Pacific Journal of Medical and Health Sciences
ISSN: 2456-7450 | Volume 2, Issue 1, January to March 2026, pp - 13-23

PJMHS
PACIFIC JOURNAL OF MEDICAL AND HEALTH SCIENCES

Research Paper

Subacute Exposure to Diethyl, Dibutyl and Di-(2-ethylhexyl) Phthalates Disrupts Systemic Bioenergetics, Lipid Homeostasis and Spermatogenesis in Male Rats

Fatima Omowunmi Hamzat[1,2], Ismail Adetayo Lawal[2], Aisha Abdulrauf[2]*, Abdulmuiz Oluwatobi Adebiyi[2], Aminat Temitayo Atoyebi[2], Kamaldeen Kabirat Opeyemi[2], Soliu Ibrahim Olashile[2], Sulaiman Muhammad Eze[2] and Babatunde Joseph Dare[1,2]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University, Ilorin, Nigeria.

[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Al-Hikmah University, Ilorin, Nigeria.

Received : 09 September 2025
Revised : 23 September 2025
Accepted : 27 September 2025

***Corresponding Author Email:** aabdulrauf@alhikmah.edu.ng

ABSTRACT

***Background**:*

Phthalate esters are pervasive endocrine-disrupting chemicals implicated in metabolic and reproductive dysfunction. However, integrated evidence linking systemic bioenergetics, lipid dysregulation, and spermatogenic impairment under equidose subacute exposure remains limited.

***Objective**:*

This study investigated the effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on systemic bioenergetics, lipid homeostasis, and testicular function in male rats.

***Methods**:*

Fifty male Sprague–Dawley rats were randomized into five groups (n=10) and administered DEP, DBP, or DEHP (50mg/kg/day) orally for 30 days. Serum bioenergetic markers (G6PDH, LDH-X), lipid profile (TC, TG, LDL, HDL), semen parameters, and testicular histology were assessed.

***Results**:*

Phthalate exposure induced a coordinated metabolic–reproductive disruption. G6PDH activity was significantly reduced, while LDH-X levels were elevated in DBP and DEHP groups, indicating impaired redox balance and cellular integrity. Atherogenic dyslipidaemia was evident, with increased TC, TG, and LDL alongside decreased HDL, most pronounced in

DEHP-treated rats. Semen analysis revealed significant declines in sperm concentration, motility, morphology, and viability across exposed groups, with severity ranking DEHP > DBP > DEP. Histologically, progressive degeneration of seminiferous tubules was observed, ranging from mild epithelial disruption (DEP) to severe architectural collapse and germ cell loss (DEHP), corroborated by PAS-demonstrated depletion of glycogen-rich germ cells.

***Conclusion**:*

Subacute phthalate exposure disrupts systemic metabolic homeostasis and induces a bioenergetic–oxidative bottleneck that propagates into testicular structural degeneration and impaired spermatogenesis. These findings establish a mechanistic continuum linking metabolic dysfunction to male reproductive toxicity and highlight the heightened potency of DEHP. This integrative model underscores the need to contextualize male infertility within environmental–metabolic risk frameworks.

KEYWORDS: Phthalates, Bioenergetics, Dyslipidaemia, Spermatogenesis, Testicular toxicity

BACKGROUND

Phthalate esters such as diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) are ubiquitous plasticizers and well-documented endocrine-disrupting chemicals with weak estrogenic and anti-androgenic actions and effects on thyroid regulation[1,2]. Human and animal data link phthalate exposure with metabolic disturbance, hepatotoxicity, and male reproductive dysfunction.

At the systemic level, DBP and DEHP alter glucose and lipid metabolism, inducing dyslipidaemia, adipocyte hypertrophy, and obesity-like phenotypes, partly via inflammatory signaling and disruption of thePI3K/Akt/GLUT4and Jak/STAT pathways in the liver and adipose tissues[3,4,5,6].

DEHP alters serum cholesterol and triglycerides and promotes hepatic lipid accumulation and inflammatory responses, even at relatively lower developmental doses[4,5,7]. Mixture studies show that combined phthalates (DBP + DEHP) and bisphenol-A at 50 mg/kg can significantly alter serum lipid profiles, glucose, and thyroid hormones in rats, emphasizing synergistic toxicity at subacute, submaximal doses[8].

Regarding male reproduction, DEHP and DBP have been documented to reduce testosterone synthesis, impair spermatogenesis and cause structural tissue changes. High-dose DEHP in adult rats decreases sperm quality, quantity, and daily production, increases oxidative stress and inflammatory cytokines, and disrupts key mitochondrial and lysosomal enzymes in testicular tissue, implicating impaired cellular energetics as a mechanism of infertility[9]. DEHP similarly altered testicular weight, distorted seminiferous tubule architecture, and lowered serum testosterone, with transcriptomic data implicating oxidative phosphorylation and steroid biosynthesis pathways[10,11]. Long-term low-dose mixtures of DEP, DBP, and DEHP, among other phthalates, decrease serum and intratesticular testosterone and induce testicular histopathology via downregulation of steroidogenic proteins and promotion of apoptotic activity[12]. Duration-dependent phthalate exposure in adult rats leads to significant reductions in sperm count, motility and viability, and increased abnormal morphology within weeks[13].

Key gaps remain in understanding integrated phthalate toxicity, as most studies assess single compounds, isolate metabolic or reproductive outcomes, and rely on limited oxidative markers. Mixture studies rarely use equitoxic subacute doses aligned with NOAELs (e.g., 50 mg/kg) or integrate bioenergetics, lipid profiles, semen parameters, and testicular histology within one model. Moreover, diethyl phthalate (DEP) is underexplored relative to DBP and DEHP. An equidose (50 mg/kg) DEP, DBP, and DEHP rat model therefore enables comprehensive evaluation of combined effects on energy metabolism, lipid homeostasis, reproductive function, and testicular pathology.

METHODOLOGY

Ethical Approval

Ethical approval for this study was obtained from the Ethical Review Committee of the Faculty of Basic Medical Sciences, Al-Hikmah University, Nigeria with ethical approval number HUI-FHS-ERC-26-0046. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals. The research was carried out in the laboratory of the Department of Anatomy, Al-Hikmah University, Nigeria.

Experimental Animals

Male Sprague–Dawley rats weighing between 150 and 250 g were obtained from the Osun State Laboratory Animal Centre. The animals were housed under controlled environmental conditions at a temperature of 22±2°C, relative humidity of 40–70%, anda12-hourlight/12-hour dark cycle. The rats were provided with standard laboratory feed and reverse-osmosis water *ad libitum* throughout the experimental period.

Study Design

All animals were acclimatized for two weeks prior to the commencement of the experiment. A total of fifty rats were randomly assigned into five groups, with ten animals in each group, comprising the normal control group, vehicle control group, and three treatment groups exposed to diethyl phthalate (DEP), dibutyl phthalate (DBP), and bis(2-ethylhexyl) phthalate (DEHP), respectively, at a dose of 50mg/kg body weight. Treatments were administered once daily via oral gavage for a period of 30 consecutive days. The vehicle control group received corn oil at a dose of 5 mL/kg body weight. Food was withdrawn 12 hours prior to sacrifice, while water was made available until the time of euthanasia. At the end of the experimental period, the animals were euthanized by cervical dislocation.

Preparation and Administration of Phthalates

Diethyl phthalate, dibutyl phthalate, and bis(2-ethylhexyl) phthalate were obtained from Molychem, India (Catalogue Number: 84-74-3), Central Drug House, India (Catalogue Number: 84-66-2), and Nice Chemicals, India (Catalogue Number: 84-2800212), respectively. Each compound was prepared by dissolving the appropriate weighed quantity in corn oil. Approximately 70–80%of the required final volume of corn oil was initially added to the compound in an amber glass beaker, followed by magnetic stirring at a controlled temperature range of 40–50°C to ensure complete dissolution without compromising chemical stability or inducing oxidative degradation of the oil vehicle[14], as described by Rowdhwal and Chen (2018). The final volume was subsequently adjusted with corn oil to achieve the desired concentration. The prepared solutions were administered daily between 7:00 a.m. and 10:00 a.m. using an orogastric cannula at a dose of 50 mg/kg body weight.

Sample Collection

Following euthanasia, blood samples were collected via cardiac puncture and centrifuged at 2000×g for 10 minutes at 4°C to obtain serum, which was stored at −20°C until further biochemical analysis. The testes were carefully excised from each animal; one testis was fixed in Bouin's solution for histological examination, while the other was snap-frozen in liquid nitrogen and stored at −70°C for subsequent biochemical assays.

Biochemical Assays

The serum concentrations of glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase-X (LDH-X), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined using radioimmunoassay kits obtained from Beijing North Institute of Biological Technology, Beijing, China, in accordance with the manufacturer's instructions.

Semen Analysis

The cauda epididymis was dissected and homogenized in phosphate-buffered saline (pH 7.4) for semen analysis. Sperm count was determined using an improved Neubauer counting chamber (LABART; Germany) under a light microscope at ×40 magnification, with multiple fields examined to obtain an accurate count[15] in accordance with the method described by Omirinde et al. (2019). Sperm motility was assessed by placing a drop of sperm suspension on a pre-warmed slide, adding two drops of warm 2.9% sodium citrate, covering with a coverslip, and observing under a light microscope at ×40 magnification under low illumination[16], as described by Khatun et al. (2018). For sperm morphology assessment, a thin smear of the sperm sample was prepared on a clean slide, fixed in 95% ethanol, air-dried, and subsequently stained using Harris haematoxylin, G-6 orange, and EA-50 green stains. The stained slides were examined under a light microscope at ×40 magnification, and a total of 200 spermatozoa per sample were evaluated, with abnormalities expressed as percentages[15,17]. Sperm viability was determined using Eosin/ Nigrosin staining, and the percentage of live and dead sperm cells was assessed under a light microscope.

Histological Processing

Testicular tissues fixed in Bouin's solution were dehydrated through graded ethanol series, embedded in paraffin wax, and sectioned at a thickness of 5µm.The tissue sections were mounted on glass slides and stained using haematoxylin and eosin (H&E) as well as Periodic Acid–Schiff (PAS) stains. Histological examination of the testicular architecture was carried out using light microscopy.

Statistical Analysis

Quantitative data were analyzed using Chris Rorden'sez ANOVA (version 0.985) software, while Microsoft Excel was used for graphical representation of results. Data were expressed as mean ± standard error of the mean (SEM).Statistical differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Bioenergetics

Serum glucose-6-phosphate dehydrogenase (G6PDH) activity demonstrated a consistent reduction across all experimental groups relative to the control. This decrease was statistically significant in the vehicle ($p = 0.002$), DBP ($p = 0.008$), and DEHP ($p = 0.0001$) groups when compared with the control group. Although a reduction was also observed in the DEP group, this did not reach statistical significance. The magnitude of decline was most pronounced in the DEHP group, indicating a stronger effect relative to the other treatment groups.

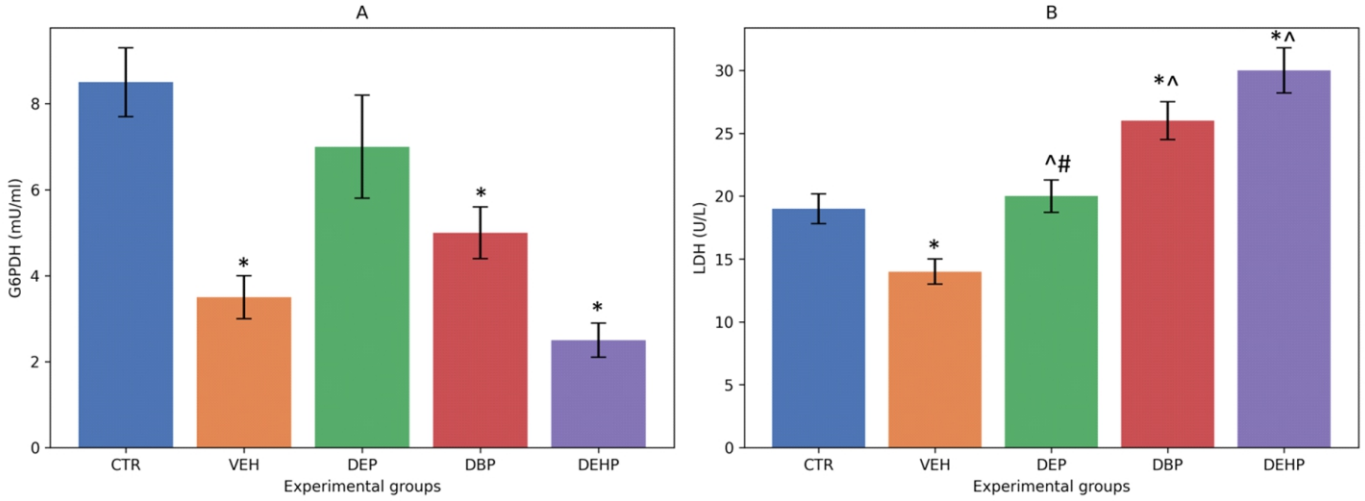

Figure 1 (A–B): Effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on serum glucose-6-phosphate dehydrogenase (G6PDH) activity (A) and serum lactate dehydrogenase-X (LDH-X) levels (B) in male rats following subacute exposure. Data are presented as mean±SEM (n=10 per group). Statistical significance is denoted as follows: *p<0.05 vs control (CTR); ^p < 0.05 vs vehicle (VEH); #p < 0.05 vs DEHP. CTR = control; VEH = vehicle (corn oil, 5 mL/kg); DEP = diethyl phthalate; DBP = dibutyl phthalate; DEHP = di-(2-ethylhexyl) phthalate

In contrast, serum lactate dehydrogenase-X (LDH-X) levels exhibited a differential pattern of change across groups. A significant reduction was observed in the vehicle group ($p = 0.005$) compared with the control. However, LDH-X levels were significantly elevated in the DBP ($p = 0.04$) and DEHP ($p = 0.0004$) groups relative to the control group. When compared with the vehicle group, all phthalate-treated groups showed significant increases in LDH-X levels (DEP: $p = 0.04$; DBP: $p = 0.04$; DEHP: $p = 0.0001$), confirming that the observed elevations are attributable to phthalate exposure rather than the vehicle effect. Additionally, LDH-Xlevelsinthe DEP group were significantly lower than those in the DEHP group ($p = 0.01$), indicating a comparatively attenuated effect. This shows a divergence in bioenergetic indices following phthalate exposure, characterized by a generalized reduction in G6PDH activity alongside selective elevations in LDH-X, particularly in DBP and DEHP groups. The magnitude and direction of these changes indicate compound-specific differences in bioenergetic disruption, with DEHP exerting the most pronounced effect, followed by DBP, while DEP shows relatively attenuated alterations.

Lipid Profile

Figures 2A–2D illustrate the effects of DEP, DBP, and DEHP on serum lipid profile parameters in experimental rats. Total cholesterol (TC) levels (Figure 2A) showed a progressive increase across all experimental groups relative to the control, with statistically significant elevations observed in the DBP ($p = 0.01$) and DEHP ($p = 0.0005$) groups. Furthermore, the DEHP group demonstrated a significant increase compared to the vehicle group (^p = 0.007), indicating a pronounced treatment-related effect.

Similarly, triglyceride (TG) levels (Figure 2B) increased across all experimental groups, with a statistically significant elevation observed in the DEHP group ($p = 0.02$) relative to the control. The DEHP group also showed a significant increase when compared with the vehicle group (^p = 0.0003), further supporting the magnitude of its effect.

Low-density lipoprotein (LDL) levels (Figure2C) followed a comparable upward trend across all treatment groups relative to the control. This increase reached statistical significance in the DBP ($p=0.002$) and DEHP ($p=0.0001$) groups. However, when compared with the vehicle group, only the DEHP group showed a statistically significant elevation(^p=0.004), indicating a stronger effect relative to the other phthalates.

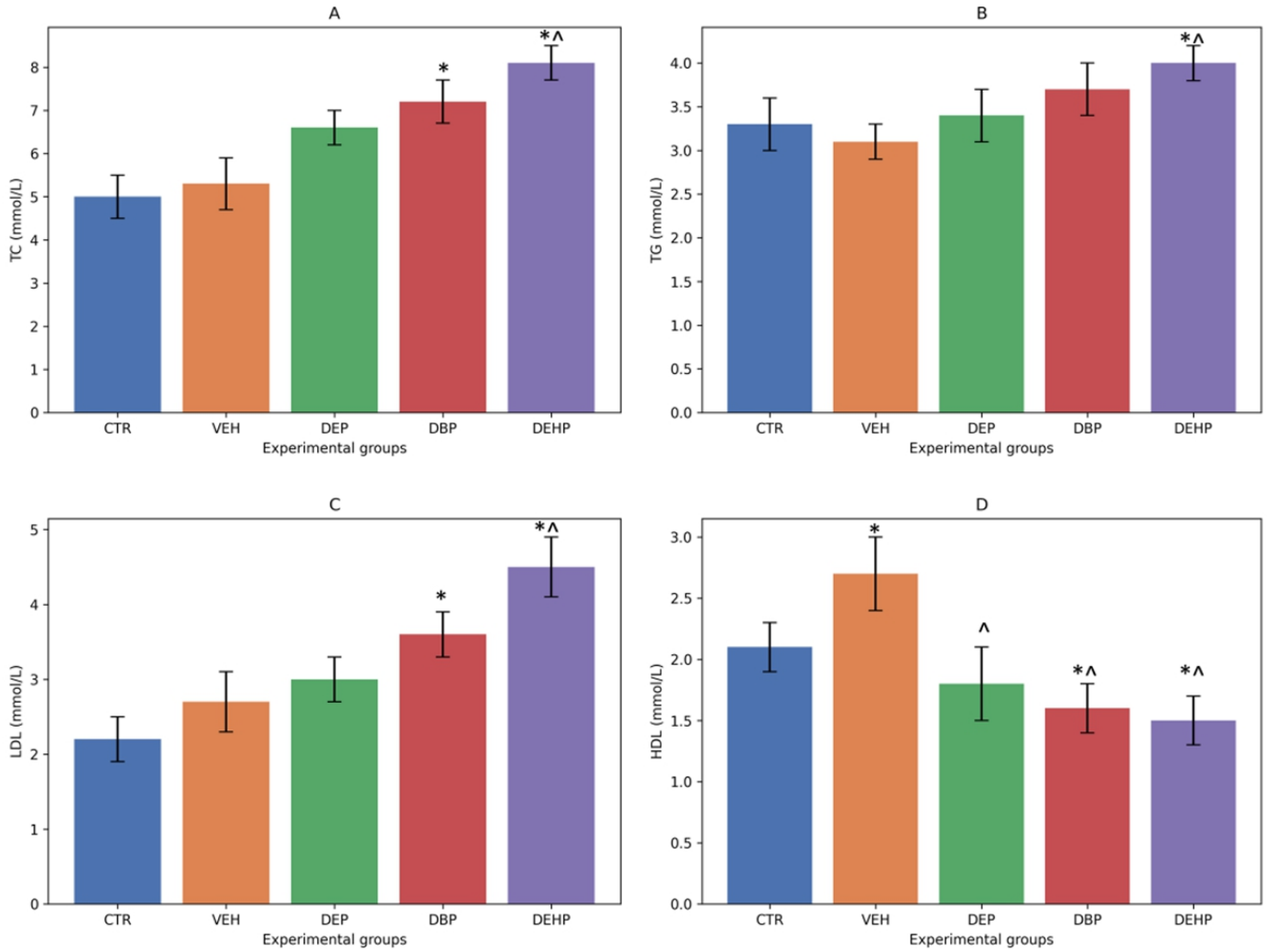

Figure 2 (A–D): Effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on serum total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein (LDL) (C), and high-density lipoprotein (HDL) (D) levels in male rats following subacute exposure. Data are presented as mean±SEM (n=10pergroup). Statistical significance is denoted as follows: *p<0.05vs control (CTR); ^p<0.05vs vehicle (VEH); #p<0.05vs DEHP. CTR = control; VEH = vehicle (corn oil, 5 mL/kg); DEP = diethyl phthalate; DBP = dibutyl phthalate; DEHP = di-(2-ethylhexyl) phthalate

In contrast, high-density lipoprotein (HDL) levels (Figure 2D) exhibited a divergent pattern. A significant increase was observed in the vehicle group ($p = 0.03$) relative to the control. However, HDL levels were significantly reduced in the DBP ($p = 0.049$) and DEHP ($p = 0.02$) groups compared to the control. Additionally, all phthalate-treated groups showed significant reductions in HDL levels when compared with the vehicle group (DEP: ^p=0.04; DBP: ^p=0.008; DEHP: ^p = 0.005), indicating a consistent downward effect of phthalate exposure relative to vehicle conditions. There is a consistent pattern of lipid profile alteration characterized by elevations in TC, TG, and LDL, alongside reductions in HDL, with the most pronounced effects observed in the DEHP group, followed by DBP, while DEP exhibits comparatively moderate changes.

Semen Analysis

Sperm concentration showed a marked reduction across all phthalate-treated groups relative to both control and vehicle groups. This decrease was statistically significant in the DEP (*p=0.004; ^p=0.0004*), DBP (*p=0.002; ^p=0.0003*), and DEHP (*p=0.002; ^p=0.0003*) groups, indicating a consistent treatment-related decline.

Table 1: Effects of diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) on semen quality and quantity in male rats

Groups	Sperm Concentration	Sperm Motility	Sperm Morphology	Live/Death Ratio (%)
	(*10^6/mL)	(%)	(*10^2/%)	
CTR	347±28.76	75.4±2.04	0.92±0.03	77.6±2.29
VEH	383.2±24.39	64.8±4.28	0.96±0.01	73±4.82
DEP	220.8±13.05*^	66±2.45*	0.77±0.1	70.4±2.48
DBP	198.8±17.14*^	59.2±3.37*	0.73±0.03*^	56.2±4.67*^
DEHP	172.6±25.75*^	49.8±1.77*^	0.61±0.01*^	53.8±2.67*^

Data are presented as mean ± SEM (n = 10 per group). Statistical significance is denoted as follows: *p < 0.05 vs control (CTR); ^p<0.05vsvehicle (VEH). CTR=control; VEH=vehicle; DEP=diethyl phthalate; DBP=dibutyl phthalate; DEHP = di-(2-ethylhexyl) phthalate

A similar trend was observed in sperm motility. Percentage sperm motility was significantly reduced in the DEP (*p=0.02*), DBP (*p=0.0003*), and DEHP (*p=0.0001*) groups compared with the control group. However, when compared with the vehicle group, only the DEHP group showed a statistically significant reduction (^p=0.012), suggesting a more pronounced effect at this level.

Sperm morphology also showed treatment-dependent alterations. Significant reductions were observed in the DBP (*p=0.004; ^p=0.0004*) and DEHP (*p=0.001; ^p=0.0001*) groups relative to both control and vehicle groups. Although the DEP group exhibited a reduction in sperm morphology, this change did not reach statistical significance (*p = 0.19; ^p = 0.09*).

Similarly, the sperm live/death ratio was significantly reduced in the DBP (*p=0.003; ^p=0.04*) and DEHP (*p = 0.0001; ^p = 0.008*) groups compared with both control and vehicle groups. In contrast, the DEP group showed a reduction that was not statistically significant (*p = 0.07; ^p = 0.64*). The semen analysis results show consistent decline in semen quality parameters across phthalate-treated groups, with the most pronounced impairments observed in the DEHP group, followed by DBP, while DEP exhibited comparatively milder effects.

Histological Observation

Histological assessment reveals an exposure-dependent gradient of testicular structural alteration. Control testes show preserved cytoarchitecture, with well-organized seminiferous tubules, intact germinal epithelium, and normal interstitial architecture. The vehicle group exhibits only mild irregularities in tubule contour and epithelial organization, with overall structural integrity maintained. In contrast, the DEP group demonstrates mild-to-moderate alterations, including reduced germinal epithelial thickness, focal disruption of spermatogenic layers, decreased cellular density, and slight luminal enlargement, indicating early structural compromise.

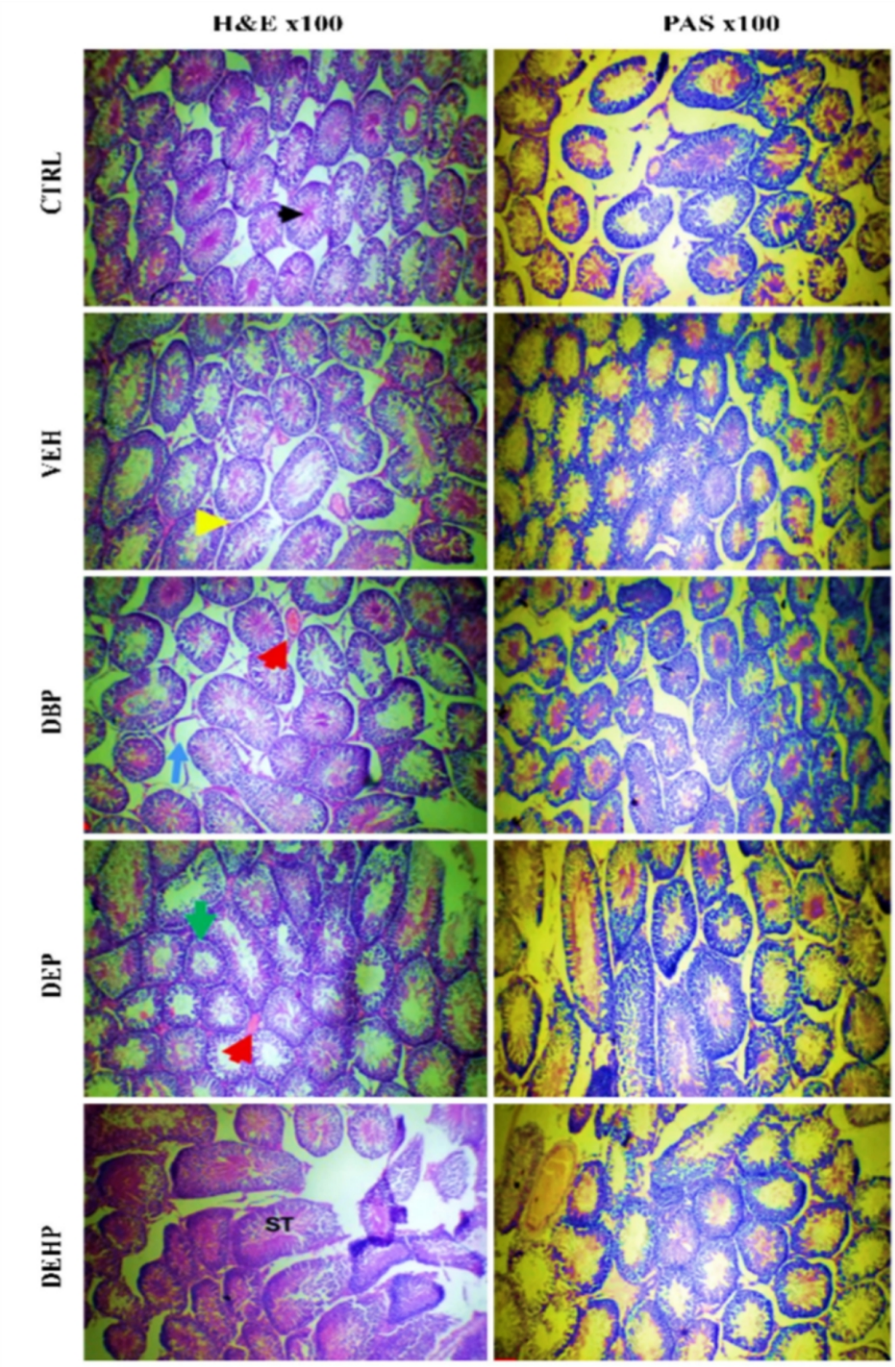

Figure 3: Histopathological alterations in testicular tissue following subacute exposure to phthalates. Representative photomicrographs of rat testicular sections stained with haematoxylin and eosin (H&E; left panels) and Periodic Acid–Schiff (PAS; right panels) at×100magnification across experimental groups: control (CTRL), vehicle (VEH), diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-(2-ethylhexyl) phthalate (DEHP). The CTRL group shows normal seminiferous tubule architecture with intact germinal epithelium, orderly spermatogenic cell layers, and well-defined lumina. The VEH group exhibits mild structural irregularities without overt disruption of testicular organization. DBP exposure is associated with moderate histoarchitectural distortion, including irregular seminiferous tubules, germinal epithelial disorganization (red arrow), and luminal widening (blue arrow). DEP-treated sections demonstrate mild-to-moderate alterations characterized by focal epithelial disruption (red arrow) and reduced cellular density (green arrow). DEHP exposure results in severe degeneration of testicular architecture, including marked seminiferous tubule distortion, epithelial sloughing, and structural collapse with reduced spermatogenic cell populations. PAS staining reveals progressive depletion and disorganization of glycogen-rich germ cells across treatment groups, most pronounced in DEHP

The DBP group shows more pronounced histopathological alterations than DEP, with irregular and distorted seminiferous tubules, disorganized germinal epithelium, reduced epithelial height, and increased luminal diameter, indicating substantial cellular loss and structural disruption.

The DEHP group exhibits the most severe damage, characterized by extensive tubular distortion, marked epithelial attenuation, widespread loss of spermatogenic cells, epithelial sloughing, and poorly defined tubular architecture with markedly dilated lumina, reflecting profound tissue compromise.

PAS staining corroborates these changes, showing strong uniform staining in controls but progressively reduced and heterogeneous patterns in treated groups, most notably in DEHP, indicating depletion of seminiferous epithelial integrity. Overall, a clear severity gradient is observed: DEHP > DBP > DEP > VEH ≈ CTRL, with increasing disruption of tubular structure, epithelial organization, and cellular density.

DISCUSSION

The present study demonstrates that subacute exposure to phthalates induces a coordinated disruption of metabolic homeostasis, cellular redox balance, and testicular structural integrity, collectively converging on impaired spermatogenesis. Importantly, these alterations do not occur in isolation but rather reflect a tightly interlinked pathological continuum in which systemic metabolic derangement propagates cellular dysfunction within the testicular micro environment[18,19].

The observed dyslipidemia characterized by elevated total cholesterol, triglycerides, and LDL alongside reduced HDL represents a fundamental perturbation in lipid handling that extends beyond systemic circulation into testicular physiology. Although cholesterol is the obligatory substrate for steroidogenesis, its accumulation in circulation in parallel with impaired reproductive indices indicates a failure of effective intracellular trafficking and utilization[20]. This suggests that phthalate exposure disrupts lipid partitioning and mobilization, thereby uncoupling substrate availability from functional steroidogenic output. Such dysregulation is consistent with PPAR-mediated metabolic reprogramming, which alters lipid storage, transport, and utilization dynamics, ultimately compromising steroidogenic efficiency[21,22]. Within this framework, the testis becomes functionally constrained despite apparent substrate abundance, reflecting a state of metabolic inefficiency rather than deficiency[23].

Concurrently, the consistent reduction in G6PDH activity across exposed groups indicates a systemic suppression of the pentose phosphate pathway, with direct implications for intracellular NADPH availability[24]. Given the central role of NADPH in maintaining redox homeostasis and supporting biosynthetic processes, its depletion establishes a pro-oxidant intracellular environment. Germ cells, by virtue of their high proliferative and differentiation demands, are particularly susceptible to such redox imbalance[25,26]. The resulting oxidative vulnerability compromises cellular integrity, predisposing spermatogenic cells to damage and loss. This redox imbalance is further compounded by the observed elevation in LDH-X in DBP and DEHP groups, which serves not as a marker of enhanced glycolytic flux but rather as an indicator of cellular membrane compromise and leakage from the seminiferous epithelium[27]. Together, these bioenergetic alterations define a state of metabolic–oxidative bottleneck, wherein energy production, antioxidant defense, and cellular stability are simultaneously impaired[28,29].

Within the testicular milieu, these systemic and cellular disturbances manifest structurally as progressive degeneration of seminiferous tubule architecture. The reduction in germinal epithelial height, luminal dilatation, and eventual tubular distortion reflect a continuum of germ cell attrition and epithelial destabilization[30,31]. The PAS-demonstrated depletion of glycogen-rich germ cells further reinforces the presence of impaired intracellular energy storage and utilization, indicating that the seminiferous epithelium is not only structurally compromised but also metabolically depleted[32]. The integrity of the seminiferous epithelium is critically dependent on coordinated interactions between germ cells and supporting Sertoli cells; thus, disruption of cellular energy balance and redox status inevitably translates into architectural disorganization and loss of functional capacity[8,33].

These structural changes are directly mirrored by the marked decline in semen quality parameters, including sperm concentration, motility, morphology, and viability. Rather than representing independent endpoints, these functional impairments are the downstream expression of cumulative metabolic, oxidative, and structural insults[34,35]. The emergence of a mixed oligo-astheno-teratozoospermic profile reflects disruption across multiple stages of spermatogenesis, from germ cell proliferation to maturation and structural integrity[36,37]. Notably, the observation that DEP induces measurable functional impairment despite relatively modest histological disruption highlights the sensitivity of spermatogenic processes to early metabolic perturbations, preceding overt structural collapse[38,39].

The graded severity of effects observed across phthalates (DEHP>DBP>DEP) further supports a dose-potency relationship in which the extent of metabolic disruption dictates the magnitude of downstream reproductive impairment. DEHP, in particular, appears to exert a more profound capacity to disrupt metabolic–bioenergetic coupling, thereby amplifying structural and functional degeneration within the testis[40,41,42].

These findings support a unifying model in which phthalate exposure initiates systemic metabolic dysregulation that

propagates through impaired lipid handling and redox imbalance, culminating in bioenergetic insufficiency within the testicular microenvironment. This, in turn, destabilizes seminiferous epithelial architecture and compromises spermatogenic output. In the Nigerian context, where background metabolic risk factors such as dyslipidemia are increasingly prevalent, such environmental exposures may exert additive or synergistic effects, thereby amplifying the burden of male reproductive dysfunction. These results therefore underscore the necessity of conceptualizing male infertility within a broader metabolic and environmental health framework, rather than as an isolated reproductive disorder.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

REFERENCES

1. Baralić K, Jorgovanović D, Živančević K, Buha Djordjevic A, Antonijević Miljaković E, Miljkovic M, et al. Combining in vivo pathohistological and redox status analysis within silico toxicogenomic study to explore the phthalates and bisphenol A mixture-induced testicular toxicity. Chemosphere. 2020; 261:129296. doi: 10.1016/j.chemosphere.2020.129296.
2. Toumi D, Zoukar O, Afsa S, Gharbi N, Farhat I, Di Bella G, et al. Adverse effects on the endocrine and reproductive system of single and combined Ortho phthalates in female rats following short-term exposure. Environ Sci Pollut Res Int. 2025; 32:22651-22664. doi:10.1007/s11356-025-36970-8.
3. Jia Y, Liu T, Zhou L, Zhu J, Wu J, Sun D, et al. Effects of di-(2-ethylhexyl) phthalate on lipid metabolism by the JAK/STAT pathway in rats. Int J Environ Res Public Health.2016; 13:1085. doi:10.3390/ijerph13111085.
4. Zhou L, ChenH, Xu Q, Han X, Zhao Y, Song X, et al. The effect of di-2-ethylhexylphthalate on inflammation and lipid metabolic disorder in rats. Ecotoxicol Environ Saf. 2019; 170:391-398. doi: 10.1016/j.ecoenv.2018.12.009.
5. Zhang Y, Ge S, Yang Z, Li Z, Gong X, Zhang Q, et al. Disturbance of di-(2-ethylhexyl) phthalate in hepatic lipid metabolism in rats fed with a high-fat diet. Food Chem Toxicol. 2020;111848. doi: 10.1016/j.fct.2020.111848.
6. Cao S, Shen Y, Yang X, Zhang G, Zhang Y, Wang Y, et al. Environmental di-(2-ethylhexyl) phthalate exposure accelerates lipid metabolism disorders via the gut-fat axis in male SAMP8 mice: role of gut microbiota and thyroid hormone signaling. Environ Int. 2025; 204:109808. doi: 10.1016/j.envint.2025.109808.
7. SuH, Lai C, Lee K, Chiang Y, Chen C, Hsu P. Prenatal exposure to low-dose di-(2-ethylhexyl) phthalate (DEHP) induces potentially hepatic lipid accumulation and fibrotic changes in rat offspring. Ecotoxicol Environ Saf. 2023; 269:115776. doi: 10.1016/j.ecoenv.2023.115776.
8. Baralić K, Djordjevic A, Živančević K, Antonijević E, Anđelković M, Javorac D, et al. Toxic effects of the mixture of phthalates and bisphenol A subacute oral toxicity study in Wistar rats. Int J Environ Res Public Health. 2020; 17:746. doi:10.3390/ijerph17030746.
9. Poli V, Motireddy S. Toxicity study of di (2-ethylhexyl) phthalate (DEHP) administration on reproductive parameters in male albino rats. Comp Clin Pathol. 2023; 33:75-84. doi:10.1007/s00580-023-03524-8.
10. HanL,WangJ,ZhaoT,WuY,WeiY,ChenJ,etal.Stereological analysis and transcriptome profiling of testicular injury induced by di-(2-ethylhexyl) phthalate in prepubertal rats. Ecotoxicol Environ Saf. 2021; 220:112326. doi: 10.1016/j.ecoenv.2021.112326.
11. Liu X, Li Y, Zi T, Zhang Y, Wang Z, Peng Z, et al. Di-2-ethylhexyl phthalate exposure induces sperm quality and functional defects in mice. Chemosphere. 2022;137216. doi: 10.1016/j.chemosphere.2022.137216.
12. Gao H, Xu R, Cao W, Qian L, Wang M, LuL, et al. Effects of six priority-controlled phthalate esters with long-term low-dose integrated exposure on male reproductive toxicity in rats. Food Chem Toxicol. 2017; 101:94-104. doi: 10.1016/j.fct.2017.01.011.
13. Emojevwe V, Naiho A, Nwangwa E, Oyovwi M, Anachuna K, Agbanifo-Chijiokwu E, et al. Duration-dependent effects of high doses of phthalate exposure on semen quality in adult male rats. JBRA Assist Reprod. 2021; 26:53-61. doi:10.5935/1518-0557.20210033.
14. Rowdhwal SSS, ChenJ.Toxiceffectsofdi-2-ethylhexyl phthalate: an overview. Biomed Res Int. 2018; 2018:1750368. doi:10.1155/2018/1750368.
15. Omirinde JO, Olukole SG, Oke BO. Age-related changes in testicular and epididymal sperm parameters in the African greater cane rat. Anim Res Int. 2019; 16:3255-3264.

16. Khatun A, Rahman MS, Pang MG. Clinical assessment of male fertility. ObstetGynecol Sci. 2018; 61:179-191. doi:10.5468/ogs.2018.61.2.179.

17. Yu S, Rubin M, Geevarughese S, Pino JS, Rodriguez HF, Asghar W. Emerging technologies for home-based semen analysis. Andrology. 2018; 6:10-19. doi:10.1111/andr.12441.

18. Mondal S, Bandyopadhyay A. Antioxidants in mitigating phthalate-induced male reproductive toxicity: a comprehensive review. Chemosphere. 2024; 364:143297. doi: 10.1016/j.chemosphere.2024.143297.

19. Moghazy M, Papathanasiou M, Tzoupis H, Papavasileiou KD, Xing C, Lauschke V, et al. A systematic literature review of reproductive toxicological studies on phthalates. IntJMolSci. 2025; 26:8761. doi:10.3390/ijms26188761.

20. Zubi ZBH, Alfarisi HAH. Hyperlipidemia and male infertility. Egypt J Basic Appl Sci. 2021; 8:370-378. doi:10.1080/2314808X.2021.1977080.

21. Milinković M, Borlakova M, Krivokapic M, Bolevich S, Jakovljevic V. Male reproductive toxicity of di (2-ethylhexyl) phthalate: data from a rat model. Toxicol Environ Chem. 2024; 106:287-310. doi:10.1080/02772248.2024.2364361.

22. Deng W, Zhao J, Wang X, Li D, Wang M, Zheng X, et al. Role of ferroptosis mediated by abnormal membrane structure in DEHP-induced reproductive injury. Free Radic Biol Med. 2025; 228:04026. doi: 10.1016/j.freeradbiomed.2025.04.026.

23. Källsten L, Almamoun R, Pierozan P, Nylander E, Sdougkou K, Martin JW, et al. Adult exposure to di-n-butyl phthalate induces persistent effects on testicular cell markers and testosterone biosynthesis in mice. Int J Mol Sci. 2022; 23:8718. doi:10.3390/ijms23158718.

24. Chigrinski E, Gerunov T, Merzlikin N, Gerunova LK, Hua J. The role of the pentose phosphate pathway in metabolic disorders in testes during cypermethrin intoxication. Reprod Toxicol. 2025; 133:109084. doi: 10.1016/j.reprotox.2025.109084.

25. Hong Y, Zhou Y, Shen L, Wei Y, Long C, Fu Y, et al. Exposure to DEHP induces testis toxicity via ROS/mTOR/NLRP3 signaling pathway in immature rats. Ecotoxicol Environ Saf. 2021; 225:112889. doi: 10.1016/j.ecoenv.2021.112889.

26. Zhao T, Wang J, Shen L, Long C, Liu B, Wei Y, et al. Increased m6A RNA modification relates to inhibition of Nrf2-mediated antioxidant response in DEHP-induced testicular injury. Environ Pollut. 2020; 259:113911. doi: 10.1016/j.envpol.2020.113911.

27. Mondal S, Bandyopadhyay A. From oxidative imbalance to compromised sperm parameters: toxicological aspects of phthalate esters. Environ Toxicol Pharmacol. 2023; 97:104085. doi: 10.1016/j.etap.2023.104085.

28. Zhang L, Gao X, Qin Z, Shi X, Xu K, Wang S, et al. USP15 in DBP-induced testicular oxidative stress via Keap1/Nrf2 pathway. Sci Total Environ. 2021; 783:146898. doi: 10.1016/j.scitotenv.2021.146898.

29. Helmy H, Senousy MA, El-Sahar AE, Sayed RH, Saad M, Elbaz EM. Aberrations of miR-126-3p, miR-181a and Sirtuin1 network mediate DEHP-induced testicular damage. Toxicology. 2020; 443:152406. doi: 10.1016/j.tox.2020.152406.

30. Başımoğlu Koca Y. Effects of Di isobutyl phthalate on rat testicular tissue: histopathological evaluation. Academia J Biol. 2024;46(4). doi:10.15625/2615-9023/21119.

31. Yang L, Yang B, Lu D, Peng Z, Ren Z, Fang K, et al. Dynamic assessment of toxicity and pathology of DEHP in germ cells of rats. Reprod Biol. 2020; 20:580-588. doi: 10.1016/j.repbio.2020.07.005.

32. Gouri K, Sinha P. Impact of DEHP toxicity on testis histology in rats. Toxicol Int. 2023; 29:538-546. doi:10.18311/ti/2022/v29i4/30294.

33. Wang S, Li D, Deng W, Wu S, Wei Y, Wei G. Role of p62-Keap1-Nrf2 pathway in protecting Sertoli cells against phthalate-induced ferroptosis. J Appl Toxicol. 2025; 45:4963. doi:10.1002/jat.4963.

34. Wang H, He H, Wei Y, Gao X, Zhang T, Zhai J. Do phthalates cause poor semen quality? A systematic review and meta-analysis. Environ Sci Pollut Res. 2022; 30:12553-12567. doi:10.1007/s11356-022-24215-x.

35. Caporossi L, Viganò P, Paci E, Capanna S, Alteri A, Pigini D, et al. Effects of plasticizers exposure on male fertility: a case-control study. Int J Environ Res Public Health.2022;20:235. doi:10.3390/ijerph20010235.

36. AmjadS, Rahman MS, Pang W, Ryu D, Adegoke EO, Park YJ, et al. Effects of phthalates on mouse sperm function and fertility. Toxicology. 2021; 465:152746. doi: 10.1016/j.tox.2021.152746.

37. Zhou L, Shan T, Cai W. Correlation between urinary phthalate concentration and semen quality parameters. J Chin Physician. 2020; 22:518-521. doi:10.3760/cma.j.cn431274-20190201-00112.

38. Patel SS, Trangadia BJ, Patel U, Delvadiya RS, Makwana AA, Raval SH, et al. Toxic effects of dibutyl phthalate on testes of zebrafish. Environ Sci Pollut Res. 2024; 31:45678-45692. doi:10.1007/s11356-024-34868-5.

39. Reyes-Cruz E, Rojas-Castañeda J, Landero-Huerta D, Hernández-Jardón N, Reynoso-Robles R, Juárez-Mosqueda ML, et al. Disruption of gonocyte development following neonatal DEHP exposure. Reprod. Biol. 2024; 24:100877. doi: 10.1016/j.repbio.2024.100877.

40. Zhang Z, Han H, Ding L, Yang H, Deng Y, Shang Y, et al. DEHP induces male reproductive toxicity via mitophagy-dependent ferroptosis. Free Radic Biol Med. 2026; 219:01014. doi: 10.1016/j.freeradbiomed.2026.01.014.

41. Lin Y, Xu W, Yang L, Chen Z, Zhai J, Zhu Q, et al. Mechanism of testicular injury induced by DEHP and protective agents. Chem Biol Interact. 2023; 380:110575. doi: 10.1016/j.cbi.2023.110575.

42. Willson C. Phthalate toxicity in rats and its relation to testicular dysgenesis syndrome in humans. Toxicol Pathol. 2021; 49:1430-1440. doi:10.1177/01926233211045331.