimi, D. E., Ojo, O. A., & Adeyemi, O. S. (2024). Atrazine exposure caused oxidative stress in male rats and inhibited brain-pituitary-testicular functions. *Journal of Biochemical and Molecular Toxicology*, 38(1), e23579.
- Seiler, J. P. (1992). Toxicology of the triazine herbicides. *Environmental Health Perspectives*, 97, 163–171.
- Shahat, A. M. (2020). Testicular thermoregulation and its influence on spermatogenesis. *Andrology*, 8(5), 1102–1115.
- Siboto, A., Mothapo, M., & Motaung, S. (2023). High-dose Atrazine exposure induces compensatory hepatic and endocrine adaptations in male rats. *Environmental Research*, 228, 115768.
- Soare, L. C., Păunescu, A., & Maria, P. C. (2019). The morphophysiological, histological, and biochemical response of some nontarget organisms to the stress induced by the pesticides in the environment. In *Pesticides-Use and Misuse and Their Impact in the Environment*. IntechOpen.
- Soerensen, M., Lee, D., & Thompson, M. (2024). Associations between pesticide exposure and prostate cancer risk: Findings from the Stanford Health Study. *Environmental and Molecular Mutagenesis*, 65(2), 78–89.
- Syngenta. (2025). The economic impact of Atrazine in U.S. agriculture. Syngenta Crop Protection Report.
- Yokoi, K., & Mayi, Z. K. (2004). Substance abuse and male hypogonadism. *Journal of Clinical Medicine*, 8(5), 732.

Zlatnik, M. G. (2016). Endocrine-disrupting chemicals and reproductive health. *Current Opinion in Obstetrics and Gynecology*, 28(3), 175–180.