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SHORT COMMUNICATION

Effects of Di (2-ethylhexyl) phthalate on sperm morphology of Wistar rats

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ABSTRACT

Phthalate extensively used as plasticizers in medical devices, cosmetics, children's toys, deodorants and other industries, which is a major environmental toxicant. As they are not covalently bound to polymers and leach easily into the environment and cause a significant health risk. Hence, the present study was carried out to evaluate the effects of Di (2-ethyl hexyl) phthalate on sperm morphology in Wistar rats. Animals were exposed to DEHP at 100, 200 and 400 mg/kg b.wt in corn oil for a period of 28 days. Animals were sacrificed on 29th day and semen was collected from the cauda epididymis and stained with eosin (5%) and nigrosine (10%) staining. Results of this study revealed more sperm abnormalities in all DEHP-exposed groups with more pronounced abnormalities in the high dose group. A highly significant (P<0.01) reduction in the percentage of live sperms and an increase in dead sperms. These results indicated that DEHP has caused many sperm abnormalities and confirm its role as a potent reproductive toxicant.

Keywords: Eosin, nigrosine, phthalate, sperm abnormalities

INTRODUCTION

DEHP is frequently used as a plasticizer in a variety of products like food packaging material, beverage bottles, children's toys, nutraceutical products, cosmetics, deodorants, nail polish, shampoos and skin cleansers. Phthalates, particularly DEHP, leaches readily from products during heating, cleaning and repeated use due to their non-covalent bonding with polymers. DEHP affects endocrine, reproductive, and cardiovascular systems as well as neural development, and causes multiple organs toxicity in humans[1]. DEHP is a potent toxic compound that mainly affects male reproductive system leading to infertility and Testicular Dysgenesis Syndrome (TDS) in humans including insufficient testosterone production during in utero development, undescended testes, malformations of the penis, reduced Anogenital Distance (AGD), decreased sperm motility, poor semen quality and abnormal sperm morphology[2], infertility and testicular cancer[3]. DEHP has negative impacts on fertility by affecting spermatogenesis, sperm functionality, male reproductive hormones and also affects both qualitative and functional characteristics of semen[4].

MATERIALS AND METHODS

An experimental trial was conducted using forty male Wistar rats, which were randomly allocated into four groups as Group I, II, III and IV. Group I acted as control and received corn oil; Group II, III and IV received 100, 200 and 400 mg/kg b.wt. of DEHP respectively. DEHP was administered once daily by oral gavage for a period of 28 days. The animals were sacrificed on 29th day of experimental trial to evaluate the reproductive toxicity induced by DEHP.

The plasticizer Di (2-ethylhexyl) phthalate (50 g) and corn oil were purchased from M/s. Eswarr Scientific & Co, Trichy and stored at room temperature between 20 and 25°C. The corn oil is used as a vehicle (solvent) for the preparation of DEHP suspension.

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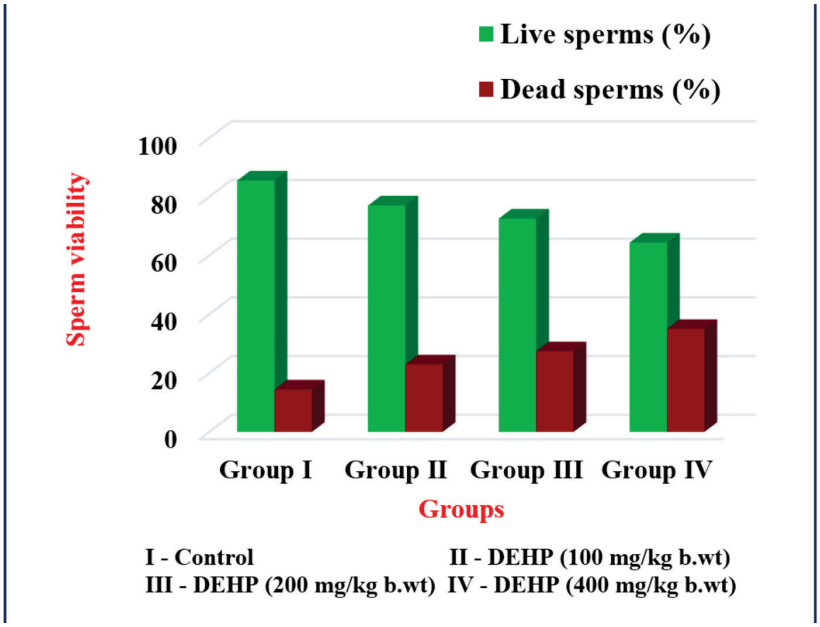
Experimental trail was carried out in Laboratory Animal House of the Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal with the approval from Institutional Animal Ethics Committee [No. IAEC/13/VCRI - NKL/2025, Dated: 28.03.2025].

All the rats were sacrificed on 29th day and necropsy was conducted to evaluate the pathological lesions. Semen was collected from the tail of the epididymis of testes from all the groups and smears were prepared on clean glass slides. A drop of eosin (5 %) mixed with four drops of nigrosine (10 %) and a drop of sperm suspension was added. The contents were gently



Graph 1. Mean sperm viability of Wistar rats in DEHP induced toxicity

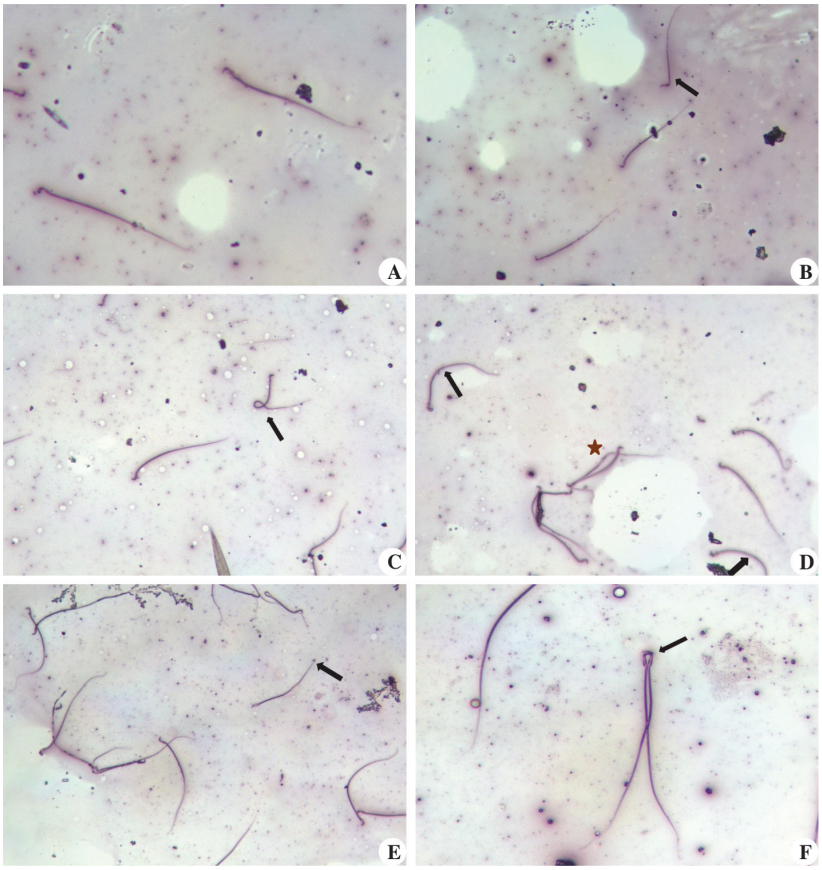


Fig. 1 (A). Control rats showing normal sperm; **(B).** Low dose - DEHP induced rat sperm showing bent neck (arrow); **(C).** Low dose - DEHP induced rat sperm depicting coiled sperm (arrow); **(D).**Medium dose - DEHP induced rat depicting angulated tail (arrows), cytoplasmic vacuolation (star); **(E).** Medium dose - DEHP induced rat sperm revealing microcephaly (arrow); **(F).** Medium dose - DEHP induced rat sperm revealing fusion of head and neck (arrow)

mixed and a small drop of suspension was placed on the slide. A thin smear was prepared using a spreader slide and allowed to air dry. Air-dried stained slides were examined under microscope to study the sperm abnormalities. Under the microscope, live sperm appeared unstained, while dead sperm appeared as pink colour.

At least 100 spermatozoa were counted in different fields of each stained slide to evaluate the proportion of viable and non-viable sperm, and to identify various morphological abnormalities, such as head, midpiece and tail deformities. The observations were recorded in a systematic manner to quantify the percentage of live and dead sperms.

The testes were collected in all the DEHP treated groups and fixed in 10 per cent neutral buffered formalin for histopathological examination. The tissue sections were cut into 4 µm thickness and subjected to routine H & E staining.

RESULTS

In Eosin-Nigrosin staining, live sperm appears as transparent due to their intact membranes, whereas dead sperm exhibit a distinct pink colour. Live and dead sperms in control and DEHP treated groups are presented in Table 1 and Graph 1. In normal healthy rats about 80 % of the sperms are viable, while approximately 20 % are non-viable. The typical sperm morphology in rats is characterized by a hook-shaped head and an elongated tail. Control rats exhibited normal sperm morphology (Fig. 1. A). In contrast, rats treated with a low dose of DEHP showed abnormalities such as bent neck (Fig. 1. B) and coiled sperm (Fig. 1. C) while the medium dose DEHP group showed defects like angulated tail (Fig. 1. D), microcephaly (Fig. 1. E) and fusion of head and neck (Fig. 1. F). Rats exposed to high dose of DEHP revealed various types of sperm abnormalities *viz.*, head fusion (Fig. 1. G), multiple angulations (Fig. 1.H), flat head (Fig. 1. I), head fusion with coiled tail (Fig. 1. J). In addition, multiple anomalies like microcephaly, angulated tail, bent neck, curved tail, coiled tail (Fig.

Table 1. Mean (±SE) of sperm viability in Wistar rats in various toxic dose levels of DEHP induced toxicity

S.No.	Sperm morphology	Group I	Group II	Group III	Group IV
1.	**Live sperms (%)**	85.52±1.08[a]	77.06±0.77[b]	72.60±1.01[c]	64.40±0.80[d]
2.	**Dead sperms (%)**	14.4±22.93[a]	22.93±0.77[b]	27.40±1.01[c]	35.0±0.86[d]

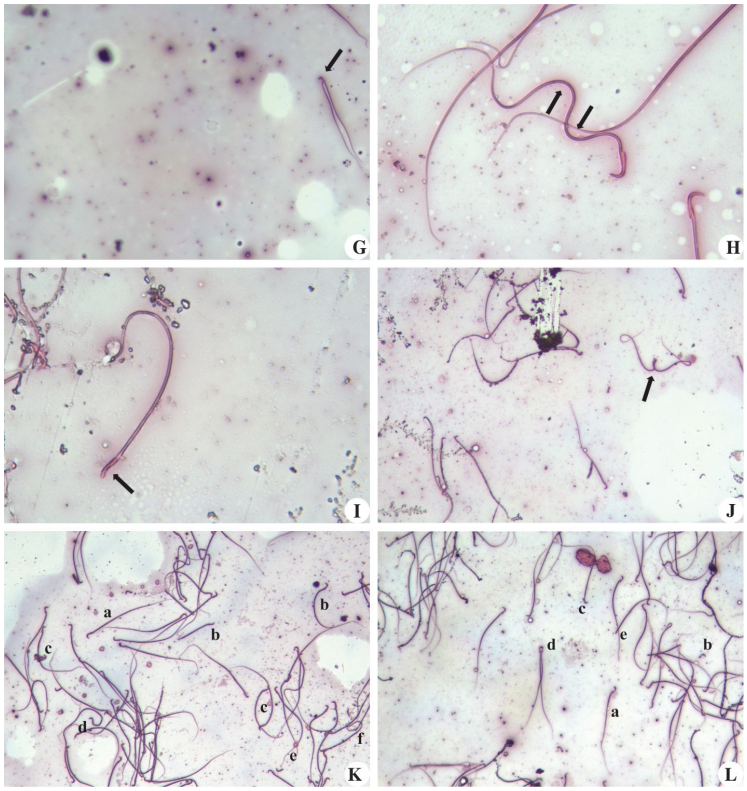
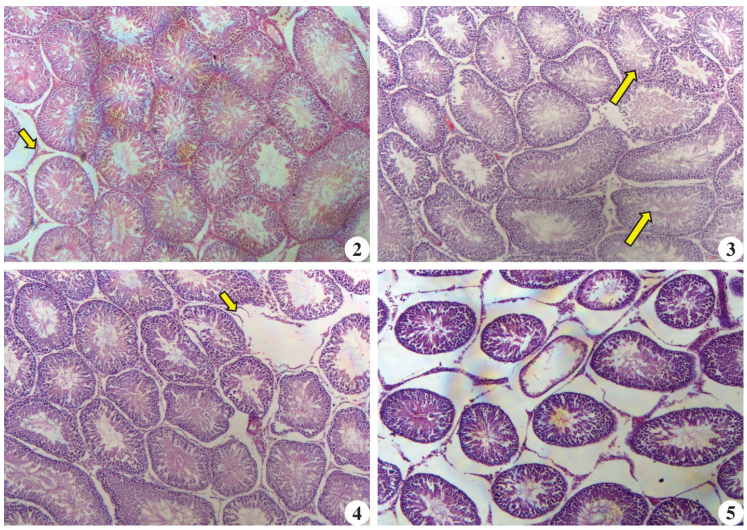


Fig. 1 (G). High dose - DEHP induced rat sperm displaying head fusion (arrow); **(H).** High dose-DEHP induced rat sperm displaying multiple angulations (arrows); **(I).** High dose - DEHP induced rat sperm revealing flat head (arrow); **(J).** High dose - DEHP induced rat sperm revealing head fusion with coiled tails (arrow); **(K).** High dose - DEHP induced rat sperms revealing multiple anomalies (a) Normal sperm (b) Microcephaly (c) Angulated tail (d) Bent neck (e) curved tail (f) Coiled tail; **(L).** High dose DEHP induced rat sperms revealing multiple anomalies (a) Normal sperm (b) Detached head (c) Bent head (d) Pairing of sperm (e) Headless tail



1. K) and combined defects such as detached head, bent head, pairing of sperm and headless tail (Fig. 1. L) were noticed in high dose group.

The control rat exhibited normal testicular architecture with well-organized seminiferous tubules with fully packed spermatozoa and interstitial Leydig cells (Fig. 2). The severity of testicular histopathological lesions in DEHP groups increased in dose-dependent manner. The low dose DEHP treated group revealed mild atrophy of seminiferous tubules with vacuolations (Fig. 3) whereas, medium dose group showed loss of few seminiferous tubules with mild reduction of spermatids in the tubular lumen (Fig. 4). The testes of rats in high dose group exhibited severe atrophy of most of the seminiferous tubules (Fig. 5).

DISCUSSION

Though there were abnormal sperms in all DEHP treated groups, a marked increase in sperm abnormalities was recorded in high dose DEHP treated groups. Similar abnormalities also recorded by[5,6]. The head, midpiece and tail abnormalities might be due to degeneration of seminiferous germinal epithelium and spermicidal action of DEHP. In addition, DEHP also leads to depletion of zinc from spermatids and resulted in sperm abnormalities as zinc plays a crucial role in sperm maturation[7] also caused reproductive toxicity through oxidative stress and lipid peroxidation in testes[8].

This might be due to generation of reactive oxygen species and thereby leading to oxidative stress. Since sperm membranes are rich in polyunsaturated fatty acids and they are highly vulnerable to damage of sperm membrane resulting in reduced sperm viability[9].

Fig. 2. Control - 29th day: Testes showing normal seminiferous tubules with fully packed spermatozoa and clumps of interstitial leydig cells (arrows) (H&E x 40); **Fig. 3**. Low dose DEHP – 29th day: Testes showing mild atrophy and vacuolations in the seminiferous tubules (arrows) (H&E x 40); **Fig. 4.** Medium dose DEHP – 29th day: Testes showing loss of seminiferous tubules (arrow) and mild reduction of spermatids in tubular lumen (H&E x 40); **Fig. 5.** High dose DEHP – 29th day: Testes showing severe atrophy of seminiferous tubules (H&E x 40)

DEHP toxicity damages testes by different mechanism such as production of reactive oxygen species (ROS), oxidative stress, lipid peroxidation, damage to DNA, disruptions in cellular functions, alterations in cellular redox mechanism and mitochondrial dysfunctions in gonocytes of testes[10].

These histopathological lesions of testes might be due to alterations in hypothalamic amino acid neurotransmitters involved in neuroendocrine reproductive regulation[11].

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