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Hormones with Sperm and Testes in Albino Male Mice Effected by Treated with Phthalate Migration from Bottled Water

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ABSTRACT: Phthalates, such as including type dibutyl phthalate (DBP), or phthalic acid esters, chemical are widely used in industry as plasticizers for many polymer products and are present in bottled water and. Effects of male infertility and testes after detected in bottled water. In this study, we aimed to examine the reproductive toxicity resulting from simultaneous exposure dibutyl phthalate (DBP) in male albino mice. The toxicity was evaluated by causing adverse effects on the male reproductive system, The mice were divided into four groups, and each group was treated for (DBP) at three concentrations (3-7-15) mg/kg for 45 days, except control group, its effect on FSH hormones in highest concentration was recorded (17.79 mIU/ml), in (2.06 mIU/ml) for LH and testosterone (1.85 ng/ml), when Changes in sperm count from (31.67) (sperm $\times 10^6$) to decrease in control group (19.67) (sperm $\times 10^6$) at 15 mg/kg were observed, along with a decrease in motility and turbidity, and an increase in mortality (23.67%) compared to the control group. It also affected testicular tissue by affecting the appearance of the seminiferous tubules, with swelling in the tissue due to exposure to phthalates at concentrations of (7 - 15) mg/kg respectively. Finally, examination of the comet revealed a toxic relationship to the DNA strand, which was at its shortest in length at the first concentration and longest comet at the second and third concentrations as a result of chronic exposure to the specified doses.

INTRODUCTION

The World Health Organization (WHO) recognizes access to safe drinking water as a fundamental human right and a cornerstone of public health, in many countries, bottled water is considered as a safer alternative to tap water, due to fears about municipal water quality and a lack of access to dependable filtration systems, the world's reliance on bottled water has grown dramatically in recent decades (Salehi et al., 2022 and Organization, 2024). The drinking water is one of the important routes for humans. During storage bottled water under sunlight effects of direct sunlight exposure on the migration of antimony and phthalates (BPA, DMP, and DBP), after the duration is examined for were detected by HPLC (Habib et al., 2023; Massahi et al., 2026). common plasticizer Di-butyl phthalate (DBP) is a used in industry to add flexibility and stability, to plastics such as polyvinyl chloride (PVC). Since the DBP is not chemically bonded to the plastic matrix, it can migrate into the environment over time and is routinely detected in bottled drinking water all over the world (Khurshid et al., 2025; Salih et al., 2026).

The exposure of drinking water kept in polyethylene terephthalate (PET) bottles to phthalates (PAEs) is causing serious worries due to their harmful impacts on human health (Khanniri et al., 2024). Of the many kinds of phthalates that have been thoroughly studied for their negative impacts on male fertility, two kinds are DEHP and DBP (Zhang et al., 2021). Dibutyl phthalate (DBP) has been considered a priority pollutant by the U.S. Environmental Protection Agency due to its possible effects on the environment and human health (Ma et al., 2023). Furthermore, the European Food Safety Authority (EFSA) has determined the TDI for DBP to be 10 $\mu\text{g/kg}$ body weight/day (EFSA Journal, 2019).

The word “sperm” originates from the Greek word sperma, which means “seed.” Sperm was first discovered under the microscope in 1677 by Antonie van Leeuwenhoek, where he referred to them as “little animalcules.” Exposure to phthalates has been proposed as one of the causes for male infertility problems, although the conflicting results from various populations

exposed to phthalates call for further studies (Dhar et al., 2026). On the other hand, mice were fed Di(2-ethylhexyl) phthalate (DEHP) and methyl paraben (MP) orally every day for 28 days. Follicle-stimulating hormone (FSH), blood testosterone, luteinizing hormone (LH), and testicular abnormalities were all impacted by this. In male mice, this modifies hormone levels and adversely affects reproductive function (Yesumanipreethi and Magadalenal et al., 2024; Abeed et al., 2024). In another study, exposed plastics and microparticles to mice and rats were leading suffer from testicular inflammation, reduced testosterone levels, and impaired sperm quality experience disrupted spermatogenesis (Amerreh et al., 2020 and Jin et al., 2021).

According to a different study, between 25% and 50% of infertility and other fertility-related issues globally are caused by phthalate. This prompted research into the reproductive effects in humans, which are spread throughout the environment and enter the body through a variety of ways (ingestion, inhalation, or skin contact) before undergoing rapid metabolism (Radke et al., 2018 and Caporossi et al., 2020).

The excessive industrialization and urbanization lead to the emergence of additional environmental contaminants, specifically endocrine disruptors. Research has demonstrated that it adversely affects sperm production, semen quality, and fertility. Semen metrics, while crucial for assessing male fundamental reproductive potential, possess diagnostic constraints by defining normative reference values for certain semen traits. This indicates that there are concerns regarding the well-being of the male reproductive system. Research, though constrained, suggests that elevated ambient temperatures may negatively influence male fertility (Dissanayake et al., 2019, Kumar et al., 2022 and Sciorio et al., 2025).

On the other hand, one study presented findings related to human exposure to phthalates, showing that it caused fertility disturbances in both males. At the hormonal level, phthalates can alter the secretion of pituitary and hypothalamic hormones. At the cellular level, they can affect reproduction and interfere with intracellular membrane receptors. Testosterone concentrations in the testes were determined using liquid chromatography-mass spectrometry, and a significant decrease in testosterone levels was observed in both groups, (Hlisníková, et al., 2020 and Källsten et al., 2022; Bustani et al., 2025).

Phthalate causes reduced semen volume, total sperm counts, sperm concentration, morphological abnormalities of sperm head including large sperm head sizes, and other variations (Caporossi et al., 2020 and Zhang et al., 2021).

When exposed to dibutyl phthalate (DBP) is a widely used phthalate plasticizer, the effects of sperm dysfunctions led to a concentration-dependent decrease in testosterone levels and another effect caused by on mitochondrial, adenosine triphosphate (ATP) levels, sperm motility. Phthalate, ester plasticizer (DBP), affects spermatozoa. Specifically, the one study focused on DBP's influence on the stability and inhibitory identified as crucial for sperm motility and seminiferous tubular degeneration in mice was associated with histopathological injury of the testis compared to control (Alm Amoun et al., 2024 and Park, 2024; 2025; Han et al., 2025).

Another study indicated a link between phthalate exposure and changes in DNA integrity in human sperm. Neutral single-cell microgel electrophoresis (the comet test) was used to measure sperm DNA integrity. In contrast, certain phthalate species (dibutyl phthalate (DBP) and benzo butyl phthalate (BBP) cause higher genotoxicity. The comet test is widely used in environmental toxicology and biomonitoring. In prepubertal rat testes, even at lower levels than those provided, long-term dibutyl phthalate exposure was associated with testicular damage, supporting its use. (Sicińska et al., 2021; He et al., 2024 and Yuan et al., 2026). This study aims to highlight the presence of phthalates in samples of tap water and samples of bottled water, specifically type (DBP), and their association with their effect on hormones, the histological characteristics of the testes, DNA parameters, sperm damage, and their negative impact on the reproductive capacity and fertility of future generations.

MATERIAL AND METHODS

Chemicals

Phthalates: Dibutyl phthalate (DBP) (99-99.5% purity) was purchased from Emscope Laboratories (Ltd.), England, by an authorized office in Bab Al-Muazzam, dissolved in corn oil, and administered orally via feeding tube.

Collection of Semen Samples

Semen was collected from epididymis of mice after that that exposure to phthalate (DBP) as control, Subjects were instructed to semen sample. Semen was left to liquefy for a minimum of 20 minutes before semen analysis was conducted. The parameters evaluated included abnormalities and count of sperm and the percentage of dead spermatozoa.

Animal dosing and regimen

After of the acclimatization 32 adult mice in the (Al-Nahrain Technology Center) two weeks in the 12/12 hour in light and night for the care in the laboratory Animals, then used in this study were split up into four groups, each with eight mice. Next, the mice were divided into different test groups at random. DBP was gavage using a proper feeding tube, at least three times a week. The first group was the control group (G1), the second group was given the lowest dose (3 mg/kg), the third group was given a dose (7 mg/kg), and the final group was given an oral dose of 15 mg/kg. This was done in order to quantify the alterations that the substance caused in the mice. The study has been approved by the Ethics Committee of the Department of Biology, College of Science, University of Baghdad (CSEC/1125/0180).

Hormone (FSH, LH, Testosterone) analysis

blood samples of approximately (0.5–1 mL) is obtained through cardiac puncture under deep anesthesia. To separate the serum, samples were centrifuged at $3000 \times g$ for 15 minutes at 4°C. Aliquots of serum were promptly kept at -80°C until examination. FSH, LH, and testosterone serum concentrations were quantitatively assessed using particular (ELISA), LH (ELISA Kit), FSH (ELISA Kit) and Elabscience® T(Testosterone) ELISA Kit Catalog No: E-EL-0155. (reference)

Measurement semen parameters (total count sperm, motility, abnormality, dead) %

total count sperm:

- 1- Epididymis extraction: The lower part of the testicle is dissected to allow sperm to exit, and a solution (PBS) is applied to facilitate this process.
- 2- Sperm fixation mixed and diluted
- 3- A very small drop of the sample is placed on both ends of the slide
- 4- examined under a microscope with the sperm heads counted at magnification (200 and 400).
- 5- counting: 10 ml put onto a Neubauer slide, counted under a microscope in the five designated squares. The final concentration (per mL or per epididymis) (Boitrelle et al., 2021)

Sperm motility, abnormality and dead %): approach by (WHO-2010).

The sample was examined under a light microscope of type (Bright-field Microscope) belonging to the company Nikon Corporation, Tokyo, Japan to determine the viability (motility or death) of the sperm.

Comet assay approach (Singh et al., 1988):

Preparing the cell and agarose mixture:

Mixed 100 microliters of cell suspension with 600 microliters of low-melting-point agarose (0.8% in PBS).

Preparing the slides:

Spread 100 microliters of this mixture onto prepared and pre-coated microscope slides.

Cytolysis (Lysis): Immersed the coated slides in lysis buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 minutes.

Electrophoresis:

Placed the slides in an electrophoresis chamber filled with the same TBE buffer, but without the addition of SDS. Set the electrophoresis conditions to 2 V/cm and 100 mA for 2 minutes.

Staining:

Stained the slides with ethidium bromide at a concentration of 20 µg/ml at 4°C.

Microscopic Examination:

Examined the samples while still moist using a fluorescence microscope (with a 40x common lens, a 420–490 nm excitation filter, and a 510 nm barrier filter). Measured the length of the comet tail from the center of the nucleus to the tail end for 100 cells per dose level.

Histology

Testicular tissue was fixed in a 10% diluted neutral formalin solution for 24 to 48 hours. They were dehydrated through the use of graded alcohol concentration, cleared using xylene, and then embedded in paraffin wax. The paraffin embedded block was cut into 4 micrometer thick sections using Tissue-Tek Auto Section microtome (China). Incubation of the sections took place in a warm water bath, followed by mounting of the sections on clean glass slides. The sections underwent deparaffinization in xylene and hydration in a sequence of ethanol from 100% to 70%. After that, the sections were stained with hematoxylin and eosin (H&E). After staining, dehydration, clearing, and mounting of the sections with coverslips took place. Finally, the prepared liver and kidney sections were observed under an Olympus light microscope (Japan) (Bancroft, 2008).

Statistical analysis

The statistics analysis was done via SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). The results were reported as mean $\pm$ SE for quantitative data of serum of hormone parameters. One-way ANOVA was used to compare the means between the control group and the three DBP groups for each parameter. In case the ANOVA found a statistically significant effect, Fisher's least significant difference (LSD) was applied to determine the difference between the pairs of groups. Statistical significance was defined as $P \leq 0.05$, while high significance was set at $P \leq 0.01$. Mean values marked with different lowercase letters were significantly different from one another. Hepatic and renal histopathology findings were qualitatively analyzed based on the existence, extent, and localization of the observed changes.

RESULTS

HPLC chromatogram

The HPLC chromatogram, two peaks appeared at the times of 4.17 minutes and 6.05 minutes. This means that the sample has two detectable peaks. The major peak was observed at 4.17 minutes having an area of 80.00% of the total area, with the value of 11250.25 mAU.s, and a height of 775.00 mAU. On the other hand, the minor peak that appeared at 6.05 minutes had a total area of 20.00%, with an area of 3544.14 mAU.s and a height of 191.08 mAU. With respect to the peak width, it is clear that high separation efficiency was obtained since the peak width for each peak, $W_{0.5}$, was 0.15 and 0.05 minutes. With regard to the retention time of this peak, as compared with standard chromatograms, it is peak (4.17) is associated with dibutyl phthalate (DBP), while the previous peak is associated with some other compound.

Analysis of hormones level

Table (1) shows the results of the hormone measurement in our study, which showed a decrease in all three concentrations (3-7-15) mg/kg from phthalate with significant differences represented by ($p > 0.01$).

Table 1: Comparison between difference treatments in Hormones level

Treatment	Means $\pm$ SE		
	FSH (mIU/ml)	LH (mIU/ml)	Testosterone(ng/ml)
Control	37.05 $\pm$ 2.25 a	4.75 $\pm$ 0.39 a	5.09 $\pm$ 0.46 a
Phthalate 3 (mg\kg)	31.14 $\pm$ 1.11 b	4.13 $\pm$ 0.23 ab	3.83 $\pm$ 0.39 b
Phthalate 7 (mg\kg)	19.12 $\pm$ 0.62 c	3.38 $\pm$ 0.26 b	2.86 $\pm$ 0.06 bc
Phthalate 15 (mg\kg)	17.79 $\pm$ 1.41 c	2.06 $\pm$ 0.08 c	1.85 $\pm$ 0.16 c
L.S.D.	4.810 **	0.8734 **	1.0227 **
P-value	0.0001	0.0006	0.0005
Means having with the different letters in same column differed significantly. ** ($P \leq 0.01$).			

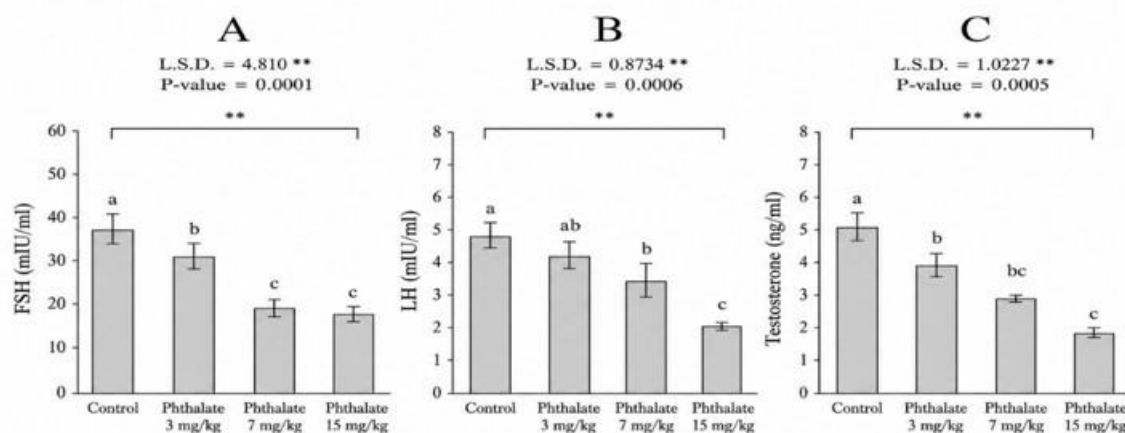


Fig 1. Effects of phthalate treatment on reproductive hormone markers. Bar charts show follicle-stimulating hormone (FSH, A), luteinizing hormone (LH, B), and testosterone (C) levels across treatment groups. Values are expressed as mean $\pm$ SE. Means with different letters within the same panel differed significantly. Significance is indicated by ** ($P \leq 0.01$).

Semen parameters

After exposure to phthalates at different concentrations as shown in Figure (2), the difference appears and the number of sperm, their motility and death decreases, with a significant difference at ($p < 0.01$). Phthalates significantly reduced sperm count at all three concentrations (27.33, 22.33, and 19.67) compared to the untreated control (31.67). Motility was lowest at the high concentration (15mg/kg), showing 66.67 compared to the control (92.33). Regarding abnormality, concentrations 7 mg/kg and 15mg/kg, showed higher percentage than the low concentration (3mg/kg). As a result, for sperm mortality, the untreated sample exhibited a low mortality rate of 7.76%, compared to 14.00% at the low concentration (3 mg/kg). The results indicate that abnormality is less sensitive to phthalates and more sensitive and affected by motility.

Table 2: Comparison between different treatments in Semen parameters

Treatment	Means $\pm$ SE			
	Total count (sperm $\times 10^6$)	Motility (%)	Abnormality (%)	Dead (%)
Control	31.67 $\pm$ 1.45 a	92.33 $\pm$ 1.45 a	6.33 $\pm$ 0.33 c	7.67 $\pm$ 0.33 c
Phthalate 3 (mg/kg)	27.33 $\pm$ 2.03 ab	86.00 $\pm$ 3.00 ab	10.67 $\pm$ 1.20 b	14.00 $\pm$ 2.31 bc
Phthalate 7 (mg/kg)	22.33 $\pm$ 1.45 bc	80.67 $\pm$ 1.20 b	13.67 $\pm$ 1.76 b	18.00 $\pm$ 2.08 ab
Phthalate 15 (mg/kg)	19.67 $\pm$ 1.45 c	66.67 $\pm$ 2.02 c	18.00 $\pm$ 1.15 a	23.67 $\pm$ 2.60 a
L.S.D.	5.269 **	6.656 **	3994 **	6.634 **
P-value	0.0034	0.0001	0.0009	0.0033
Means having with the different letters in same column differed significantly. **($P \leq 0.01$).				

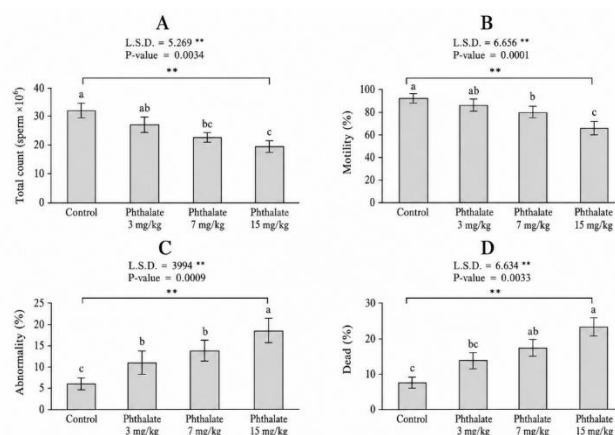


Fig 2: Effects of phthalate treatment on sperm parameters. Bar charts show total sperm count (A), sperm motility (B), sperm abnormality (C), and dead sperm percentage (D) across treatment groups. Values are expressed as mean $\pm$ SE. Means with different letters within the same panel differed significantly. Significance is indicated by ** ($P \leq 0.01$).

Histological of testes

Figures (A-B-C-D) of tissue sections taken from the testicles of mice stained with (H&E stain) reveal a clear contrast and marked gradation in histological changes, demonstrating a dose-dependent effect compared to healthy testicles. The histological figure of the testis in section (A) shows normal appearance of seminiferous tubules with normal germinal epithelium (g) and normal testicular interstitium (arrow as a result of dosing with the first concentration used after exposure to phthalates (3 mg/kg) for 45 days. In section (B), a part of the testicle exposed to phthalates at a concentration of (7 mg/kg) shows slight swelling of the epithelial tissue, while normal testicular tissue shows no effect. In the same exposure to phthalates (DBP) but treated with high dose (15 mg/ kg) as shown in (C), there is swelling in the testicular tissue (g) more than in the epithelial tissue, finally, section (D) in the figure show normal testicular, that lead to toxic effect of the (DBP) substance to the treated material, which led to negative results and affected the tissue and seminiferous tubules at both concentrations (7 and 15) mg/kg. This explains the change in hormones and the decrease in the percentage of each, which is linked to the effect on the tissue and testicle after exposure to phthalates, since the testicle is responsible for their production.

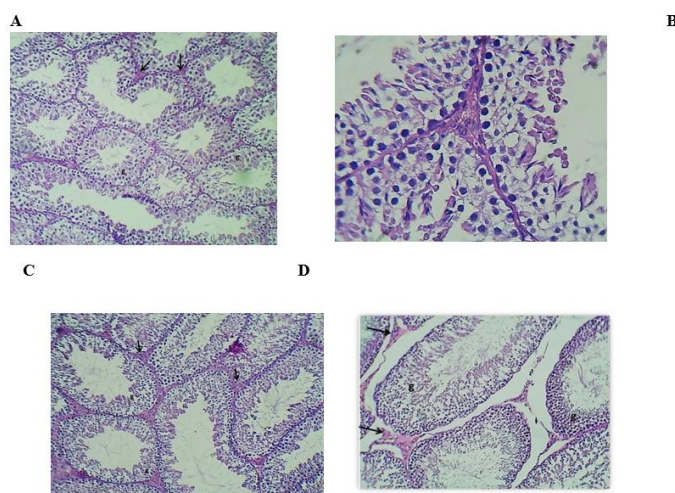


Figure 3: (A) section of testes\ treated with phthalate 3 mg\kg shows: normal appearance of seminiferous tubules with normal germinal epithelium (g) and normal testicular interstitium (arrow), (B) section of testes treated with phthalate 7 mg\kg shows: little cellular swelling of germinal epithelium and normal testicular interstitium, (c) shows normal appearance of seminiferous tubules with little cellular swelling of germinal epithelium (g) and normal testicular interstitium (black arrow). and (D)(Control) shows normal germinal epithelium (g), testicular interstitial (arrows). H&E stain.400x.

Comet assay

The potential toxicity of phthalates was determined by administering varying doses of the substance to mice for 45 days. The figures and table (3), fig (4) illustrate these effects on DNA damage and the resulting impact on white blood cells, as shown in the comate assay. Figures (A) shows a normal DNA with no breakage in the DNA strand, the highest percentage of cells without a comet was recorded at approximately 80.17%, representing the stability of genetic material under normal conditions when compared to phthalate exposure for the specified duration. The concentration of 3 mg/kg appears in the image (B). Some of the DNA breaks with contains a comet with a rate of 10.23% compared to the control. In figure (C) the percentage of DNA damage and defects has increased it reaches 4.61 %, this represents a comet that is half the number found compared to the normal image of no comet. Finally, the DNA damage stage reaches 3.18%, as shown in figure (D), where we observe the increase in the comet's length. At the highest concentration of 15 mg/kg, it is recorded at 20.37% compared with control. Phthalates (DBP), which attacked biomolecules and caused breaks in DNA strands, led to nucleotide deformation and a comet-like appearance during electrophoresis.

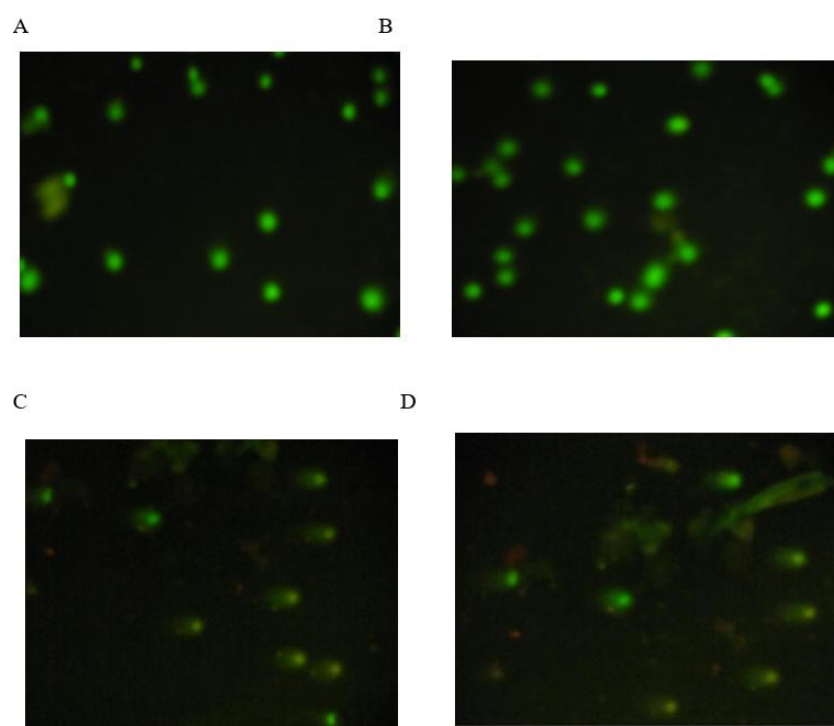


Figure 4: (A) Control group shows the normal appearance of white blood cells; no damage comet assay (B) Group treated with Phthalate 3 (mg\kg) for 45 days showing their DNA fragmentation (C) Group treated with Phthalate 7 (mg\kg) for 45 days showing more their DNA fragmentation (D) Group treated with Phthalate 15 (mg\kg) for 45 days showing their DNA fragmentation more than all.

Table (3): Effect of different concentration of phthalate on DNA damage (comet assay)

	No comet % (mean + SD)	Low comet % (mean+ SD)	Medium comet % (mean+ SD)	High comet % (mean+ SD)
Control	A 80.17±0.36	A 10.23 ±2.17	C 4.6 1±0.75	C 3.1 8±1.37
Phthalate 3 mg\kg	C	A	A	A

	58.44±2.53	34.08 ±3.20	5.22 ±0.98	4.33 ±1.07
Phthalate 7 mg\kg	C 49.50±1.40	B 40.18 ±1.42	A 7.53 ±0.63	A 4.21 ±0.50
Phthalate15 mg\kg	C 40.06±1.07	C 30.23 ±0.921	B 9.38 ±0.37	B 20.37 ±0.96

Discussion:

DBP is a known toxicant that has been linked with negative reproductive and developmental impacts on animal physiology (Anis *et al.*, 2024). Thus, this research seeks to examine the mechanism by which DBP causes male reproductive toxicity.

The levels of serum testosterone were significantly lower in DBP-treated group compared with the control group (Wang *et al.*, 2019). Moreover, DBP triggers Sertoli and Leydig cells, leading, spermatogenic failure consequently dependent sperm count decrease and also lethargy thus are lower than average levels of Sperm motility. It has confirmed a prior meta-analysis study in which we, applied a different approach, which is used to assess the effect of DBP exposure on sperm quality parameters in animals (Molele *et al.*, 2023 and Mohanty *et al.*, 2023).

For regarding the testosterone analysis results, testosterone, results were largely null (the testosterone/LH ratio showed a negative association), Suppressed testosterone concentrations and elevated LH with reduced T/LH ratios suggests that early-life phthalate exposure induces increases in luteinizing hormone (LH) drive to maintain normal testosterone levels, which may represent impaired function of the Leydig cells that presents only at higher testosterone outputs during adulthood, analysis showed a negative correlation between dibutyl phthalate (DBP) concentration and serum testosterone concentration ($p < 0.001$) in both juvenile and adult mice. Exposure to a 1 mg/kg dose of DBP may result in a 21.38% decrease in testosterone concentration, while the addition of 1 mg/kg DBP may result in a 0.03% decrease (Nielsen *et al.*, 2025). Conversely, other studies indicate decreased blood testosterone levels and increased levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). However, some studies suggest that endocrine disruption caused by phthalate exposure may stimulate elevated follicle-stimulating hormone levels (Poli *et al.*, 2024; Yesumanipreethi and Magadalenal, 2024). underscoring DBP's detrimental effects on Leydig cells. Therefore, it affected the hormone testosterone compared with control (Kamel *et al.*, 2025).

In addition, another study exposing mice to DBP phthalates showed severe pathological tissue damage in testicular sections compared to control groups. Marked changes included degeneration, necrosis, and shrinkage of spermatozoa, as well as their separation into sperm-free luminal spaces. Germline epithelium and Sertoli cells appeared indistinct, and the seminiferous tubules had irregular histological appearance. Furthermore, were affected on Leydig cells (responsible for hormone production), we agree with this study's finding of necrosis, degeneration, programmed cell death, and accumulation of necrotic debris within the seminiferous tubules of phthalate-exposed mice, accompanied by azoospermia. In addition, DBP exposure has been found to cause significant decreases in sperm count and vitality along with increases in the proportion of abnormal sperm. Also, in the same experiment, the level of DBP was inversely related to sperm count in young adult rats ($p < 0.001$). With 1 mg/kg DBP exposure, there is a 5.87% decrease in sperm count, whereas with every 1 mg/kg increase in DBP exposure, the sperm count decreases by 0.05% (Ibrahim, 2025; Bakeer *et al.*, 2025).

It was further noted that there were significant decreases in sperm number, sperm motility, and sperm viability in both the DBP-exposed and the combined treatment groups exposed to dibutyl phthalate (DBP) and polystyrene nanoplastic particles (PSNPs), while there were significant increases in sperm abnormalities in all exposed groups relative to the control group (Sharma *et al.*, 2023). Another study indicated potential effects on the male reproductive system, including a significant decrease in epididymal weight and sex hormone levels, while testicular tissue was damaged with impaired sperm count and a decrease in spermatogenesis. Testicular tissue damage and the formation of cavities were also observed following exposure to dibutyl phthalate. (Xu *et al.*, 2023). DBP exposure has been found to negatively influence sperm motility, which is one of the vital parameters of sperm function that is necessary for sperm movement towards the egg cell. Decreased sperm motility could thus hinder its fertilization capacity and is viewed as a major factor in male infertility (Dhar *et al.*, 2026). also conducted a second study, which used another type of phthalate this type of substance is the general term for a large class of chemicals, and the

specific categories used in different studies vary. Prior epidemiological studies that specifically examine the association between population health and disease have pointed out that if women are exposed to di-ethylhexyl phthalate (DEHP) exposure to this substance will be associated with three categories of health problems, namely abnormal sexual development, diseases in adult males, and reproductive system aging. The root cause of these associations is that phthalates themselves produce toxicity, and they exert this toxic effect by inhibiting the normal function of the gonads (Xie et al., 2024).

Phthalates have destroyed approximately 15–20% of male infertility and males having low sperm count approx. 36–40%. The researchers suggest that these chemical exposures may interrupt normal spermatogenesis, either through modifications in hormonal signaling or directly at the level of sperm development and function (Hlisníková et al., 2020). Phthalates are widely used plasticizers related to male reproductive toxicity, such as dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP). The effects of DBP and DEHP (100, 200, and 400 mg/kg for 15 days) on testicular shape and function in immature Swiss albino mice were evaluated in this study (Ali et al., 2026). This study is consistent with the results regarding the effect of phthalates (DBP) treated rats on measurement comet length and their effect on DNA fragmentation, particularly at high concentrations, through measured tail DNA percentage and tail length as decreased significantly, was greater in compared with the control group, but another study found exposure to phthalate caused testicular disruption (Li et al., 2021 and Radha et al., 2022).

CONCLUSION

Administering a substance (DBP) to male albino mice for 45 days, which was found in bottled water by increased contact time, resulted in a significant change in hormone levels. This included affecting sperm count, motility, and mortality. Histological examination revealed damage to testicular and seminiferous tissues, and finally, DNA comet showed a variation in DNA strand tail. Finally, based on the findings of the study, we recommend replacing plastic containers of water with biosafe alternatives to reduce the migration and impact of chemical phthalate on hormones, sperm, and even DNA levels for sustainable solutions that prioritize safety and human well-being.

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