

To cite this article: Anil K Chandolia, Priya Khangrawat, Sharey, Kumari Pragya and Seema Srivastava (2025). IMPACT OF PS-MPS ON THE FUNCTIONING OF EPIDIDYMISS AND SEMINAL VESICLE IN WISTAR ALBINO RATS, International Journal of Current Research and Applied Studies (IJCRAS) 4 (2): Article No. 118, Sub Id 206

IMPACT OF PS-MPS ON THE FUNCTIONING OF EPIDIDYMISS AND SEMINAL VESICLE IN WISTAR ALBINO RATS

Anil K Chandolia, Priya Khangrawat, Sharey, Kumari Pragya and Seema Srivastava

Department of Zoology, Reproductive Toxicology Lab,
University of Rajasthan, Jaipur-302004,
Rajasthan, India

Corresponding author
Dr. Seema Srivastava, Ph.D.
Professor, Department of Zoology
Reproductive Toxicology Lab University of Rajasthan
Jaipur-302004, Rajasthan, India.

Tel. No.: +91 9214026350

Email: drseemaa07@gmail.com

Running title: Impact of PS-MPs on epididymis and seminal vesicle.

DOI : <https://doi.org/10.61646/IJCRAS.vol.4.issue2.118>

ABSTRACT

Microplastics enter the human body through plastic food packaging, food products, drinking water, and contaminated air. Microplastics produce negative impact on fertility of both males and females. In males, microplastics have been found to reduce sperm quality, testicular function, and overall fertility. The present study investigated the effects of polystyrene microplastics (PS-MPs) on the epididymis and seminal vesicle. Male Wistar albino rats were divided into groups based on administered doses of PS-MPs: 5, 50, 500, and 5000 µg per kilogram of body weight. A sham control group was included for comparison. The study assessed organ weight, sperm characteristics, histological and biochemical parameters. The results revealed a dose-dependent decline in epididymal weight, ranging from 3% to

11%. Sperm analysis showed a significant decline in sperm count, viability, and motility across all test groups. Morphological assessments of cauda epididymal sperm revealed higher prevalence of defects, including, bent tails, coiled tails, headless tails, and tail-less heads. Histological examination highlighted pronounced structural aberrations in the epididymis and seminal vesicle, particularly in animals treated with the highest dose of PS-MPs. Biochemical analysis showed a 35% reduction in sialic acid levels and diminished L-carnitine activity, indicating functional impairment of the epididymis. Additionally, reduced fructose levels in the seminal vesicle suggested a decline in the energy available in seminal fluid for ejaculated sperm. Overall, the study concluded that PS-MPs have a significant negative impact on the epididymis and seminal vesicle, likely affecting sperm maturation and post-ejaculation efficiency.

Keywords:Microplastics, Male fertility, Epididymis, Seminal vesicle

INTRODUCTION

Microplastics pose a credible threat to the environment and human health. These particles are the result of our continuous use of plastics for over a century. When left in the environment, plastics degrade into small particles that spread across marine ecosystems, soil, and air (Ziani et al., 2023). Previous reports suggest that once ingested, microplastics can accumulate in various organs, including the liver, reproductive organs, kidneys, brain, lungs, heart, and liver. There is credible evidence indicating that microplastics interfere with the blood-testis barrier, causing disturbances in the endometrium (Sun et al., 2024), placental dysfunction (Dibbon et al., 2024), ovarian fibrosis (Adhikari et al., 2024), and impaired spermatogenesis (Jin et al., 2024).

Microplastics play significant gender-specific roles in the reproductive system, affecting its functioning and fertility outcomes. In females, they disrupt the performance of the reproductive system (Balali et al., 2024) by inducing ovarian inflammation, oxidative stress, endoplasmic reticulum stress, and apoptosis in germ cells (Wang et al., 2024). In males, microplastics have deleterious effects on sperm quality (D'Angelo and Meccariello, 2021), sperm count, and the growth of reproductive organs (Daniels and Eberhardt, 2024). A study by Zhang et al. (2022) highlighted the potential role of microplastics in male infertility. Previous studies have also reported that microplastics reduce sperm count, cause hormonal imbalances (Jin et al., 2022), increase testicular oxidative stress (Hu et al., 2024), and negatively impact overall fertility outcomes.

A study by Zhao et al. (2023) revealed the presence of microplastics in both semen and testicular samples. Additionally, it has been established that microplastics can enter semen through the epididymis and seminal vesicle (Yang et al., 2024). However, there is limited information on how these microplastics interact with the biochemistry of epididymal tissues and the seminal vesicle. In the present study, we aimed to investigate the primary role of polystyrene microplastics (PS-MPs) in the functioning of the epididymis and seminal vesicle. Furthermore, this study seeks to evaluate sperm characteristics during their transit through the cauda epididymis.

MATERIALS AND METHODS

Test chemical

Polystyrene microparticles (0% cross-linked, density: 1.05 g/cm³, size: 5 μm, SD < 0.1 μm, CV < 2%) were procured from Sigma Aldrich, Merck, Darmstadt, Germany. The chemical was stored at 4 °C throughout the investigation period.

Animal model

Male Wistar albino rats (*Rattus norvegicus*) with proven fertility, weighing 250–280 g and aged 3–4 months, were used for this study. The rats were housed at the departmental facility of the University of Rajasthan under carefully controlled conditions. A 12-hour light/dark photoperiod was consistently maintained for all experimental groups housed in polypropylene cages (43×27×15 cm). The animal house temperature was kept at 21–25 °C with humidity

levels between 32–70%. The rats were provided with a pellet diet sourced from Ashirwad Industries Ltd., Chandigarh, India, and had ad libitum access to drinking water.

Ethical approval

Approval from the Institutional Animals Ethics Committee (IAEC) was obtained well in advance of the experimental work. The study strictly adhered to the guidelines outlined by the Indian National Science Academy (INSA), New Delhi, for the care and use of animals. All experiments were conducted under the supervision of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2010).

Experimental design

Graded concentrations of polystyrene-based microplastics (PS-MPs) were prepared using the manufacturer's suspension (1 ml containing 1.05 g of MPs). The doses - 5, 50, 500, and 5000 µg of PS-MP/kg body weight - were administered orally, dissolved in olive oil. An equivalent volume of olive oil was given to the control group for comparison. The control group was designated as Group I, while Groups II–V represented the graded doses of PS-MPs, with each group comprising seven male rats of proven fertility. Doses were administered once daily for 120 days, and on the 121st day, the animals were sacrificed. Epididymis and seminal vesicle were collected for further analysis. The doses used were selected based on the study by Ijaz et al. (2021) and adhered to OECD guidelines for chemical testing, Section 4 - Protocol 423: Acute Oral Toxicity (OECD, 2001).

Organ weight

The epididymis and seminal vesicle were carefully dissected, cleared of extraneous tissues, and weighed individually.

Sperm characteristics

Sperm parameters, such as count, motility, viability, and abnormalities, were evaluated according to the guidelines specified in the WHO manual (1999). Samples were obtained by excising a segment of the cauda epididymis and gently disintegrating it in 1 ml of normal saline (pH 7.0).

Cauda epididymal sperm morphology

A portion of cauda epididymal tissue was dissected and agitated gently in normal saline. The sample was washed in distilled water and examined using Papanicolaou staining for morphological observation, as described in the WHO (1999) guidelines.

Histological evaluation of epididymis and seminal vesicle

A small segment of the epididymis and seminal vesicle was fixed in 4% paraformaldehyde overnight. The tissues were then dehydrated using ethanol, cleared with xylene, and embedded in paraffin wax. Thin sections of 5 µm were prepared and stained with Harris's hematoxylin and eosin. The stained specimens were subsequently examined under a light microscope at different magnifications.

Epididymal biochemistry

Estimation of sialic acid

Sialic acid levels in the epididymis were estimated using method explained in Svennerholm et al. (1957). Briefly, 2 mL of samples containing 10–30 µg of N-acetylsialic acid or equivalent sialic acids were prepared. Two test tubes received 2 mL of resorcinol reagent, while a third (sample blank) received blank reagent. Standard solutions of 0, 15, and 30 µg of N-acetylsialic acid were also prepared. The tubes were heated at 110°C for 15 minutes, cooled, mixed with 5 mL of amyl alcohol, shaken, and chilled in ice water. After centrifugation, the amyl alcohol phase was transferred to microcells and analyzed at 450 nm and 580 nm using a spectrophotometer. Absorbance values from the blank were subtracted, and N-acetylsialic acid concentrations were calculated using standard curves.

Estimation of L-carnitine

L-Carnitine was determined using the spectrophotometric method described by Marquis and Fritz (1964). A 0.1 mL aliquot of supernatant was added to a reaction medium containing 0.1 mol/L Tris-HCl, 0.05 µmol/L AcCoA, 0.1 µmol/L DTNB, and 0.05 mL CAT (19 U/mL). The mixture was incubated at 37°C for 30 minutes, after which 2 mL of Tris buffer was added. The absorbance was measured at 412 nm using a spectrophotometer. The L-carnitine content in caudal epididymal fluid is expressed as mmol/mg protein.

Estimation of α -glucosidase activity

Neutral α -glucosidase activity in the epididymis was measured using the method described by Wang et al. (1999). The reaction system included 1.2 mL of buffer (69 mmol/L citric acid, pH 6.8), 0.2 mL of p-nitrophenyl- α -D-glucopyranoside (PNPG, 23 mmol/L), and 0.2 mL of supernatant. The reaction mixture was incubated at 37°C for 4 hours, and the reaction was stopped by adding 0.25 mL of Na₂CO₃ (0.1 mol/L). Absorbance was measured at 405 nm using a UV/VIS spectrophotometer (Systronics 108, Ahmedabad, India). PNPG content was quantified using a standard curve, and neutral α -glucosidase activity was expressed as mg PNP/mg protein of tissue.

Seminal vesicle biochemistry

Estimation of fructose in seminal vesicle

Fructose concentration in the seminal vesicle was determined using the method of Mann (1964). The tissue sample was homogenized, and the homogenate was deproteinized by mixing 0.1 mL of seminal vesicle homogenate with 1.9 mL of distilled water. Subsequently, 5% zinc sulfate was added, with the mixture being shaken after each addition. The sample was then centrifuged at 2000 rpm for 30 minutes. The resulting deproteinized supernatant was reacted with acidic resorcinol to produce a colored product, and its absorbance was measured at 520 nm.

Statistical analysis

The numerical data from the parametric analysis are expressed as Mean \pm SEM. Comparisons

between the mean values of the test and control groups were performed using the Student's t-test (MS-EXCEL, Microsoft, US) and One-Way Analysis of Variance (ANOVA) with Tukey's multiple comparison tests (MINITAB, Pennsylvania, US). The significance of variance was evaluated based on confidence intervals (CIs), with levels of 95%, 99%, and 99.99% interpreted as significant, highly significant, and extremely significant, respectively.

RESULTS

Epididymal and seminal vesicle weight

The epididymal weight showed a significant reduction in response to PS-MPs exposure. Specifically, Groups III to V exhibited a 3–11% decline in organ weight, with Group V, which received the highest dose of PS-MPs, demonstrating the most pronounced decrease. Group II also showed an approximate 2% reduction, although this was not statistically significant compared to the control group (Group I). A dose-dependent trend was evident among the test groups, with lower doses associated with relatively smaller declines in epididymal weight, while higher doses caused greater reductions, as shown in Table 1.

In contrast, the response of the seminal vesicle weight differed distinctly from the pattern observed for epididymal weight. The seminal vesicle weight increased substantially (10%) in Group II but decreased significantly (4%) in Group V. Notably, Groups III and IV exhibited a slight but consistent decline of approximately 1.5% (Table 1).

Sperm characteristics

Cauda epididymal sperm abnormalities increased substantially across all test groups, demonstrating a clear dose-dependent pattern. The percentage increase in abnormalities was 13%, 14%, 36%, and 54% in Groups II through V, respectively, compared to the control (Table 2). A significant decline in sperm motility ($p < 0.01$) was observed only in Groups IV (15%) and V (31%). Similarly, a reduction in sperm viability was noted exclusively in Group IV ($p < 0.01$) and Group V ($p < 0.001$). Likewise, sperm count showed a significant decline in Groups III through V, following a dose-dependent trend. The most pronounced reduction was recorded in Group V, with a count of 17.66 ± 2.02 million/ml compared to 44.76 ± 1.43 million/ml in the control group (Group I). A gradual decline in sperm count across the test groups, correlated with increasing doses of PS-MPs, ranged from a minimum of 6% to a maximum of 60% (Table 2).

Sperm morphological alterations

Abnormalities are common; however, a significant increase in specific deformities suggests distinct extraneous actions. The most common abnormalities observed in the test groups included bent tails, headless tails, tailless heads, coiled tails, pinheads, and flattened heads. The highest occurrence of these abnormalities was recorded in Group V, while Group II exhibited the fewest abnormalities compared to the control group. Broken heads detached from tails were also more prevalent in Group V, indicating damage in the neck region. Tail-related abnormalities were predominantly observed at the terminal end, significantly affecting the overall motility of spermatozoa (Figure 1).

Alteration epididymal histoarchitecture

The histological attributes of the control (Group I) epididymal tissues showed normal epithelium, smooth muscle, and stereocilia. In contrast, the epididymal histology of the test groups revealed a significant reduction in the number of spermatozoa within the lumen. Although the epithelial lining arrangement was comparable to the control (Figure 2), the basal cells in the test groups appeared relatively more disorganized and size were varied significantly compared to the control. Smooth muscle fibers also exhibited disintegration in multiple areas, along with the formation of crests, particularly pronounced in Groups IV and V (Figure 2). The sperm density in the epididymal lumens of these two groups showed a marked decline.

Histological architecture of seminal vesicle

The histology of the seminal vesicle in Group I showed normal glandular components and an intact epithelial lining. Group II also exhibited normal histological attributes; however, an increased presence of fat cells was noted in this group (Figure 4). Slight disruptions in the crypts and epithelial cells were observed, which became progressively more pronounced in Groups III through V. In Group V, extensive loss of glandular components was evident, along with the loss of intricate folding and lumen dilation. These characteristics strongly suggest the onset of seminal vesicle atrophy. Additionally, Group V displayed a noticeable loss of mucosal branching at various locations (Figure 4).

Biochemical assay of epididymal tissues

The level of sialic acid in the epididymal tissues was slightly lower in all test groups compared to the control (Figure 5A). However, a significantly reduced level of sialic acid was observed in Group V, recorded at 0.61 ± 0.09 mg/g; approximately 35% lower than the control group (Group I), which measured 0.93 ± 0.07 mg/g of epididymal tissue. Similarly, the activity of L-carnitine showed depletion in most of the PS-MPs-exposed rats (Figure 5B). The most pronounced reduction in L-carnitine activity was noted in Group V (0.71 ± 0.08 mM/mg protein), while the other groups showed changes that were not statistically significant. Likewise, the activity of neutral- α -glucosidase in the epididymal tissue showed a slight decline; however, the values remained statistically non-significant (Figure 5C).

Biochemistry of seminal vesicle

The level of fructose in the seminal vesicle showed significant alterations across the test groups. Although no dose-dependent trend was observed with PS-MPs exposure, a notable decline in fructose levels was recorded in Group V compared to the control group (>25%). Specifically, the fructose level in Group V was measured at 4.88 ± 0.42 mg/g tissue, whereas the control group recorded 6.54 ± 0.46 mg/g (Figure 6).

DISCUSSION

Several studies have reported the adverse effects of PS-MPs on the male reproductive system (Fang et al., 2024; Sun et al., 2022; Jin et al., 2021). Most of these studies have consistently confirmed a strong association between PS-MPs and reproductive dysfunction, reproductive

toxicity, and oxidative stress in testicular cells. However, the dynamic activity of PS-MPs, ranging from inducing apoptosis in spermatogonial cells (Fang et al., 2024) to producing inefficient sperm (D'Angelo et al., 2021), suggests potential roles in other major reproductive organs, such as the epididymis, vas deferens, seminal vesicle, and ventral prostate. This study aimed to identify specific changes in two key reproductive organs: the epididymis, which stores mature sperm before ejaculation, and the seminal vesicle, which provides a supportive microenvironment for sperm during transit, as well as in a female reproductive organ.

Epididymal weight in the current study showed a clear dose-dependent decline, likely due to reduced sperm production in the testis. Li et al. (2021) reported that high spermatogenic cell apoptosis significantly decreases sperm density in the lumen of seminiferous tubules. Similarly, a recent study demonstrated that a decline in sperm production leads to a proportional regression in epididymal weight (Endo et al., 2024). Thus, one possible reason for the reduced epididymal weight could be the lower sperm count in the epididymal tubules. However, other factors may also contribute to the reduction in epididymal weight, such as an increased rate of apoptotic cell loss, reduced epididymal fluid, and/or epididymal atrophy. Grechi et al. (2024) reported the presence of microplastics in epididymal tissue, highlighting the proven negative effects of PS-MPs. These effects may similarly impact epididymal cells, including inducing inflammatory responses, necroptosis, and apoptosis (Wu et al., 2023; Wang et al., 2022; Hou et al., 2022).

Seminal vesicles are accessory male reproductive glands that produce fluids essential for the transportation of sperm during and after ejaculation. They provide the necessary energy and a supportive microenvironment for ejaculated sperm to survive and fertilize the egg. Although the present study did not show a significant impact of PS-MPs in most test groups, two distinct alterations were observed. Rats exposed to 5 µg of PS-MP/kg body weight daily showed a substantial increase in seminal vesicle weight, whereas those exposed to 5000 µg of PS-MP/kg body weight daily exhibited a significant decline in weight. This highlights a unique differentiation in the effects of PS-MPs at low and high doses. The findings contradict an earlier study by Ijaz et al. (2021), where the authors reported a differential effect on seminal vesicle weight in the opposite direction. In their study, an increase in seminal vesicle weight was observed in groups exposed to 2000 µg/l of PS-MPs, while a partial decline was noted in animals administered 2 µg/l of PS-MPs. It appears that high doses of PS-MPs have a negative impact on reproductive organs, whereas low doses primarily interfere with the metabolism and functioning of these organs.

The cauda epididymal sperm characteristics indicated a pronounced negative impact of PS-MPs at higher doses, specifically at 500 and 5000 µg of PS-MP/kg body weight. Almost all parameters, including sperm abnormality, motility, viability, and count, deviated significantly compared to the control group. Sperm count declined by nearly 60% in the group administered 5000 µg of PS-MP/kg body weight, indicating a clear impact on the overall fertility of exposed rats. Several studies have reported a decline in sperm count following PS-MP exposure (Hamza et al., 2023; Li et al., 2021). Notably, the findings of Ijaz et al. (2021) align with the current results, as their study also observed a gradual decline in sperm count. Additionally, the viability and abnormality percentages reported in their study were

consistent with the present findings. Interestingly, the decline in motility and viability was not statistically significant in the groups administered 5 and 50 µg of PS-MP/kg body weight, possibly due to the dose-dependent toxicity of PS-MPs in both the testis and epididymis.

Morphological characteristics revealed unique oxidative damage to cauda epididymal sperm, including bent tails, head-tail separation, and coiled tails. These abnormalities clearly indicate oxidative stress-induced damage to the neck piece and/or interference during spermiogenesis, resulting in cytoplasmic droplets remaining within the plasma membrane of newly formed sperm. The observed abnormalities in cauda epididymal sperm strongly suggest oxidative stress and/or psychological stress (Walke et al., 2023; Clarke et al., 1999; Alam and Chander, 2024). Thus, it can be inferred that PS-MPs may induce oxidative stress in epididymal tissues, leading to a high prevalence of sperm abnormalities. Additionally, the significant damage observed in cauda epididymal sperm could also be attributed to the psychological stress associated with the administration of high daily doses of PS-MPs.

The histological attributes of epididymal tissue clearly indicated a decline in sperm density as the doses of PS-MPs increased. This corresponds to the earlier observation of reduced epididymal weight in rats exposed to 50, 500, and 5000 µg of PS-MP/kg body weight. Changes in the epididymis are often an indirect consequence of testicular function; therefore, lower sperm density does not necessarily reflect the direct impact of PS-MPs. However, reproductive toxicants can lead to increased cellular debris and/or sloughed germ cells, as well as a depletion of luminal content (Vidal and Whitney, 2014). The present study also observed disorganization of basal cells and the presence of cellular debris in the lumen. The histological adversities observed in the higher dose groups suggest epididymal toxicity induced by PS-MPs. Multiple studies have reported that PS-MPs exhibit toxicity in various cell types (Dusza et al., 2022; Hu et al., 2022).

The present study demonstrated that PS-MPs also cause alterations in the histological attributes of the seminal vesicle. Animals treated with 500 and 5000 µg of PS-MP/kg body weight exhibited disorganization of mucosal folds and desquamation of the epithelium. These specific adversities are indicative of seminal vesicle toxicity. Previous studies have linked the presence of microplastics in semen to their accumulation in the seminal vesicle and epididymis (Zhang et al., 2024; Montano et al., 2023). Evidence suggests that PS-MPs induce oxidative stress in the testis (Fang et al., 2024; Zangene et al., 2024), making it highly likely that similar activities occur in the seminal vesicle and potentially in epididymal tissues. An imbalance in the antioxidative mechanism can lead to apoptosis (Halliwell and Cross, 1994; Lohiya et al., 2014), and excessive oxidative stress in the mucosal folds of the seminal vesicle may result in cellular apoptosis or, eventually, necrosis.

Alterations in the biochemistry of epididymal tissue highlight the significant role of PS-MPs in epididymal function and sperm maturation. The present study showed a significant change in the level of sialic acid, particularly in the group of animals exposed to 5000 µg of PS-MP/kg body weight, which suggests a reduced availability of sialoproteins in the epididymis.

It is important to note that sialoproteins, as anti-agglutinins, prevent head-to-head agglutination of sperm and are therefore crucial for sperm maturation during the capacitation process in the proximal epididymis (Harayama et al., 2000). Additionally, the activity of L-carnitine, a key component in sperm metabolism and maturation during epididymal transit, also declined significantly in animals administered 5000 µg of PS-MP/kg body weight. These findings suggest that PS-MPs impair sperm maturation in the epididymis, potentially affecting sperm quality. Although the level of fructose in seminal tissue showed limited reduction in animals exposed to 500 and 5000 µg of PS-MP/kg body weight, the change was not statistically significant. Nevertheless, this may still indicate a subtle impact on the functional efficiency of the seminal vesicle.

CONCLUSION

Based on the results, the present study affirmatively highlights the exclusive primary effects of PS-MPs on the epididymis and seminal vesicle. These effects appear to include both morphological and maturational adversities on spermatozoa during their transit through the epididymis. The study also concludes that at higher doses (>500 µg/kg body weight), PS-MPs may potentially interfere with the process of capacitation in the epididymis and the post-ejaculation maintenance of sperm. Furthermore, since the seminal vesicle also exhibited damage at higher doses of PS-MPs, this may impair the ability to meet the energy demands of sperm post-ejaculation, thereby affecting the overall rate of fertility success.

Acknowledgement

The authors are thankful to the Department of Zoology, University of Rajasthan for providing necessary facilities.

Conflict of interest

Authors have declared no competing interests.

REFERENCES

- Adhikari, M., Biswas, C., Mazumdar, P., Sarkar, S., & Pramanick, K. 2024. Evaluating the potential of daily intake of polystyrene microplastics via drinking water in inducing PCOS and its ovarian fibrosis progression using female zebrafish. *NanoImpact*, 34, 100507.
- Alam, I., & Chander, P. 2024. Exploring the Complex Relationship between Psychological Stress and Fertility: Implications for Population Growth in Developing Countries. *Journal of Pharmaceutical Research International*, 36(11), 139–149.
- Balali, H., Morabbi, A., & Karimian, M. 2024. Concerning influences of micro/nano plastics on female reproductive health: focusing on cellular and molecular pathways from animal models to human studies. *Reproductive biology and endocrinology : RB&E*, 22(1), 141.
- Clarke, R. N., Klock, S. C., Geoghegan, A., & Travassos, D. E. 1999. Relationship between psychological stress and semen quality among in-vitro fertilization patients. *Human reproduction (Oxford, England)*, 14(3), 753–758.

CPCSEA. Guidelines on the regulation of scientific experiments on animals. New Delhi: Ministry of Environment and Forests, CPCSEA Standard Operating Procedures for Institutional Animals Ethics Committee (IAEC); 2010.

D'Angelo, S., & Meccariello, R. 2021. Microplastics: A Threat for Male Fertility. *International journal of environmental research and public health*, 18(5), 2392.

Daniels D, Berger Eberhardt A. 2024. Climate change, microplastics, and male infertility. *Curr Opin Urol*, 34(5):366-370.

Dibbon, K. C., Mercer, G. V., Maekawa, A. S., Hanrahan, J., Steeves, K. L., Ringer, L. C. M., Simpson, A. J., Simpson, M. J., Baschat, A. A., Kingdom, J. C., Macgowan, C. K., Sled, J. G., Jobst, K. J., & Cahill, L. S. 2024. Polystyrene micro- and nanoplastics cause placental dysfunction in mice†. *Biology of reproduction*, 110(1), 211–218.
<https://doi.org/10.1093/biolre/ioad126>

Dusza, H. M., Katrukha, E. A., Nijmeijer, S. M., Akhmanova, A., Vethaak, A. D., Walker, D. I., & Legler, J. 2022. Uptake, Transport, and Toxicity of Pristine and Weathered Micro- and Nanoplastics in Human Placenta Cells. *Environmental health perspectives*, 130(9), 97006.

Endo, T., Kobayashi, K., Matsumura, T., Emori, C., Ozawa, M., Kawamoto, S., Okuzaki, D., Shimada, K., Miyata, H., Shimada, K., Kodani, M., Ishikawa-Yamauchi, Y., Motooka, D., Hara, E., & Ikawa, M. 2024. Multiple ageing effects on testicular/epididymal germ cells lead to decreased male fertility in mice. *Communications biology*, 7(1), 16.

Fang, Q., Wang, C., & Xiong, Y. 2024. Polystyrene microplastics induce male reproductive toxicity in mice by activating spermatogonium mitochondrial oxidative stress and apoptosis. *Chemico-biological interactions*, 396, 111043.

Grechi, N., Ferronato, G. A., Devkota, S., & Ferraz, M. A. M. M. 2024. Microplastics are detected in bull epididymal sperm and polystyrene microparticles impair sperm fertilization†. *Biology of reproduction*, 111(6), 1341–1351.

Halliwell, B., & Cross, C. E. 1994. Oxygen-derived species: their relation to human disease and environmental stress. *Environmental health perspectives*, 102 Suppl 10(Suppl 10), 5–12.

Hamza, A., Ijaz, M. U., & Anwar, H. 2023. Rhamnetin alleviates polystyrene microplastics-induced testicular damage by restoring biochemical, steroidogenic, hormonal, apoptotic, inflammatory, spermatogenic and histological profile in male albino rats. *Human & experimental toxicology*, 42, 9603271231173378.

Harayama, H., Liao, P. C., Gage, D. A., Miyake, M., Kato, S., & Hammerstedt, R. H. 2000. Biochemical characterization of sialoprotein "anti-agglutinin" purified from boar epididymal and seminal plasma. *Molecular reproduction and development*, 55(1), 96–103.

Hou, L., Wang, D., Yin, K., Zhang, Y., Lu, H., Guo, T., Li, J., Zhao, H., & Xing, M. 2022. Polystyrene microplastics induce apoptosis in chicken testis via crosstalk between NF-κB and Nrf2 pathways. *Comparative biochemistry and physiology. Toxicology & pharmacology: CBP*, 262, 109444. 4

Campen, M. J., & Yu, X. 2024. Microplastic presence in dog and human testis and its potential association with sperm count and weights of testis and epididymis. *Toxicological sciences: an official journal of the Society of Toxicology*, 200(2), 235–240.

Ijaz, M. U., Shahzadi, S., Samad, A., Ehsan, N., Ahmed, H., Tahir, A., Rehman, H., & Anwar, H. 2021. Dose-Dependent Effect of Polystyrene Microplastics on the Testicular Tissues of the Male Sprague Dawley Rats. *Dose-response: a publication of International Hormesis Society*, 19(2), 15593258211019882.

Jin, H., Ma, T., Sha, X., Liu, Z., Zhou, Y., Meng, X., Chen, Y., Han, X., & Ding, J. 2021. Polystyrene microplastics induced male reproductive toxicity in mice. *Journal of hazardous materials*, 401, 123430.

Jin, H., Xue, B., Chen, X., Ma, T., Ma, Y., Zou, H., Zhu, J., Tong, X., Song, R., Meng, W., & Liu, Z. 2025. Polystyrene microplastics induced spermatogenesis disorder via disrupting mitochondrial function through the regulation of the Sirt1-Pgc1 α signaling pathway in male mice. *Environmental pollution (Barking, Essex: 1987)*, 364(Pt 2), 125364.

Jin, H., Yan, M., Pan, C., Liu, Z., Sha, X., Jiang, C., Li, L., Pan, M., Li, D., Han, X., & Ding, J. 2022. Chronic exposure to polystyrene microplastics induced male reproductive toxicity and decreased testosterone levels via the LH-mediated LHR/cAMP/PKA/StAR pathway. *Particle and fibre toxicology*, 19(1), 13.

Li, S., Wang, Q., Yu, H., Yang, L., Sun, Y., Xu, N., Wang, N., Lei, Z., Hou, J., Jin, Y., Zhang, H., Li, L., Xu, F., & Zhang, L. 2021. Polystyrene microplastics induce blood-testis barrier disruption regulated by the MAPK-Nrf2 signaling pathway in rats. *Environmental science and pollution research international*, 28(35), 47921–47931.

Lohiya, N. K., Alam, I., Hussain, M., Khan, S. R., & Ansari, A. S. 2014. RISUG: an intravasal injectable male contraceptive. *The Indian journal of medical research*, 140 Suppl(Suppl 1), S63–S72.

Marquis, N. R., & fritz, I. B. 1964. Enzymological determination of free carnitine concentrations in rat tissues. *Journal of lipid research*, 5, 184–187.

Mann T. Fructose, Polyols and Organic acids. In *Biochemistry of semen and the male reproductive tract*. London: Methuen & Co. 1964;237-239.

Montano, L., Giorgini, E., Notarstefano, V., Notari, T., Ricciardi, M., Piscopo, M., & Motta, O. 2023. Raman Microspectroscopy evidence of microplastics in human semen. *The Science of the total environment*, 901, 165922.

OECD Guidelines for the Testing of Chemicals, Section 4. Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. https://www.oecd-ilibrary.org/environment/test-no-423-acute-oraltoxicity-acute-toxic-class-method_9789264071001-en

Sun, J., Sui, M., Wang, T., Teng, X., Sun, J., & Chen, M. 2024. Detection and quantification of various microplastics in human endometrium based on laser direct infrared spectroscopy. *The Science of the total environment*, 906, 167760.

Sun, S., Jin, Y., Luo, P., & Shi, X. 2022. Polystyrene microplastics induced male

reproductive toxicity and transgenerational effects in freshwater prawn. *The Science of the total environment*, 842, 156820.

Svennerholm L. (1957). Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method. *Biochimica et biophysica acta*, 24(3), 604–611.

Vidal, J. D., & Whitney, K. M. (2014). Morphologic manifestations of testicular and epididymal toxicity. *Spermatogenesis*, 4(2), e979099.

Walke, G., Gaurkar, S. S., Prasad, R., Lohakare, T., & Wanjari, M. 2023. The Impact of Oxidative Stress on Male Reproductive Function: Exploring the Role of Antioxidant Supplementation. *Cureus*, 15(7), e42583.

Wang, M., Wu, Y., Li, G., Xiong, Y., Zhang, Y., & Zhang, M. 2024. The hidden threat: Unraveling the impact of microplastics on reproductive health. *The Science of the total environment*, 935, 173177.

Wang, X., Zhang, X., Sun, K., Wang, S., & Gong, D. 2022. Polystyrene microplastics induce apoptosis and necroptosis in swine testis cells via ROS/MAPK/HIF1 α pathway. *Environmental toxicology*, 37(10), 2483–2492.

Wang, Y., Ye, J., Ganapathy, V., & Longo, N. 1999. Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. *Proceedings of the National Academy of Sciences of the United States of America*, 96(5), 2356–2360.

WHO. 1999. Laboratory Manual for Examination of Human Semen and Sperm Cervical Mucus Interaction. Cambridge University Press, New York.

Wu, D., Zhang, M., Bao, T. T., & Lan, H. 2023. Long-term exposure to polystyrene microplastics triggers premature testicular aging. *Particle and fibre toxicology*, 20(1), 35.

Yang, W., Wu, L., Li, G., Shi, L., Zhang, J., Liu, L., Chen, Y., Yu, H., Wang, K., Xin, L., Tang, D., Shen, Q., Xu, C., Geng, H., Wu, H., Duan, Z., Cao, Y., & He, X. 2024. Atlas and source of the microplastics of male reproductive system in human and mice. *Environmental science and pollution research international*, 31(17), 25046–25058.

Zangene, S., Morovvati, H., Anbara, H., Hye Khan, M. A., & Goorani, S. 2024. Polystyrene microplastics cause reproductive toxicity in male mice. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 194, 115083.

Zhang, C., Chen, J., Ma, S., Sun, Z., & Wang, Z. 2022. Microplastics May Be a Significant Cause of Male Infertility. *American journal of men's health*, 16(3), 15579883221096549.

Zhang, C., Zhang, G., Sun, K., Ren, J., Zhou, J., Liu, X., Lin, F., Yang, H., Cao, J., Nie, L., Zhang, P., Zhang, L., Wang, Z., Guo, H., Lin, X., Duan, S., Cao, J., & Huang, H. 2024. Association of mixed exposure to microplastics with sperm dysfunction: a multi-site study in China. *EBioMedicine*, 108, 105369.

Zhao, Q., Zhu, L., Weng, J., Jin, Z., Cao, Y., Jiang, H., & Zhang, Z. 2023. Detection and characterization of microplastics in the human testis and semen. *The Science of the total*

environment, 877, 162713.

Ziani, K., Ioniță-Mîndrican, C. B., Mititelu, M., Neacșu, S. M., Negrei, C., Moroșan, E., Drăgănescu, D., & Preda, O. T. 2023. Microplastics: A Real Global Threat for Environment and Food Safety: A State-of-the-Art Review. *Nutrients*, 15(3), 617.

Table 1: Weight of epididymis and seminal vesicle in animals treated with PS-MPs against control. Level of significance was compared against Group I and approved when *p<0.05, and *p<0.001.**

	Group I	Group II	Group III	Group IV	Group V
Epididymis (mg)	74.11±1.12	72.71±0.92	71.97±0.93*	70.43±1.47*	65.83±1.14***
Seminal vesicle (mg)	226.85±1.78	250.75±2.01*	224.11±2.15	224.15±2.77	218.70±1.92*

Table 2: Abnormality, motility and viability percentage of cauda epididymal sperm in PS-MPs treated rats. Sperm count was also evaluated. All values were compared against control (Group I). Level of significance was set at *p<0.05, **p<0.01 and *p<0.001.**

	Group I	Group II	Group III	Group IV	Group V
Abnormality (%)	22.92±0.85	26.05±1.18*	27.32±1.44*	31.36±0.92**	35.28±1.55***
Motility (%)	68.05±1.95	67.90±1.96	64.63±2.33	57.49±1.81**	46.93±2.24**
Viability (%)	83.37±1.64	80.94±0.99	74.61±2.37	65.79±2.27**	60.57±2.86***
Count (mil/ml)	44.76±1.43	41.96±1.53	39.69±1.17*	34.90±1.93**	17.66±2.02***

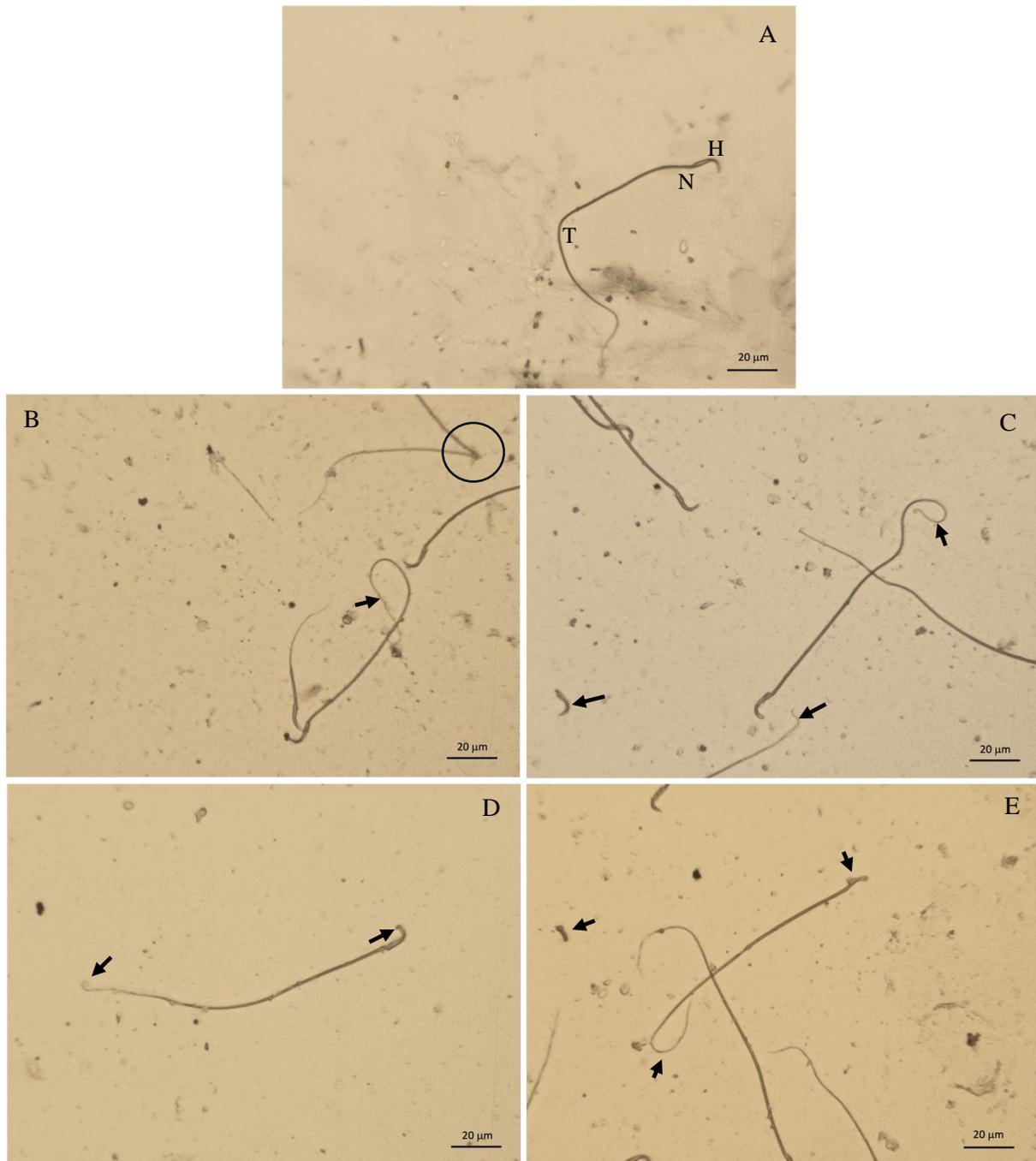


Figure 1: Morphological evaluation of Pap-stained cauda epididymal sperms. The representative images of each group (Groups I-V labelled as A-E) are showing types of abnormalities found exclusively and in large number in the test groups. Major abnormalities observed in PS-MPs treated rats were coiled tail, twisted tail, pinhead, headless tails, tail-less heads, and other deformities in head. The pictures are scaled at 20 μm and observed under light microscope.

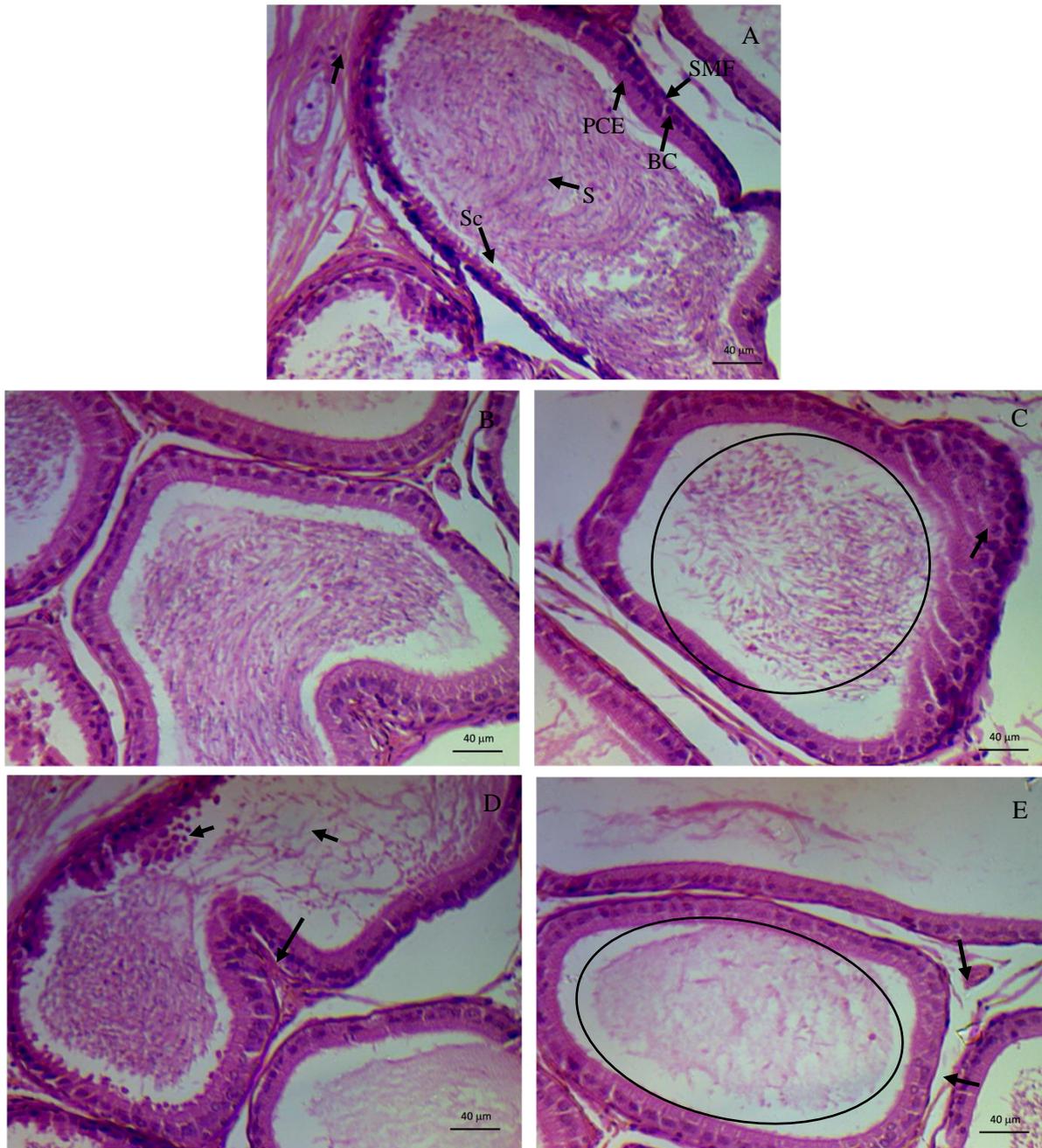


Figure 2: A. Group I indicated normal columnar epithelium, smooth muscle fiber, and stereocilia. Lumen within PCE was filled with spermatozoa. B. histological architecture of Group II was comparable to control, well defined long columnar cells with short basal cells. Lumen was filled with spermatozoa. C. In Group III the number of spermatozoa in the lumen was comparatively lower than control (Group I), whereas, some disorganization was evident in PCE. D. In Group IV basal cells were

found disorganized and appeared to fall into the lumen. Cellular debris were also found in the lumen. E. In Group V lumen within epithelium indicated decline in number of sperms relative to other test groups and control (Group I). Basal cells were disorganized at many occasions, Sc also disappeared at various location in columnar epithelium. SMF: Smooth muscle fiber; PCE: Pseudostratified columnar epithelium; S: Spermatozoa; Sc: Stereocilia; BC: Basal cell.

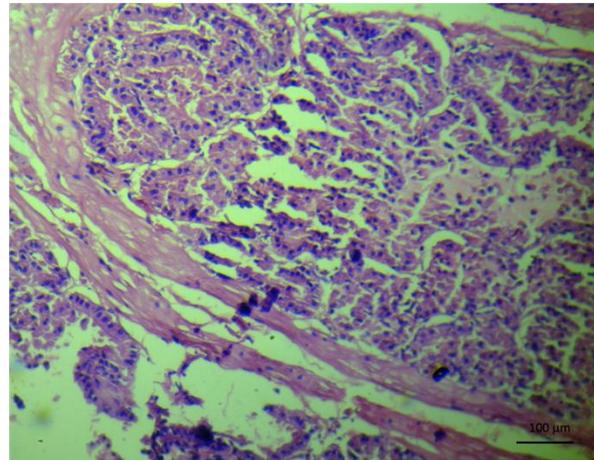
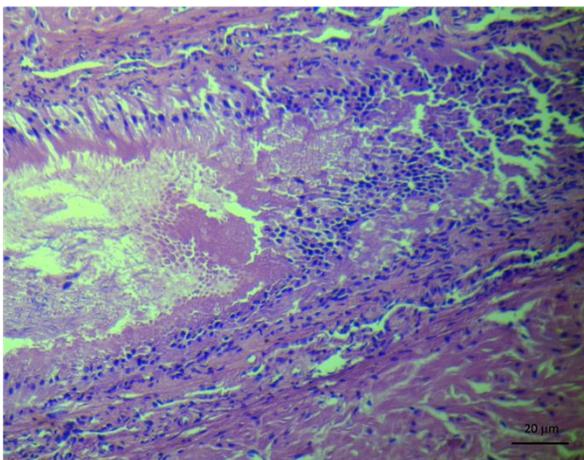
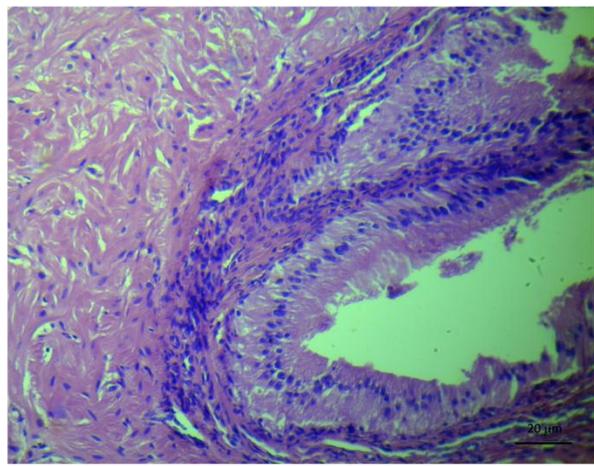
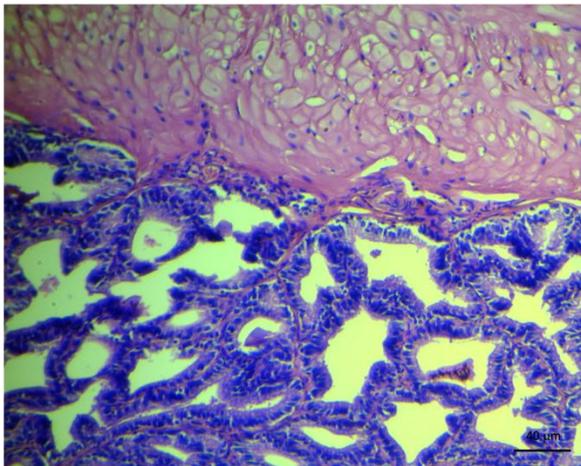
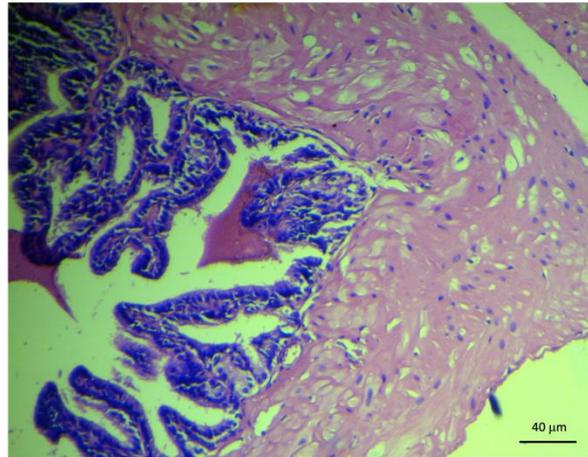
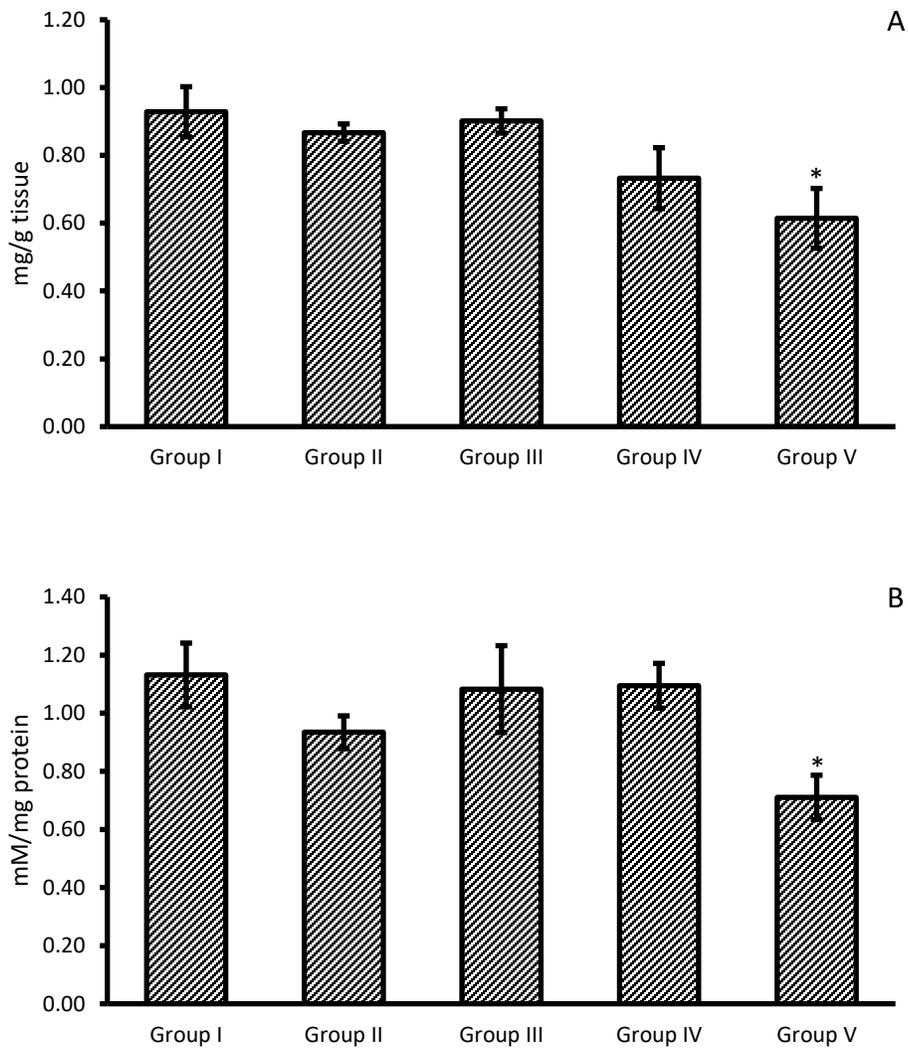


Figure 4: A. The control (Group I) seminal vesicle histology indicated complex glandular structure, where lumen contained highly irregular crypts. The pseudostratified epithelia were columnar resting on lamina muscularis. B. Group II also indicated complex folding however some irregularities were observed relative to control. the epithelium was still columnar. Smooth muscles had relatively higher fat deposition C. Group III indicated loss of intricate folding, though lumen remained dilated. D. Group IV showed comparatively lower complexity in the folding of crest. Dilation of lumen was comparable to Group III. E. Group V showed significant loss of branching of mucosa of seminal vesicle. Crest was limited and lumen space was considerably reduced. Desquamation of covering epithelium and cracks in the luminal secretions. SM: Smooth muscle; LP: Lamina muscularis; PSE: Pseudostratified epithelia; C: Crypts; L: Lumen.



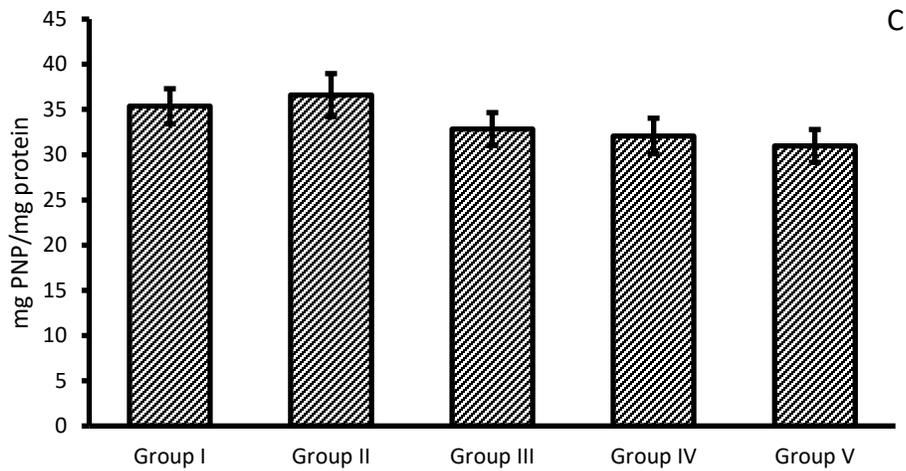


Figure 4: Biochemical test for activity of A. sialic acid, B. L-carnitine, and level of C. Neutral α glucosidase in epididymal tissues of control and test groups. Level of significance was set at $*p<0.05$.

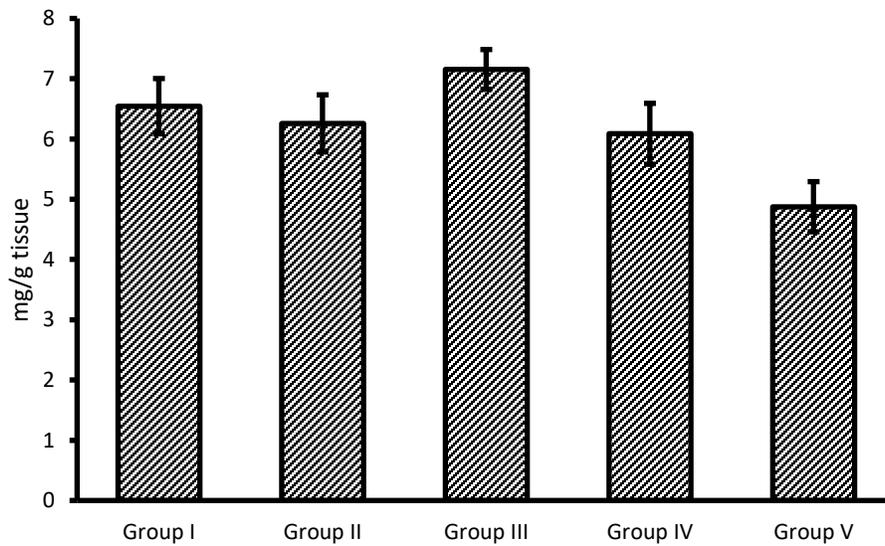


Figure 5: Level of fructose in seminal vesicle following administration of PS-MPs against control (Group I). Level of significance was set at $*p<0.05$.