Chronic Toxic Effects of Polystyrene Nanoplastic on Testis of Adult Male Albino Rats: Histopathological and Biochemical Study

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ABSTRACT

Background: A significant environmental health issue is the exposure to polystyrene nanoparticles (PS-PNs), degradation products of plastic waste into nanoparticles (< 100 nm), that are present in soil, water, and the atmosphere. Chronic exposure to PS-PNs produces toxic effects in tissues due to oxidative stress and induced inflammation, ending in organ damage. **Current work aimed** to assess possible chronic toxic effects of PS-PNs on testicular tissue, associated mechanisms, and the effect of PS-PNs withdrawal on tissue recovery. **Methods:** The total experiment period was 6 months, with one month for withdrawal. 28 adult male albino rats were grouped into a negative control, a positive control (given Tween 80), a PS-PNs treated group (given 10 mg/kg orally of Ps-NPs for six months), and a recovery group (given 10 mg/kg orally of Ps-NPs for six months and then left without exposure for a further month). The experiment assessed semen analysis, follicular stimulating hormone (FSH), luteinizing hormone (LH), and testosterone serum levels, malondialdehyde (MDA) and glutathione (GSH) levels in testicular tissue homogenate, and examination of testicular tissue by light microscope. **Results of the work** showed a decrease in sperm parameters, serum FSH, LH, and testosterone, and testicular GSH and an increase in testicular MDA associated with degenerative changes in testicular tissue in PS-PNs treated rats. These adverse effects were significantly ameliorated in the recovery group. **Conclusion:** PS-PNs induce chronic toxic effects on the testes that are reversible following cessation of exposure.

KEYWORDS

polystyrene nanoparticles, testicular toxicity, oxidative stress.

INTRODUCTION

Plastics are typically synthetic or semi-synthetic organic materials with high molecular weight, composed of long-chain polymer molecules. Among these polymers, polystyrene (PS) is a key thermoplastic, accounting for a significant portion of plastic demand due to its versatility, especially in single-use packaging, containers, personal care products, and electronics. [1. 2.]

Global plastic production has now exceeded 400 million tons annually, even though plastic pollution remains a critical environmental problem and one of the greatest challenges of the 21st century, as plastic waste persists in the environment for extended periods. [3]

Due to limited biodegradability and poor recyclability, large amounts of plastics accumulate in soil, water and the atmosphere. A single microparticle can break down into billions of nanoparticles which are even more harmful due to their ability to penetrate cellular membranes, accumulating in various tissues and impairing organ function. [4.5.]

The primary route of exposure to micro-nanoplastics (MNPs) for humans is oral ingestion, with exposure occurring through three main routes: digestive, respiratory, and dermal contact. [6]

Recently, there has been growing concern about the harmful effects of environmental plastic particles on male reproductive health, as they are associated with inflammatory responses, oxidative stress, genetic toxicity in reproductive cells, and reduced fertility. Most research has not examined whether the toxic effects can be reversed after stopping exposure. This gap is important for evaluating the effectiveness of environmental sanitation strategies aimed at reducing toxicity [7.8].

Environmental sanitation strategies focus on both public health and individual actions. Reducing the source of polystyrene (PS) by phasing out single-use items like food containers and packaging. Using non-plastic containers for storing or heating food, as heat increases the leaching of nanoplastics. Using advanced water filtration systems, such as reverse osmosis, which can effectively remove nanoplastic particles from drinking water. Regular cleaning and using vacuums with HEPA filters to reduce the inhalation of plastic-laden dust shed from synthetic textiles and building materials. [8]

Therefore, this study aimed to assess the possible chronic toxic effects of polystyrene nanoplastics (PS-NPs) on the testis of adult male albino rats, using physical, biochemical, sperm study, and histopathological parameters. The role of oxidative stress as a mechanism in these toxic effects, and the effect of withdrawal of polystyrene nanoplastics on the studied rats (recovery group).

MATERIAL AND METHODS

A. Study Materials

1. Animals:

A 24 healthy adult male albino rats with an average body weight (BW) of between 250 g to 300 grams (at the start of the experiment) were involved in our study. Animals were housed at the animal bread house in the Benha Faculty of Medicine, Benha University. Rats used in the study were given a week to adapt in order to guarantee their health and screen out any sick ones. All of the animals were fed milk, bread, and wheat for twelve hours at night and twelve hours during the day. Chemicals were administered to every animal simultaneously in the afternoon. Twenty-

four hours after the final treatment, the animals were humanely euthanized under ether anesthesia.

Chemicals:

a. **Styrene monomer powder (99.9%)** was obtained from Sigma-Aldrich, St. Louis, Missouri, USA.
b. Tween 80 (polyoxyethylene sorbitan monoleate), as an emulsifying liquid agent purchased from Win Lab, India.
c. Deionized water was purchased from PIOCHEM (6th October City, Giza, Egypt).
d. Dimethyl sulfoxide (DMSO) as a liquid was also obtained from PIOCHEM (6th October City, Giza, Egypt).

Preparation of polystyrene (PS) Nanoemulsion:

PS microspheres were initially made and subsequently converted into a nanoemulsion using the following procedure: 250 mg of PS microspheres were dissolved in 100 mL of DMSO, followed by continuous stirring with a magnetic stirrer for one hour. In parallel, 200 mg of Tween 80 were dissolved in 50 mL of deionized water by an ultrasonic homogenizer. After complete dissolution, the Tween 80 solution was added dropwise to the PS microsphere solution at a rate of approximately 1 mL/s, with continuous stirring for an additional hour. Following mixing, the organic solvent DMSO was removed by an evaporator.[9]

Morphology of the prepared Polystyrene nanoemulsion:

The prepared PS nano-emulsion morphology was done by transmission electron microscopy (TEM). The TEM images demonstrate the morphological characteristics of polystyrene nanoplastics (PS-NPs). The particles appear predominantly spherical to near spherical in shape, with relatively smooth and well-defined margins. Particle size analysis indicates that the diameter of PS-NPs ranges approximately from 30 to 60 nm, as shown in **Fig. (1 & 2)**, confirming their classification within the nanoplastics size range.

2. Animal grouping and Study design:

The animals were divided into 3 groups, each of which had eight rats:

I. **Control group (Group Ia)**: included:
a- Negative Control rats (Group Ia): four rats had unrestricted access to food and distilled water throughout the research.
b- Positive control rats (Group Ib): four rats were given tween 80 orally once daily via a gavage tube for the duration of the trial.
II. **The PS-NPs treated group (Group II):** rats were given a single dosage of PS-NPs 3% (10 mg/kg body weight) orally through a gavage tube for 6 months according to **Babaei et al. (2022).**[10]
III. **Recovery Group (Group III):** Rats in this group were treated as the same as the PS-NPs-treated group, i.e., with a single oral daily dose of PS-NPs 3% (10 mg/kg body weight) via a gavage needle for 6 months duration. Then they were left without exposure to PS-NPs for a further one month.

B. Measured Parameters:

1. Physical parameters:

The body weight of the rats was measured prior to the treatment, recorded monthly, and reassessed at the end of the trial using a sensitive balance. After dissection, the testes were excised, cleared of surrounding adipose tissue and blood vessels, and gently blotted dry before weighing. The relative testicular weight (RTW) was then estimated by:

Relative organ weight = [organ weight/ BW] ×100" [11]

2. Hormonal parameters:

Blood samples were taken from their hearts by five ml syringes to be tested later for follicular-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels that were calculated using commercial ELISA Kits (CUSABIO Company, USA) by RT – 2100 microplate reader (PIOWAY Company, Japan).[12]

3. Oxidative stress markers parameters:

Tissues from the testicles were homogenized. The following parameters were determined using the supernatant that was obtained from centrifuging the homogenates: Malondialdehyde (MDA), and Glutathione (GSH) levels in the testicular tissue using commercially available colorimetric methods (diagnostic kits supplied by Biodiagnostic; Dokki, Giza, Egypt) following the manufacturer instructions. [13,14,15]

4. Sperm study:

a- Sperm motility:

On a heated slide, one drop of 2.9% sodium citrate solution was mixed with a tiny droplet of epididymis semen. The prevalence of more progressive motile sperm was measured and documented in a number of domains. [16]

b- Sperm viability:

The percentage of colored (non-intact membrane, or non-viable sperm) and unstained (full membrane, or viable sperm) sperm was determined. The findings were presented as spermatozoa percentage with an intact membrane (viable) (white head sperm) in relation to the total sperm count[17].

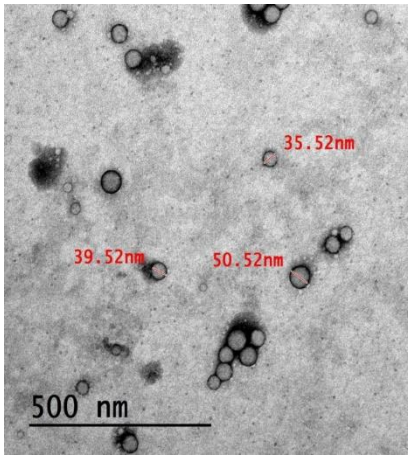
c- Sperm morphology:

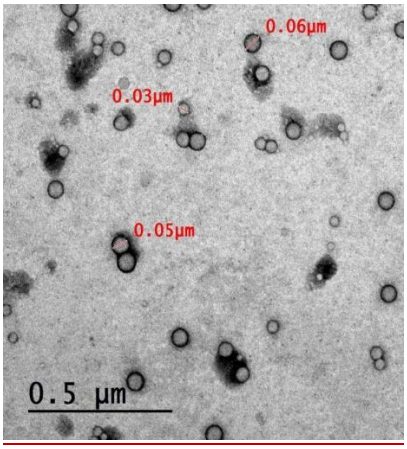
After slide drying, sperm abnormalities were expressed as percentage of normal and abnormal sperm morphology / total sperm number.[18]

d- Sperm count:

Concentration of sperm cell in cubic milliliters was estimated by multiplying the counted number of sperms by 10, which represents the depth, and by 1000, which represents the dilution. [16]

Slide films prepared by 1: 2: 4 ratio of sperm suspension: eosin: nigrosine stains or 10 µl of sperm suspension, 20 µl eosine, 40 µl nigrosine. Then the following parameters are measured





Figures (1&2): The transmission electron microscopy (TEM) images of the prepared polystyrene (PS) nanoemulsion

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E) Histopathological study (light microscopic examination):

After dissecting rats' testicular tissues, they were collected, trimmed and fixed in 10% buffered neutral formalin, dehydrated in ascending grades of ethyl alcohol (70-100%), cleared in xylene, and embedded in paraffin wax. About 4-5 µm- thick then washed in tap water and dehydrated using graded alcohol concentrations and subsequently cleared in xylol and embedded in paraffin wax. Serial sections 3-5-micron thickness were cut using a rotating microtome and stained with Mayer's hematoxylin and eosin for histopathological examination. The stained sections were examined using a light microscope and photomicrographs were obtained using an attached Microscope digital camera (Amscope™).[19]

Ethical considerations

The ethical approval was provided to the experimental design study by Ethical Committee for Research (Institutional Review Board, or "IRB"), Faculty of Medicine, Benha University with an approval letter **(ms 45-12-2024).**

III- Statistical analysis:

The collected data entry, coding, and analysis were undergone using PSW (20), IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. Data of this study were qualitative type expressed as means ± Standard deviation. Statistical analysis for parametric distributed data between three groups was performed by ANOVA (analysis of variance) test (F). p value < 0.05 was considered a statistically significant and P<0.003 was considered statistically highly significant, according to Bonferroni correction[20].

Table (1): The mean values of body weight and relative testis weight of adult male Wistar albino rats at the end of the experiment as well as the mean value of body weight gain among the different studied groups (n. = 24, 8 for each):

Group	Control		Treated		Recovery		Test of Significance	P-Value	Post-Hoc
	Mean	±SD	Mean	±SD	Mean	±SD			
Body Weight	398.63	±21.84	318.13	±46.75	314	±32.66	F=267.24	<0.001**	P1 <0.001** P2 <0.001** P3 =0.970
Weight Gain	179.75	±21.53	112.13	±46.59	112.63	±29.29	F= 10.395	<0.001**	P1 <0.001** P2 <0.001** P3 <0.977
Relative testis Weight%	0.24	±0.041	0.43	±0.06	0.44	±0.029	F=0.316	0.732	

*SD: standard deviation, F: ANOVA (analysis of variance) test, **: Highly significant at P<0.003, according to Bonferroni correction. Pair-wise comparison: P value of Post hoc LSD test, P_1: comparison between control group versus PS-NPs treated group, p_2: control group versus recovery group, p_3: PS-NPs treated group versus recovery group.*

Table (2): The mean values of different serum hormones levels [follicular Stimulating hormone (FSH); luteinizing hormone (LH) and testosterone hormone] among different studied rat groups (n. = 24, 8 for each):

Group / Hormone	Control n = 8		Treated n = 8		Recovery n = 8		F test	P-Value	Post-Hoc
	Mean	±SD	Mean	±SD	Mean	±SD			
Follicular Stimulating Hormone (FSH) (U/ml)	7.79	±1.28	0.56	±0.10	4.04	±0.92	126.217	<0.001**	P1<0.001**P2<0.001**P3<0.001**
Luteinizing Hormone (LH) (U/ml)	25.72	±2.56	4.79	±1.41	13.92	±1.18	267.240	<0.001**	P1<0.001**P2<0.001**P3<0.001**
Testosterone (Free) (pg/ml)	5.17	±0.82	0.69	±0.11	2.58	±0.79	92.796	<0.001**	P1<0.001**P2<0.001**P3<0.001**

*SD: standard deviation, F: ANOVA (analysis of variance) test, **: Highly significant at P<0.003, according to Bonferroni correction. Pair-wise comparison: P value of Post hoc LSD test, P_1: comparison between control group versus PS-NPs treated group, p_2: control group versus recovery group, p_3: PS-NPs treated group versus recovery group.*

RESULTS

In the current work, negative and positive control rats showed a non-significant difference as regards all studied parameters, also histopathological sections from testicular tissues, showed nearly normal structure, without any significant changes. So, they were represented in tables and figures as one group (control group).

1- *Physical parameters (body weight and relative testis weight):*

There was a non-significant (p=0.063) difference between all studied rat groups regarding mean values of body weight at the beginning of the study (baseline measurement). Repeated measurements of body weight (at the end of each month of the study period) revealed significant differences between the studied groups, as follows: there was a significant decrease in mean values of body weight among the PS-NPs-treated group and among the recovery group, as compared to the control group. Meanwhile, there was a non-significant difference between the recovery group and the PS-NPs-treated group. In addition, there was a non-significant difference in the mean of body weight values for the recovery group at the 7th month compared with measured values at the 6th month (after recovery month), as shown in **table (1)** and **Fig. 3.**

As regards the mean values of body weight gain at the end of the experiment, the present study showed that there was a significant (p<0.001) decrease in body weight gain among the PS-NPs-treated group as compared to control group. The recovery group showed a non-significant (p<0.977) rise in the mean value of body weight gain as compared to the PS-NPs-treated group, although it's still significantly low, as compared to control group, as shown in **table (1).**

As regards the mean values of relative testis weight, the present study showed that there was a non-significant (p=0.732) difference among all studied groups as shown in **table (1).**

2- Hormonal assay:

Regarding measured follicular stimulating hormone (FSH), luteinizing

hormone (LH) and testosterone serum levels, the mean values of FSH, LH and testosterone serum levels at the end of the experiment showed a highly significant decrease in the PS-NPs-treated group compared to the control group. However, in the recovery group, there was a highly significant increase in FSH, LH and testosterone serum levels when compared to the PS-NPs-treated group. Despite this increase, all hormones' serum levels remained significantly lower than those in the control group, as shown in **table (2)**.

3- Oxidative stress parameters:

a- ***Malondialdehyde (MDA) testicular tissue levels:***

In the current study, the mean values of MDA levels in testicular tissue homogenate showed a highly significant increase in the PS-NPs-treated group, as compared to the control group. Meanwhile, the recovery group showed a highly significant decrease in mean MDA levels in testicular tissues as compared to the PS-NPs-treated group**,** as shown in **table (3)**.

b- Glutathione (GSH) testicular tissue levels:

The present study showed a highly significant decrease in mean values of GSH levels in testicular tissue homogenate in the PS-NPs treated group, as compared to the control group. Meanwhile, the recovery group showed a highly significant increase in mean GSH levels in testicular tissues as compared to the PS-NPs-treated group**,** as shown in **table (3)**.

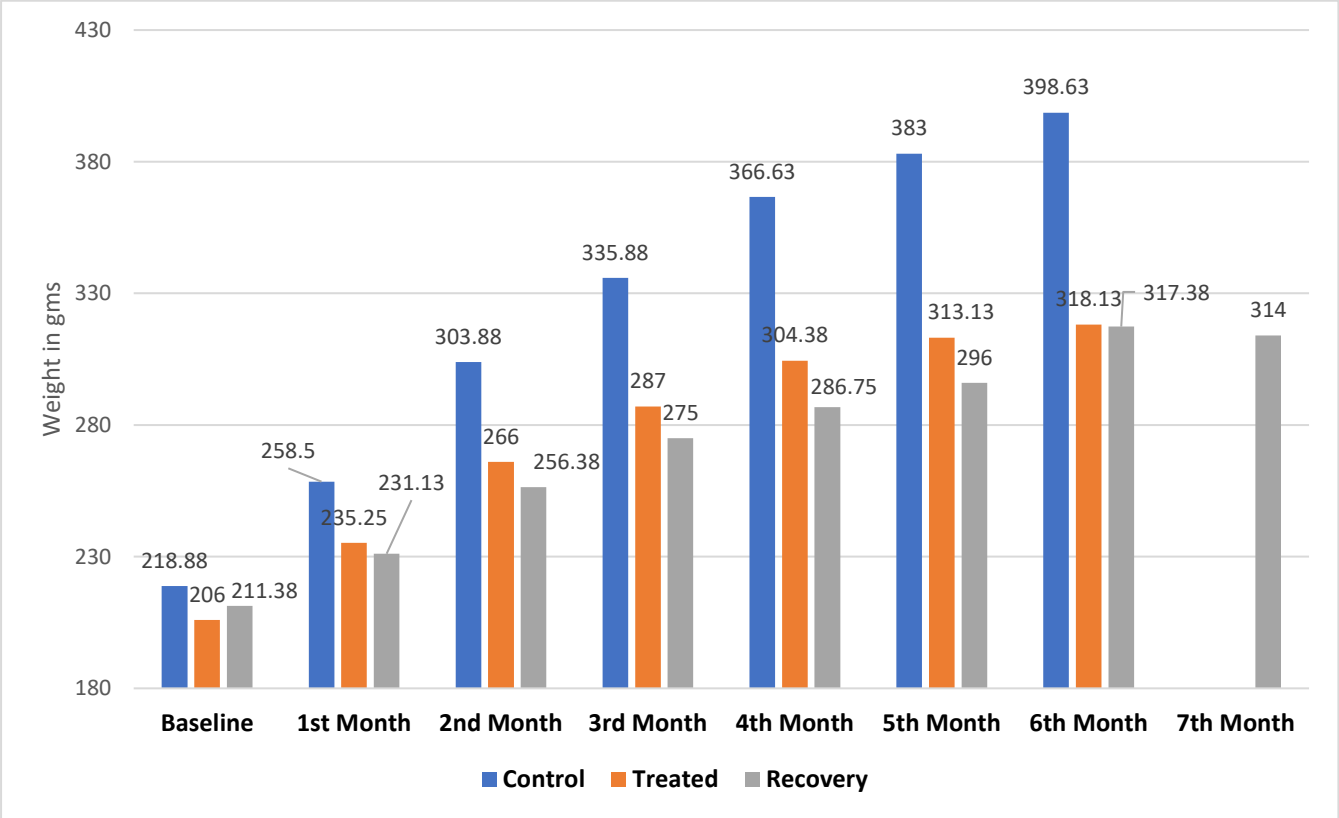

Figure (3): Bar chart showing mean values of the repeated measurements of the body weight of the studied rat groups (n. = 24, 8 for each).

Table (3): The mean values of oxidative stress indices [malondialdehyde (MDA) and glutathione (GSH)] levels in testicular tissues homogenate among different studied rat groups (n. = 24, 8 for each):

Group / Oxidative Parameters	Control n.= 8		Treated n.= 8		Recovery n.= 8		F test	P-Value	Post-Hoc
	Mean	±SD	Mean	±SD	Mean	±SD			
Malondialdehyde (MDA) (nmol/gm)	0.77	±0.05	4.91	±0.54	3.08	±0.31	264.263	<0.001**	P1 <0.001** P2 <0.001** P3 <0.001**
Glutathione (GSH) (mg/gm)	10.58	±0.79	5.49	±0.89	7.34	±1.88	32.066	<0.001**	P1 <0.001** P2 <0.001** P3 =0.009*

*SD: standard deviation, F: ANOVA (analysis of variance) test, **: Highly significant at P<0.003, *: significant at P<0.017, according to Bonferroni correction. Pair-wise comparison: P value of Post hoc LSD test, P_1: comparison between control group versus PS-NPs treated group, p_2: control group versus recovery group, p_3: PS-NPs treated group versus recovery group.*

Table (4): The mean values of semen analysis parameters [sperm motility percentage; sperm viability; sperm morphology percentage and sperm count] among different studied rat groups (n. = 24, 8 for each):

Group / Semen Analysis	Control group n= 8		Treated group n= 8		Recovery group n= 8		F test	P-Value	Post-Hoc
	Mean	±SD	Mean	±SD	Mean	±SD			
Motility %	80.63	±7.76	55	±15.12	65.0	±5.35	12.612	<0.001**	P1 <0.001** P2 =0.006* P3 =0.065
Viability %	93.25	±1.92	69.13	±19.90	78.0	±4.34	8.536	<0.001**	P1 <0.001** P2 =0.017* P3 =0.148
Morphology %	97.875	±0.954	64.688	±6.89	75.0	±3.16	118.551	<0.001**	P1 <0.001** P2 <0.001** P3 <0.001**
Sperm Count (Million)	77.5	±5.98	37.88	±4.94	53.0	±5.29	108.898	<0.001**	P1 <0.001** P2 <0.001** P3 <0.001**

*SD: standard deviation, F: ANOVA (analysis of variance) test, **: Highly significant at P<0.003, according to Bonferroni correction. Pair-wise comparison: P value of Post hoc LSD test, P1: comparison between control group versus PS-NPs treated group, p2: control group versus recovery group, p3: PS-NPs treated group versus recovery group.*

4- Sperm study:

Sperm motility and viability:

The mean values of sperm motility and viability percentages showed a highly significant decrease in the PS-NPs-treated group when compared to the control group. In contrast, the recovery group showed only a minimal improvement, as evidenced by a non-significant increase in mean sperm motility and viability values relative to the PS-NPs-treated

group. Additionally, mean sperm motility and viability values were still significantly lower than mean values for the control group, as presented in **table (4)**.

Sperm morphology and total sperm count:

There was a highly significant decrease in the mean values of normal morphology sperm percentage and total sperm count among the PS-NPs-treated group, as compared to the control group. However, the recovery group showed a highly significant (<0.001) rise in mean values of normal morphology sperm percentage and total sperm count, as compared to the PS-NPs-treated group**,** as shown in **table (4).**

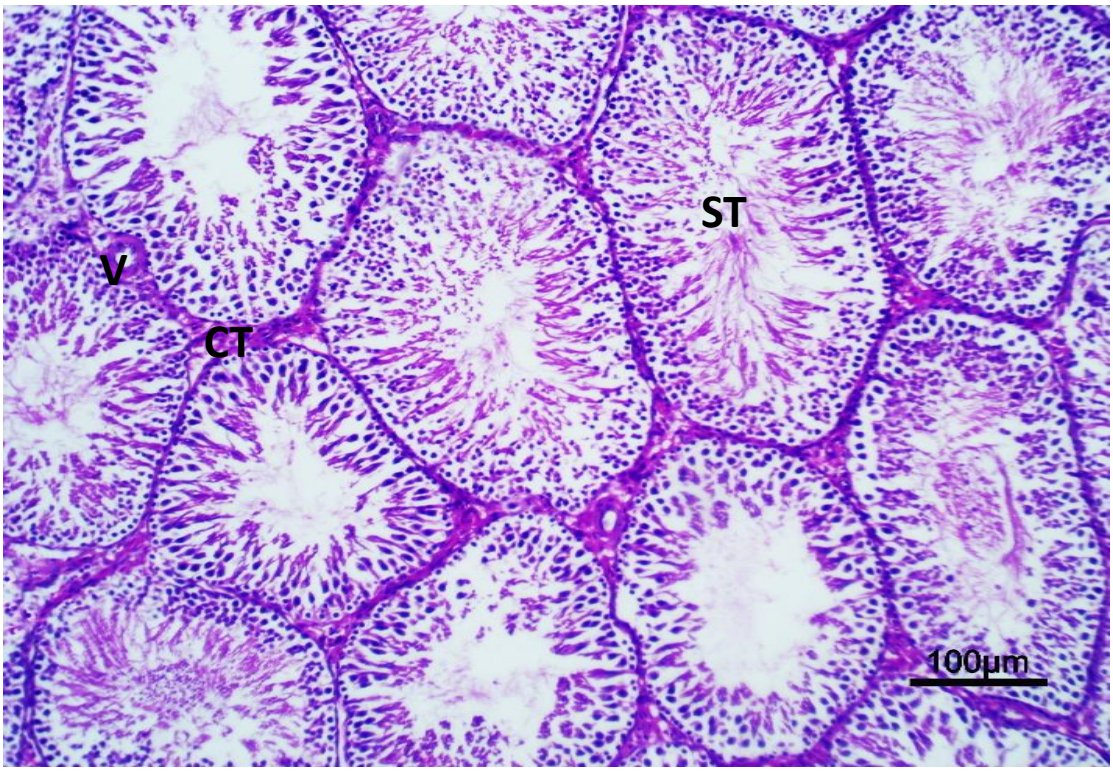

Figure (4): A photomicrograph of a transverse section of rat's testis of control group showing normal well organized seminiferous tubules (ST) with full spermatogenesis, normal blood vessels (V) and normal interstitial tissue (CT), **(Scale bar =100µm).**

5- Histopathological study (light microscopic examination):

The examination of the testis by H&E-stained sections examined under the power of 100 showed that the control group testis had a normal histological structure with a preserved histological microarchitecture of the testicular parenchyma. The seminiferous tubules (ST) were characterized by regular rounded or oval contours. The germinal epithelium demonstrated active mitotic maturation, with spermatogenic cells organized in well-defined layers extending from the basement membrane toward the tubular lumen. All maturation stages, including differentiated spermatogonia, were identifiable, with the lumina densely populated by spermatids and mature spermatozoa. Each tubule was delineated by a distinct fibrous tunica propria. The narrow interstitial spaces contained clusters of Leydig cells showing no signs of vacuolization, edema, or vascular congestion, as shown in **fig. (4).**

Significant morphological distortions within the testicular tissue are seen in testis of

the PS-NPs treated group, as follows: seminiferous tubules displayed disorganized spermatogonia layers, their cells appeared scattered and failed to form a continuous layer along the basement membrane. There was a pronounced germ cell hypocellularity across all maturation stages, often resulting in a complete absence of spermatids and mature spermatozoa. Furthermore, tubular degeneration, atrophy, and extensive intercellular vacuolization were prominent. The interstitial connective tissue was severely compromised, exhibiting complete destruction in some areas. Pathological findings included interstitial edema, severe vascular congestion, and focal cellular infiltration. Occasional partial splitting of the testicular capsule was also noted, as shown in **fig. (5-10).**

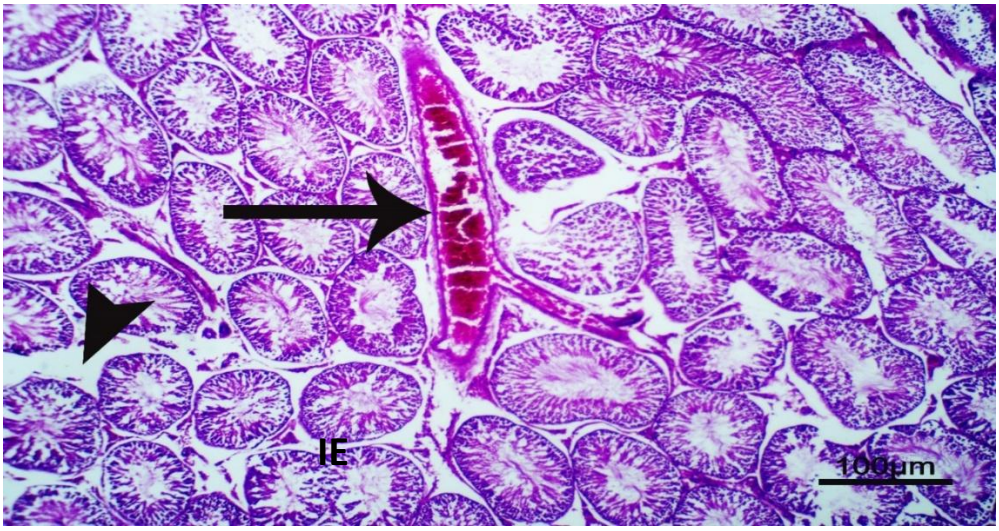

Figure (5): A photomicrograph of a transverse section of rat's testis of polystyrene nanoplastics (PS-NPs) treated group showing focal partial destruction of interstitial connective tissue (arrowhead), interstitial edema (IE) with severe vascular congestion (arrow), **(Scale bar =100µm).**

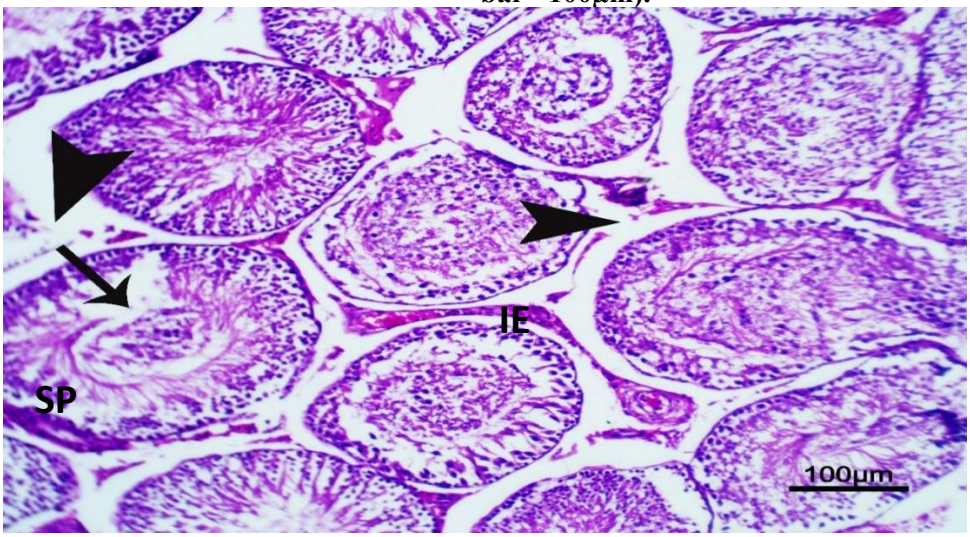

Figure (6): A photomicrograph of a transverse section of rat's testis of polystyrene nanoplastics (PS-NPs) treated group showing partial destruction of spermatogenesis (arrow) with moderate destruction of interstitial connective tissue (arrows head), interstitial edema (IE) and partial destruction of spermatogenesis with apoptosis of some spermatogonia and spermatids (SP), **(Scale bar =100µm).**

The microscopic examination of testis of the recovery group demonstrated a substantial amelioration of the histopathological lesions previously observed. The seminiferous tubules regained an apparently normal architectural organization. However, despite the overall improvement, evidence of mild interstitial destruction and focal, low-grade vascular congestion remained detectable, as shown in **fig. (11&12).**

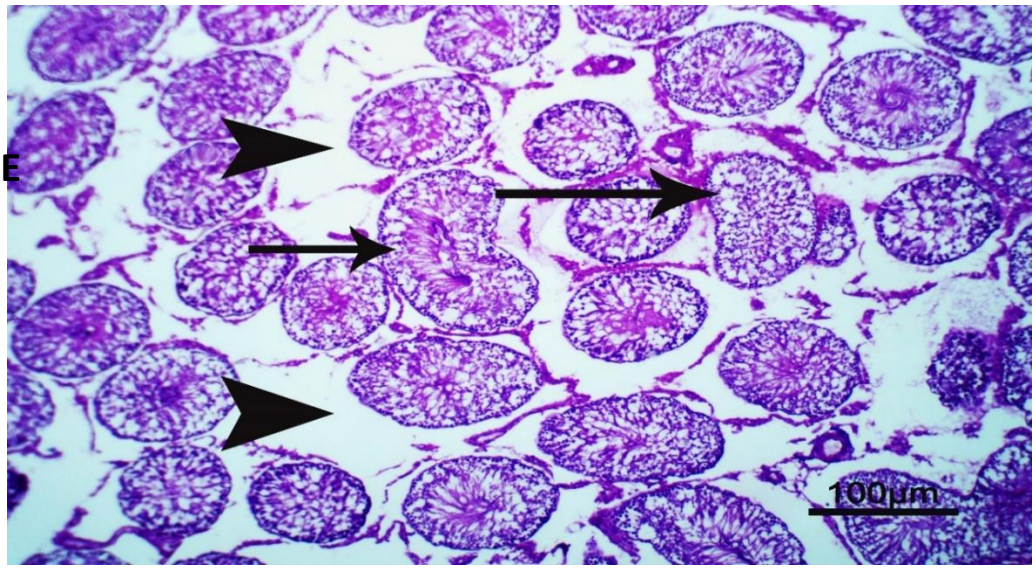

Figure (7): A photomicrograph of a transverse section of rat's testis of polystyrene nanoplastics (PS-NPs) treated group showing severe destruction of interstitial connective tissue (arrows head), interstitial edema (IE) with atrophy of seminiferous tubules and focal vacuolation of some spermatogenic cells (arrows)**, (Scale bar =100µm).**

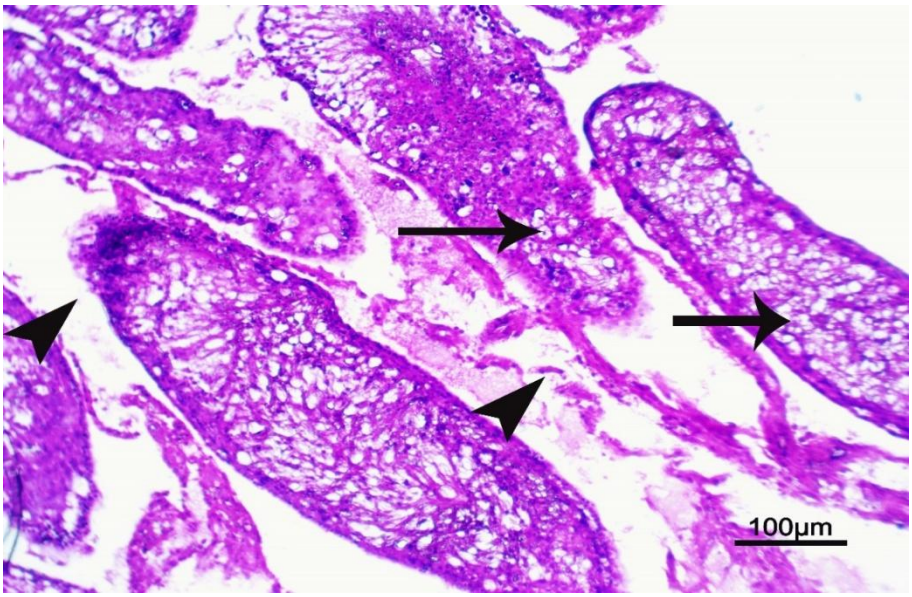

Figure (8): A photomicrograph of a transverse section of rat's testis of polystyrene nanoplastics (PS-NPs) treated group showing spermatogenic cells vacuolation with elongation of seminiferous tubules (arrows) and complete destruction of interstitial connective tissue (arrows head), **(Scale bar =100µm).**

DISCUSSION

Recently, male fertility has been on the decline, influenced by diet, lifestyle, and exposure to environmental chemicals and hazards such as polystyrene nanoplastics (PS-NPs). [21]

Meanwhile, most previous studies have primarily focused on short-term exposure, which does not accurately reflect the chronic nature of real-life environmental exposure. The present study was conducted on 24 Wistar male albino rats, which were administered PS-NPs orally at a dose of 10 mg/kg, equivalent to human environmental exposure levels as estimated by **Senathirajah et al. (2021)**, over six months to evaluate the effects of chronic polystyrene ingestion on the testis of adult male albino rats. Also, to the best of our knowledge, this is the first study to investigate the potential for recovery following chronic exposure to PS-NPs and to assess the reversibility of their adverse effects.[22]

The current work revealed a notable reduction in body weight in the PS-NPs -treated group as compared to the control group, while the recovery group exhibited a non-significant gain in the mean body weight.

The present results corroborate those of ***Meng et al. (2022)*** in mice, in which PS-NPs were administered by oral gavage at a dose of 5 mg/day for four weeks, which documented a significant decrease in body weight following exposure to PS-NPs. [23]

The observed reduction in body weight may be attributed to multiple underlying mechanisms, including oxidative stress, disruption of lipid and glucose metabolism, intestinal barrier damage leading to impaired nutrient absorption, and systemic inflammation, induced by polystyrene nanoplastics exposure. [24]

Regarding relative testis weight, the absence of a statistically significant difference among the control, treated, and recovery groups suggests that polystyrene exposure did not significantly affect testicular mass.

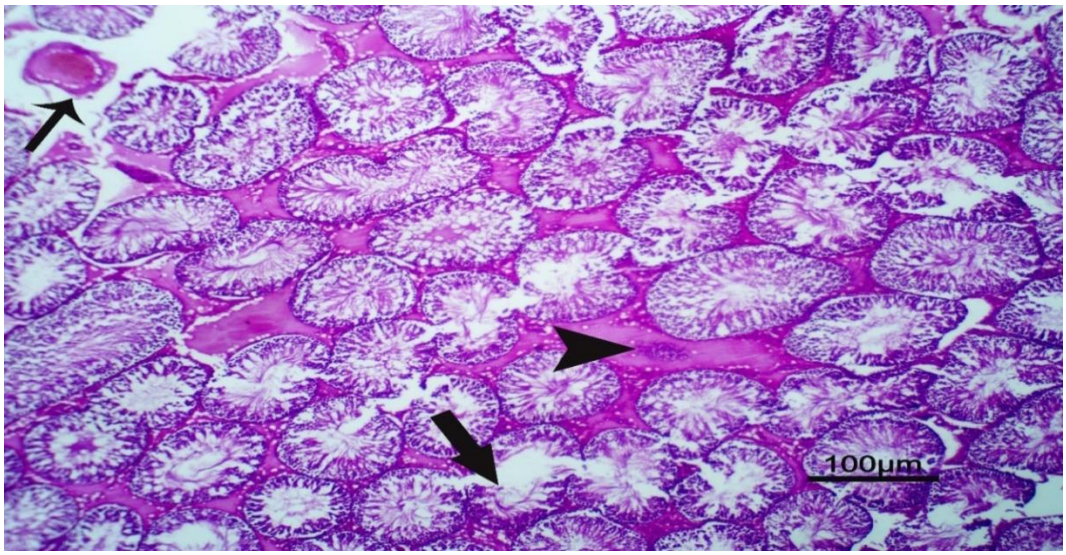

Figure (9): A photomicrograph of a transverse section of rat's testis of polystyrene nanoplastics (PS-NPs) treated group showing vascular congestion with perivascular edema (thin arrow) with partial thickening of interstitial connective tissue by focal cellular infiltration (arrow head) and focal destruction of some tubules (thick arrow)**, (Scale bar =100µm).**

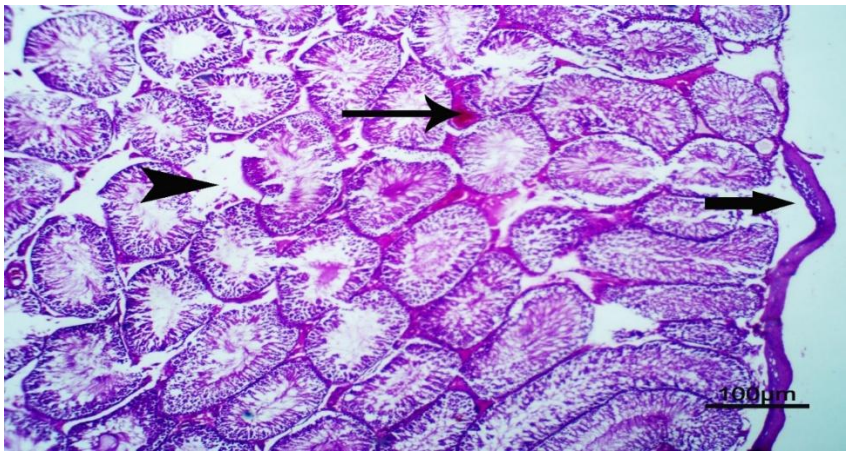

Figure (10): A photomicrograph of a transverse section of rat's testis of polystyrene nanoplastics (PS-NPs) treated group showing partial capsular splitting (thick arrow) with vascular congestion (thin arrow) and focal destruction of some tubules (arrowhead), **(Scale bar =100µm)**

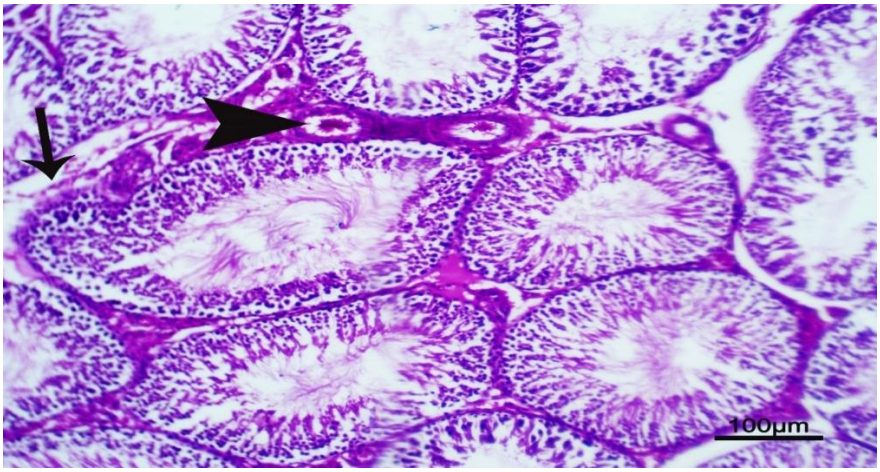

Figure (11): A photomicrograph of a transverse section of rat's testis of recovery group showing mild vascular congestion (arrowhead) with partial destruction of interstitial connective tissue**, (Scale bar =100µm).**

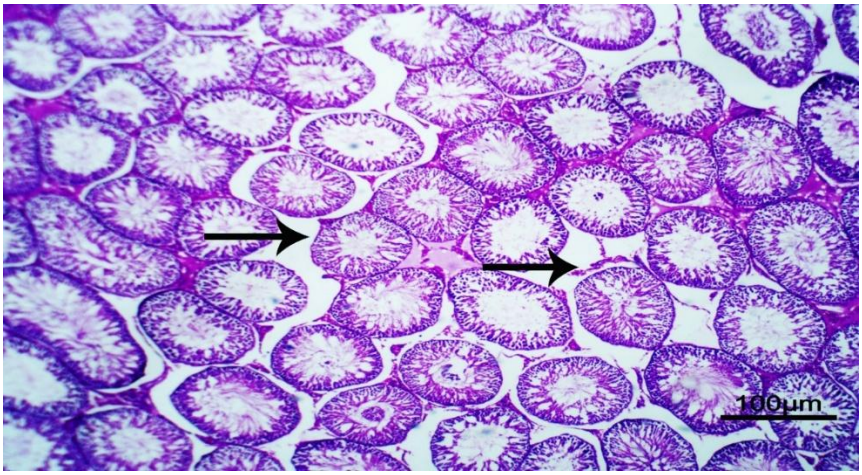

Figure (12): A photomicrograph of a transverse section of rat's testis of recovery group showing apparently normal seminiferous tubules with mild destruction of interstitial connective tissue (arrows)**, (Scale bar =100µm).**

This may be explained by the compensatory effect induced by toxicant, as some toxicants are known to cause a transient increase in testicular size and weight as a result of interstitial edema, which is further supported by histopathological results. This finding indicates that testicular weight alone may not be a sensitive indicator of reproductive toxicity, as substantial functional and cellular damage may occur without an apparent reduction in organ weight [25].

These findings corroborate those of **Amereh et al. (2020)**, who reported that oral gavage of PS-NPs for 35 days in Wister rats at doses of 1, 3 and 10 mg/kg showed no changes in testis weight. Furthermore, other studies reported a negligible change in testis weight following 35 days of oral PS-NPs administration in mice, corroborating our findings [26. 27. 28].

Luteinizing hormone, FSH, and testosterone are crucial for starting and maintaining the early stages of spermatogenesis. Normally, when the production of testosterone is adequately suppressed, the pituitary produces more LH as a result of a positive feedback mechanism triggered by activating the interstitial cells; LH raises testosterone output. Over time, and using the same process, LH production returns to normal [29.30]

In the current study, hormonal analysis further confirmed the adverse effects of PS-NPs on the hypothalamic- pituitary - gonadal axis (HPG). The PS-NPs- treated group showed a significant reduction in serum FSH, LH, and testosterone levels compared to the control group.

The decrease in FSH and LH despite low testosterone level may reflect impaired pituitary secretion or altered negative feedback regulation due to interference with central endocrine signaling, resulting in inadequate compensatory hormonal response, while decreased testosterone level indicates Leydig cell dysfunction either by dropping in LH (primary driver of testosterone) or direct toxicity on Leydig cells [31].

The current findings are in line with those obtained by **Amereh et al. (2020); Jin et al. (2022); Wei et al. (2022); Xu et al. (2023)**, in their studies of toxic effects of either PS-NPs or polystyrene microplastics (PS-MPs) on the testis of rats or mice. [26.32.33.34.].

Studies by **Amran et al. (2023)** and **Wang et al. (2025),** which measured the gonadotropins FSH and LH in female rats exposed to PS-MPs, offer clear examples in this regard. FSH decreased after exposure to PS-MPs in both investigations. However, LH increased in the study by **Wang et al. (2025)** but decreased in the study by **Amran et al. (2023)**. which was explained by a compensatory hormonal response. Therefore, part of these adverse effects on reproduction may be caused by gonadotropin levels, which are sensitive to exposure to nanoplastics. [35.36].

In the recovery group, FSH, LH, and testosterone levels were significantly higher than in the PS-NPs-treated group, demonstrating partial restoration of endocrine function upon exposure cessation. However, hormonal levels did not entirely return to control levels, indicating that Leydig cell function is still subclinically impaired or that pituitary regulation has not totally recovered. This indicates that chronic exposure to PS-NPs may cause long-term structural damage to Sertoli cells, therefore other mechanisms that directly damage both cells, like oxidative stress is suggested.

In the current work, oxidative stress markers revealed a significant increase in malondialdehyde (MDA) levels and a significant reduction in reduced glutathione (GSH) levels in the treated group. These findings confirm that oxidative stress plays a central role in PS-induced testicular toxicity.

Elevated MDA level indicates lipid peroxidation, which damages cell membranes and compromises Sertoli cell, germ cell integrity, and sperm cell membranes [37].

Reduction of GSH is explained by its nature as a potent reductant, which acts as the major intracellular antioxidant that participates in the detoxification of toxic peroxides, maintenance of protein SH groups, and elimination of xenobiotics. So, the antioxidant defense mechanisms were exhausted trying to fight off nanoplastic toxicity. This is thought to be a precursor to cell death caused by different apoptotic triggers, making cells or tissues more vulnerable to oxidative damage [38].

The current findings are in line with **Hamza et al. (2023)**, who evaluated oxidative stress-mediated toxicity induced by PS-MPs exposure in an experimental rat model and demonstrated a significant elevation in MDA levels. Also, studies by **Babaei et al. (2022)** on Wistar rats, **Wan et al. (2019)** on larval zebrafish, and **Yu et al. (2018)** on juvenile Eriocheir sinensis, showed a reduced GSH content.[39. 10.40.41].

However, **Jeong et al. (2016)** revealed that fluorescently tagged PS microbeads of varying sizes (0.05, 0.5, and 6 μm) on Monogonont Rotifer (Brachionus koreanus) increased GSH content following twelve hours in a size-dependent manner. This can be explained by the short duration of study, as early exposure to stress, GSH content may show high levels due to compensatory upregulation. Furthermore, the use of a rotifer model, which is different from mammals, may also contribute to this discrepancy [42].

The present study confirmed that the sperm functional parameters were significantly impaired following polystyrene exposure. The PS-NPs-treated group showed a significant decrease in sperm motility, viability, morphology, and total sperm count compared to the control group. These findings reflect disruption of spermatogenesis and deterioration of sperm quality, which are common indicators of testicular toxicity.

Spermatogenesis is vulnerable to oxidative stress due to high rate of cell division and the unique structural characteristics of spermatozoa. Sperm membranes are rich in polyunsaturated fatty acids, which are highly susceptible to lipid peroxidation, while sperm cells possess limited cytoplasmic antioxidant capacity. Consequently, oxidative imbalance can impair sperm motility and viability, ultimately compromising male fertility [43]

In line with this, **Hu et al. (2024)** reported that PS-NPs exposure in a dose-dependent manner led to significant abnormalities in semen parameters, including decreased sperm motility and count, along with an increased percentage of abnormal sperm forms.[21]

The results of the current work corroborate with a study done by **Feng et al. (2025),** who administered PS-NPS (50 nm) orally to male albino rats for 90 days at a concentration of 2.5 mg/kg. The results showed a significant decrease in sperm motility and count as well as an increased proportion of aberrant sperm [44].

According to **Amereh et al. (2020)**, oral gavage of PS-NPs for 35 days in rats caused significant aberrations in sperm morphology and a significant reduction in sperm viability, motility, and progression as compared to control rats [26].

Also, the current results are in good agreement with a previous study confirming that administering PS-MPs to mice for five weeks dramatically reduced sperm count and raised sperm abnormal rates **(Xie et al., 2020)**. Additionally, another study by **Jin et al. (2021)** found that exposure to various PS-MP diameters (0.5, 4, and 10 μm) caused changes in sperm

morphology, which is consistent with the current findings [45, 46].

In this study, the recovery group demonstrated a variable degree of recovery. Sperm motility and viability showed only minimal non-significant improvement compared to the treated group. In contrast, sperm normal morphology and total sperm count showed significant improvement in the recovery group, indicating partial restoration of spermatogenic activity following withdrawal of toxic insult.

These findings suggest that polystyrene exposure induced partially irreversible damage or functional recovery of sperms may require longer recovery period than gonadotropin hormones do. This may be attributed to the complex nature of the spermatogenesis process, in which spermatogonia differentiate into spermatocytes, undergo meiosis to form spermatids, and eventually mature into spermatozoa. Each of these developmental stages may have differing sensitivities to NPs exposure [47]

The spermatogenic process and the regenerative dynamics of the endocrine system differ fundamentally, which explains why reproductive hormone levels recovered more quickly than semen parameters. Since spermatogenesis is a long, multistage process that depends on the renewal and differentiation of spermatogonial stem cells, and restoration of the HPG axis may start quickly following the removal of a toxic insult. Therefore, improvement in semen quality may be delayed even with partial hormonal normalization [48].

Testicular histopathological analysis is considered a sensitive indicator of reproductive toxicity and offers direct morphological proof of spermatogenic integrity [49].

Significant variations were seen in the control, polystyrene-treated, and recovery groups in the current study, indicating the degree of reversibility after cessation and the progressive effects of long-term polystyrene exposure.

The current work exhibited significant histopathological changes, including disorganized spermatogonia layers, germ cell loss, detachment of the basement membrane, and severe disruption of the seminiferous epithelium.

The study done by **Feng et al. (2025)** was in alignment with the present study, as they showed a significant testicular damage characterized by separation from the basement membrane, interstitial edema and germ cell loss [44].

In addition, the study by **Değermenci et al. (2025)** agreed with the current results as the histopathological analysis in the testis of rats treated with 25 mg/kg Ps-NPs in low dose group and 50 mg/kg in high dose group showed significant vacuolization of the germinal epithelium and splitting from the basement membrane, as well as the presence of immature germ cells shedding into the lumen[50].

The present work showed significant edema, severe vascular congestion, focal inflammatory cell infiltration, and even total destruction in certain areas were all present in the PS-NPs- treated group's interstitial tissue.

This was in agreement with **Liu et al. (2024)** who found that animals exposed to polystyrene, Leydig cell disruption and interstitial damage have been associated with impaired testosterone synthesis, offering a morphological explanation for the decreased testosterone levels frequently observed in such models [51].

Histopathological alterations in testicular tissue may be explained by the accompanying changes in antioxidants' parameters that suggest the presence of oxidative stress as it is well known that testis and spermatozoa are extremely sensitive to oxidative stress-induced damage. Free radicals induce lipid peroxidation of

membrane-bound polyunsaturated acids of mammalian testis and other biological membranes, leading to impairment of the membrane integrity and degeneration of seminiferous tubules. As a result, testicular atrophy and tissue degeneration occur [52].

Significant improvement in testicular histopathological changes was noted in Group III (recovery group) after polystyrene exposure was stopped. The germinal epithelium was reestablished and spermatogenic activity resumed, giving the seminiferous tubules an apparently normal architectural organization although there were some interstitial edema and vascular congestion. These results suggest that testicular tissue can partially regenerate after the harmful insult is eliminated.

CONCLUSION

The present study confirmed that chronic PS-NPs exposure resulted in toxic effects in testis of adult Wistar albino rats manifested by physical parameters changes (decrease in body weight & relative testis weight), biochemical parameters (decrease levels of FSH, LH, testosterone) hormones, sperm parameters (decrease in sperm motility, viability, and count with abnormal morphology) and degradation changes at the testicular tissue. These toxic effects were induced by oxidative stress in the testicular tissue and disturbance of the pituitary hormonal axis. The stoppage of PS-NPs exposure can diminish these toxic effects.

RECOMMENDATIONS

Depending on the obtained results, the present study recommended the followings:

1. Establishing widespread public education regarding the health hazards of chronic polystyrene nanoplastics exposure, with a special concern about its reproductive toxic effects.
2. Regular evaluation of testicular functions, such as testosterone, FSH and LH levels, sperm count, and sperm motility, is highly recommended in workers exposed regularly to these hazards.
3. Further studies are needed to:

• Assess the role of antioxidants for their potential to mitigate PS NPs-induced testicular damage.

• Study the chronic toxic effects of polystyrene nanoplastics on the ovary to assess the possible toxicity on the female reproductive organs.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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