Original Research Article

Study of reproductive toxicity elicited by bisphenol A exposure in male *Mus musculus*

ABSTRACT

Bisphenol A (BPA) is a common endocrine disrupting chemical that is utilized in the manufacturing of plastic products, shows adverse effects on reproductive health of male. This study investigates the toxic impacts of BPA on reproductive health of male Swiss albino mice. For this research work, adult male mice weighing about 30±5 grams were divided into three groups (control, 55 mg and 110 mg/kg body weight) and after the completion of 35 days of treatment, animals were sacrificed and results were made. The findings suggests that both lower and higher dosage of BPA significantly ($p<0.001$) reduced the sperm count, testicular weight and seminal weight in a dose dependent manner as compared to the control group. Treated group of mice also represented remarkable abnormalities in sperm morphology such as hookless heads, banana shaped head, broken tail and coiled tails. Thus, the present study concluded that BPA is a toxic chemical widely present in the environment that adversely affect the reproductive efficiency through multiple biological processes.

Keywords: bisphenol A, BPA, reproductive toxicity, sperm count, morphology, testes, *Mus musculus.*

INTRODUCTION

Bisphenol A or BPA is widely recognized as an endocrine dysregulator and it is extensively utilized in the plastic producing industries (Palsania et al., 2024; Stavridis et al., 2022). BPA serves as a monomer in the industrial processing of

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plastic containers for edible products, linings of beverage cans, baby bottles, dental sealants, electric gadgets, water bottles, medical tubing, thermal paper receipts, toys, ophthalmic lenses and many other everyday products (Kumari & Thakur, 2026; Manzoor et al., 2022; Michałowicz J., 2014; Flint et al., 2012). People are exposed to BPA by means of oral pathway (Chapin et al., 2008), inhalation, & cutaneous absorption (Geens et al., 2012). It has been detected in serum, saliva, renal sample of human (Zimmers et al., 2014; Vandenberg et al., 2007; Yoshimura et al., 2002), breast milk, placenta, amniotic fluid, & in the blood of umbilical cord supporting the encounter of BPA to pregnant women (Usman & Ahmad, 2016; Cao et al., 2012).

Its exposure leads to multiple serious health issues including endocrine dysregulation, reproductive abnormalities, cardiovascular disorder, carcinogenicity, neurotoxicity, and metabolic toxicity (Kumari & Thakur, 2026).

The present research work was pursued to evaluate the reproductive toxicity elicited in male *Mus musculus* by the exposure of BPA.

MATERIALS & METHODS

Animal Model

For this research work, adolescent healthy male Swiss albino mice 10-12 weeks old & weighing around 30±5 gm were used. The experimental animals were kept in scientific cage and maintained under standardized condition in the animal house of University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, Bihar.

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Figure1: Experimental animal, *Mus musculus*.

Chemicals

The experimental chemicals such as bisphenol A, ethanol, & all other chemicals and reagents were purchased from authorized scientific supplier of Bhagalpur, Bihar, India. All chemicals employed in this study were of analytical grade.

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Reproductive Organs Weight

Weight of testes and seminal vesicles of all the control and treated groups were measured after the completion of 35 days of dosage by using electronic weighing scale.

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Sperm Count

Sperm count of control and treated group of mice were estimated by using the Neubauer's chamber at 40× magnification, by the method proposed by Wyrobek et al., (1984).

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Sperm Morphology

Sperm morphology was also studied in accordance to the method proposed by Wyrobek et al., (1984).

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EXPERIMENTAL DESIGN

Mice were separated into three groups, group I (control), group II (55 mg/kg of body weight) and group III (110 mg/kg of body weight) including ten mice in each group. Group I mice were administered with distilled water, group II mice were orally administered with 55 mg/kg body weight of BPA and group III mice were orally administered with 110 mg/kg body weight of BPA for 35 consecutive days through gastric gavage. At the end of treatment period, mice were sacrificed with cervical dislocation and all experimental parameters were investigated according to the protocol mentioned.

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STATISTICAL ANALYSIS

Values of each experimental groups are represented as Mean ± S.E.M. (standard error of mean). Statistical analysis of data were performed using one-way ANOVA to compare the differences between the control and treated groups.

RESULT

After the completion of treatment period, mice were sacrificed by cervical dislocation, all tests were performed and results were collected. All the values are represented in Mean ± SEM.

Effects of BPA on Reproductive Organs Weight

Oral administration of BPA at both lower (55 mg/kg of body weight) and higher (110 mg/kg of body weight) dosage demonstrated significant reduction in the weight of reproductive organs namely testes and seminal vesicles. Testicular and seminal vesicle weight of group I were 120.2 ± 1.82 & 314.2 ± 3.13 mg, group II were 105.7 ± 3.88 & 297.1 ±1.40 mg and group III were 82.6 ± 3.66 & 280.4 ± 3.51 mg. The weight of both the reproductive organs were decreased

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significantly ($p<0.001$) as compared to control group in a dose dependent manner as shown in table- 1.

Table 1: Effects of bisphenol A on testicular weight, seminal vesicle weight and sperm count after 35 days.

Experimental Groups	Dosage	No. of mice	Testicular weight (in mg)	Paired Seminal vesicle weight (in mg)	Sperm count ($\times 10^4$ /ml)
Group I: Control	Distilled Water	10	120.2 ± 1.82	314.2 ±3.13	138.9 ± 3.12
Group II: Lower Dose	55 mg/kg B.W.	10	105.7 ± 3.88	297.1 ±1.40	127.3 ± 1.97
Group III: Higher Dose	110 mg/kg B.W.	10	82.6 ± 3.66	280.4 ±3.51	88.6 ± 2.01

Values are represented as Mean ± S.E.M., Statistical significance: One-way ANOVA revealed highly significant difference among the three higroups ($p < 0.001$).

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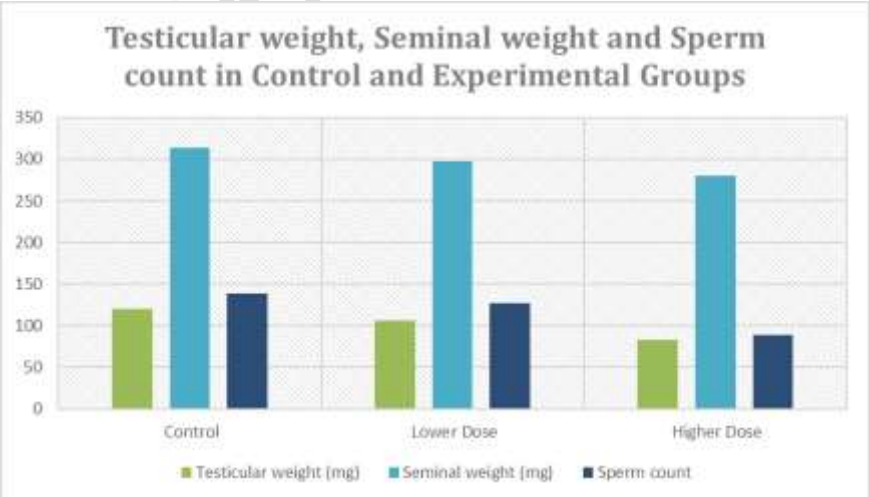

Figure 2: Histogram showing testicular weight, seminal weight and sperm count in control and experimental groups.

Effects of BPA on Sperm Count

Sperm count of group I was 138.9 ± 3.12, group II was 127.3 ± 1.97 and group III was 88.6 ± 2.01 ($\times 10^4$ sperms /ml) as shown in table- 1. The result showed significant ($p < 0.001$) dose related declination in the sperm count of BPA treated mice as compared to that of control group.

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Effects of BPA on Sperm Morphology

Treatment of mice with BPA demonstrated significant abnormalities in sperm morphology as compared to the control group of mice as shown in figure 2. Types of abnormalities in the head and tail of sperm cells such as hookless, banana shaped head, round headed, broken tail and coiled tail were recorded. Occurrence of abnormal sperms were very high in group III of mice treated with higher dosage of BPA as compared to that of group II (low dose).

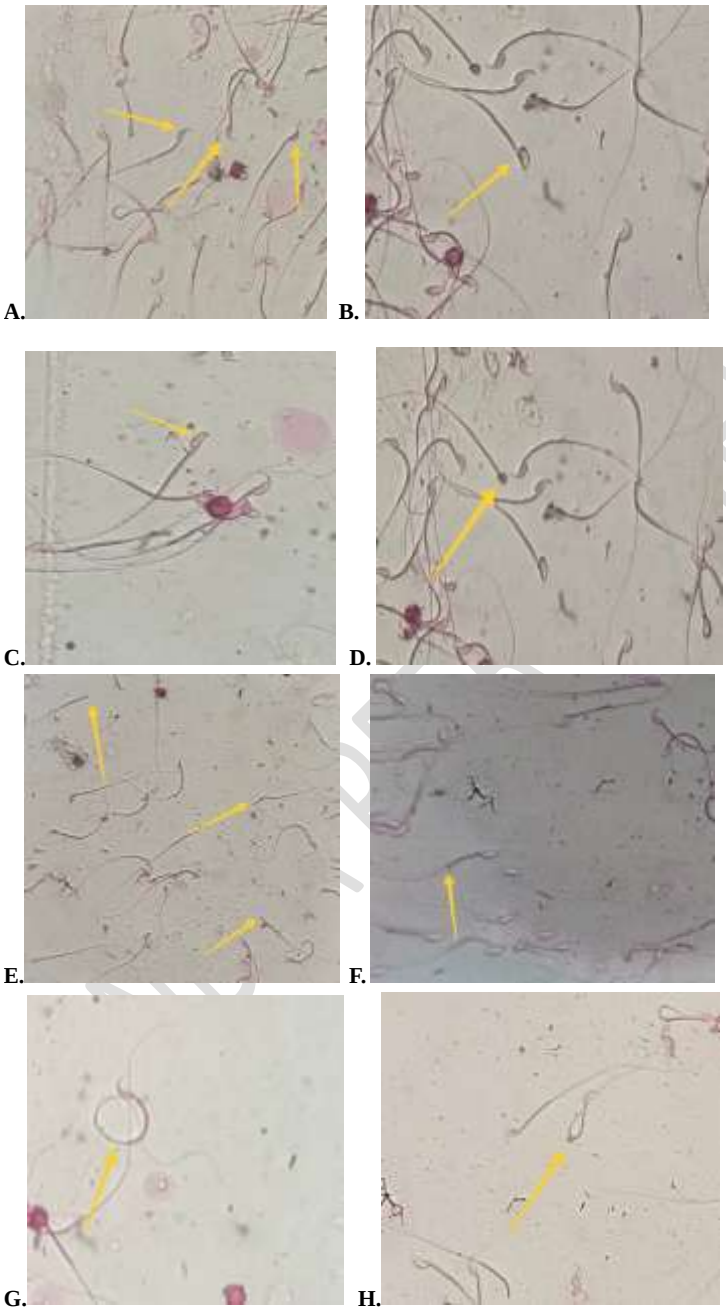

Figure.3: Sperm morphology, A- normal sperms, B- hookless, C- banana shaped, D-

round headed, E- headless, F- broken tail, G, H- coiled tail. (A- control. B, C, D, E, F, G, H- treated).

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DISCUSSION

Study of reproductive organs weight are one of the essential benchmark for the evaluation of the effects of dosage on fertility. Testicular size is the most important measure for the initial examination of spermatogenesis. Seminiferous tubules and germinal components comprise about 98 percent of the total mass of testes (Salman et al., 2010). In the present research work significant reduction in the weight of testes and seminal vesicles of the mice treated with bisphenol A in comparison to the control group were noted. The effects of BPA were dose dependent as mice treated with higher dose of BPA showed more pronounced declination in the weight of testes and seminal vesicles as compared to the mice treated with lower dosage of BPA.

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The observed reduction in testicular weight and seminal weight in the present study is consistent with previous studies (Zang et al., 2016 and Khazaeel et al., 2021) that also reported a reduction in the weight of reproductive organs and accessory reproductive organs in male mice after the administration of BPA.

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Reduction in testicular weight might be due to the decrease in the concentration of serum testosterone, reduction in germinal cells, downregulation of steroidogenic enzymes, and alteration in the process of spermatogenesis. Declination in the level of androgens may also be responsible for the reduction in the weight of seminal vesicles because male accessory reproductive organs are primarily dependent on androgens for their maturation, structure development and functional activity (Desai et al., 2016). Declination in the activity of dehydrogenases (primarily 3-beta hydroxysteroid dehydrogenase and 17-beta hydroxysteroid dehydrogenase) possibly contribute to the declined production of serum testosterone (Gupt not a et al., 2004).

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In the present investigation, a significant reduction in sperm count and significant morphological abnormalities in sperm cells in both group II and group III mice were observed in relation to that of control group of mice (group I) and the effects were in a dose related manner. Mice treated with higher dose of BPA (group III) depicted more declination in the sperm count and the occurrence of morphological damages in sperm cells were more frequent than the group II mice treated with lower dose of BPA.

A similar dose dependent decrease in sperm count in male mice were reported by Cao et al., (2020), Shi et al., (2017) & Qiu et el., (2013) and the findings might be associated with compromised spermatogenesis & declined expression of androgen receptors. Another study conducted by Pan et al., (2019) also reported a significant reduction in sperm count, hampered sperm motility and significantly impaired sperm morphology in mouse model treated with BPA. An investigation carried out by Li et al., (2011) reported that BPA exposure is associated with poor semen quality (including total sperm count, sperm concentration, sperm motility & viability) in human population and similar results were found in multiple animal studies (Li et al., 2023; Adegoke et al., (2022); Hong et al., 2022; Chitra et al., 2003; Kumari and Thakur 2026).

Decreased level of serum testosterone might be responsible for the reduced sperm counts, sperm motility and abnormalities in the morphological structure of sperm cells (Presunto et al., 2023; Zhang et al., 2007).

Prenatal exposure to BPA in Swiss albino male mice might results into significantly decreased sperm count and sperm motility, compromised spermatogenesis and intensified abnormalities in sperm cells. These detrimental effects on reproductive parameters may be due to oxidative damages and diminished antioxidant potential caused by the exposure of BPA (Al-Griw et al., 2021). Maternal exposure to BPA may contribute to reduced sperm count,

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motility, testosterone level, diminished testicular weight, and reduced acrosomal integrity in offspring of mice. It suggests that impaired male fertility is associated with disrupted steroidogenesis, dysfunction of Leydig cells and meiotic arrest during the process of spermatogenesis (Zhang et al., 2025).

CONCLUSION

In conclusion, the findings of the present study supported that bisphenol A (BPA) is an environmental toxicant that produced significant reproductive toxicity in male *Mus musculus.* BPA treated mice revealed significant reduction in sperm count, weight of testes & seminal vesicles and remarkable abnormalities in sperm cells morphology.

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