Original Article

PROTECTIVE EFFECT OF VITAMIN E ON PHTHALATE INDUCED TOXICITY ON DIAMETER AND BASEMENT MEMBRANE OF SEMINIFEROUS TUBULES

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Objective: To investigate toxic effects on basement membrane and diameter of seminiferous tubules caused by phthalates and protection by vitamin E.

Methods: Twenty four male albino rats were divided into three groups of eight animals each. Group A was given 0.4 ml of corn oil daily for 15 days. Group B was given 0.15 ml of Dioctyl phthalate (DOP) dissolved in 0.4 ml of corn oil daily for 15 days. Group C was given 0.15 ml Dioctyl phthalate and 10 mg of vitamin E, each dissolved in 0.4 ml of corn oil respectively, daily for 15 days. The mode of administration was oral gavage.

Results: On histological examination the testes of animals of group B showed disrupted and ruffled basement membrane. The diameter of seminiferous tubules in different groups did not show much variation. Co-administration of vitamin E and DOP to group C showed that DOP in this group had less deleterious effect as it did in group B, where DOP was given alone.

Conclusions: Phthalate induced testicular toxicity, effect on basement membrane and diameter of seminiferous tubules was prevented by co-administration of vitamin E and DOP.

Keywords: phthalates, dioctyl phthalate (DOP), vitamin E.

Introduction

Phthalates are synthetic chemical esters of phthalic acid and serve multifunctional roles in a variety of consumer products resulting in ubiquitous daily exposures in adults and children.[1] DOP also known as di (2-ethylhexyl) phthalate (DEHP), is one of the commonly used compound. These compounds impart flexibility, transparency and durability to PVC plastics, which are then used in an extensive range of products including toys, clothing, building material, paints, curtains, wall papers, food packaging and plastic wraps. They are also used in cosmetics, including perfumes, soaps, shampoo, hairspray, nail polish and skin moisturizers.[2] Fast food may be a source of exposure to DEHP.[3] They are, therefore, an unavoidable part of contemporary living.[4,5] Phthalates are not chemically bound in the polymers. Therefore, migration or emission of phthalates from the products into the environment is likely to occur.[6] DEHP is also used in many medical devices including intravenous (IV) tubing, IV fluid bags, total parenteral nutrition bags/tubes, and catheters[7]. Certain chemical in personal care and consumer products, including low molecular weight phthalates, or their precursors, are associated with altered pubertal timing in animal studies.[8] Some phthalate exposures from personal care products are associated with menopausal hot flashes in women.[9] Thereafter, considerable concern was raised regarding toxic effects of all PAEs, especially DOP.

Plastic materials used in food processing and storage may also increase the phthalate content of some foods.[10] Aging disposable plastic food wraps and bottles particularly at high environmental temperatures lead to transfer of phthalates into the environment. Elevated temperatures of microwave oven used in many households may lead to transfer of phthalate compounds from plastic packaging and crockery into the food being warmed. After exposure the route of entry into the body can be ingestion, inhalation or dermal contact.

DOP is rapidly absorbed and converted to mono (2-ethylhexyl) phthalate (MEHP), which also leads to male reproductive organ damages in animals, chiefly the testes when administered orally to rats.[11,12,13] Di-ester phthalates are hydrolyzed into monoester phthalates in the intestine and parenchyma, i.e., phthalates are converted in the body to a metabolite, a break-down substance produced by metabolism.[14]

Phthalates have been linked to adverse reproductive effects in male pubertal and adult rodents exposed in utero and during lactation, such as reduction in the weights of reproductive organs and a reduction in sperm count.[15] Oral administration of DOP to rats has been reported to significantly increase the lipid peroxidation by generating ROS which in turn leads

to testicular degeneration and infertility.[16] Free radicals in the cell, such as hydroxyl radical, superoxide ion and hydrogen peroxide, attack lipids, sugars, proteins and DNA. Oxidative injury to these molecules may impair a range of biomolecular processes[17, 18]. Since testicular physiology is impaired by reactive oxygen species (ROS)-dependent mechanism, suggestive of the fact that antioxidant enzymes are important in the testes. Considering the fact that phthalates also have a role in reducing the male fertility by causing seminiferous tubules atrophy and seminiferous epithelial cells disintegration, it is suggested that the mechanism behind it is oxidative stress in testes of adult rats.[19]

These studies suggest that antioxidant enzymes are customarily important in testes.[20] Antioxidants such as vitamins C and E have been shown to guard tissues against ROS[21]. Supplementation of vitamins with antioxidant effects enhanced the regeneration of damaged seminiferous epithelium in DOP treated animals, suggesting that these vitamins have a beneficial effect on DOP-induced spermatogenetic toxicity.[22] Cells are normally fortified with antioxidant defense system to counter the effect of ROS, but when the generation of ROS exceeds the capacity of cells to counteract these, additional help is required. Therefore, excess ROS generated in testicular tissues due to DOP require antioxidants such as vitamins C and E.

Methods

This study was carried out at the Experimental and Research Laboratories of University of Health Sciences, Lahore and rat was used as an experimental model, after approval from the ethical committee of the University. Twenty four healthy adult male Albino rats of Wistar strain, aged 6-8weeks and weighing 200-250 gm were used; housed in cages of appropriate size, kept in a controlled environment with room temperature of 23±2°C, and humidity of 55±5%, light and dark cycles were maintained for 12 hours each. They were fed on normal rat chow, given water ad libitum and allowed to acclimatize for a period of two weeks. The rats were divided into three groups of eight animals each. Each animal in every group was labelled with eosin stain on their back. **Group A** was given 0.4 ml of corn oil daily for 15 days by oral gavage. **Group B** was given 0.15 ml of Dioctyl phthalate dissolved in 0.4 ml of corn oil daily for 15 days by oral gavage. **Group C** was given 0.15 ml Dioctyl phthalate and 10 mg of vitamin E, each dissolved in 0.4 ml of corn oil respectively, daily for 15 days. Animals were sacrificed on the sixteenth day and testes were removed under anesthesia, cut into two pieces each and kept in Bouin's fixative for 48 hours. Each half was then washed with 70% alcohol for 72 hours to remove yellow color of Bouin's fixative; processing was done in an automatic tissue processor and paraffin blocks were prepared. Sections 4µm thick were obtained using rotary microtome. The slides were stained with hematoxylin and eosin and then examined under light microscope using X10 and X40 magnification. Diameter of seminiferous tubule was measured with the help of Leica, DM 1000 microscope with 10X objective lens and ocular micrometer; the method described by Culling, 1974 was used. The ocular micrometer was inserted into the eye piece of the microscope and calibrated using 10X objective lens, the eyepiece micro-metered and adjusted so that the scale on the linear micrometer seemed sharply focused. A stage micrometer was placed on the microscope stage and brought into focus. The area at which both ocular and stage micrometers exactly matched was observed. The factor calculated by calibration of ocular micrometer with the stage micrometer. The stage micrometer was then removed and both vertical and horizontal diameter of seminiferous tubules measured using ocular micrometer, and a mean diameter of each of these seminiferous tubules was calculated and was multiplied with the factor calculated;

100 stage divisions = 1mm =1000µm
1 stage division = 1000/100 =10 µm
1 division of ocular micrometer was = 1 stage division
1 division of ocular micrometer = 10 µm
The calibration factor =10µm

The cross sectional profiles of ten seminiferous tubules were identified in each section. The mean diameter of each seminiferous tubule was calculated by measuring the diameters twice at right angles to each other and calculating their mean. Six sections from every animal were observed and a total of 144 sections from 24 animals were examined; thus the diameter of 1440 seminiferous tubules were recorded. The basement membranes of seminiferous tubules in each PAS stained section of the Twenty four animals were observed regarding their thickness and regularity using 40X objective. The basement membranes showing irregularities were counted as ruffled and those showing breaks were counted as disrupted. SPSS 20 was used for statistical analysis. Mean±SD and Median with interquartile range was given for diameter of seminiferous tubules. Normality of the data was checked by Shapiro Wilk test. A comparison was done for the outcome, both in

to testicular degeneration and infertility.[16] Free radicals in the cell, such as hydroxyl radical, superoxide ion and hydrogen peroxide, attack lipids, sugars, proteins and DNA. Oxidative injury to these molecules may impair a range of biomolecular processes[17, 18]. Since testicular physiology is impaired by reactive oxygen species (ROS)-dependent mechanism, suggestive of the fact that antioxidant enzymes are important in the testes. Considering the fact that phthalates also have a role in reducing the male fertility by causing seminiferous tubules atrophy and seminiferous epithelial cells disintegration, it is suggested that the mechanism behind it is oxidative stress in testes of adult rats.[19]

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tubules; one with vertical and horizontal diameter of 247µm as measured in cross-sectional profile, from group B showing several seminiferous tubules; one with vertical and horizontal diameter of 245 µm as measured in cross-sectional profile and from group C showing several seminiferous tubules; one with vertical and horizontal diameter of 247 µm as measured in cross-sectional profile. Reference line measures 100 µm. H&E stain. X100.

Discussion

In the present study the rats of group A showed thin and regular basement membrane indicating a normal pattern. After 15 days of administration of DOP to the rats of group B,the basement membrane of the seminiferous tubules revealed the detrimental effect of DOP. Many tubules showed ruffled basement membrane, others showed disruption. The ruffled effect was seen due to the sloughing of the germinal epithelium which lead to the collapse of the tubule, resulting into ruffled appearance of the basement membranes. The disrupted basement membrane however was due to possibly the oxidative stress mechanism of the DOP which caused lipid peroxidation of the membranes.

There was not much variation in diameter of seminiferous tubules in different groups. A few tubules in groups B showing some atrophy is attributed to the sloughing of the germinal epithelium by the DOP. However, when the results were compared between different groups they were not statistically significant (p=0.200).

The results observed in the present study are in accord with the previous reports which revealed that Phthalate esters are associated with tissue damage,[23] which is responsible for the disruption of the basement membrane. In other areas atrophy of the seminiferous tubules was shown by decreased diameter of the tubules and ruffled basement membrane. However the rats in group C were given both DOP and vitamin E, it showed that DOP in this group did not have the same deleterious effect as it did in group B, where DOP was given alone. The basement membrane remained thin and regular indicating lesser or no damage to the cells in group C as compared to those in group B. This reveals a preventive effect of Vitamin E which is due to its antioxidant properties. These results of present study are in accordance with the previous study which suggests that one of the important mechanisms of testicular damage is oxidative stress caused by phthalates which dissociate easily from their compounds, particularly at high temperatures.[24] The increase in global warming, therefore poses a great threat to human health by causing the release of such chemicals into the environment. To reduce the oxidative stress, supplementation of antioxidant vitamin E is highly beneficial.[25]

It is evident from the discussion that phthalates are toxic to the normal health of testes and the use of antioxidants can forestall its deleterious effects. The facts that phthalates are being used widely in the manufactured goods;[26] the increasing utility of such items in the modern lifestyle[24]and that they are easily and constantly being ejected into the environment from the products they are used in, increase the problem many folds. The last and the most important fact is that they are a constant source of health hazard, affecting not only the present but the future generations also. Consequently this complex problem needs to be tackled elaborately at the environmental level.

Conclusion

It is concluded that our observations confirmed the previous findings on phthalate induced toxicity on rat testes. The results also show the vitamin E has a beneficial effect in preventing the histological changes in the testes of rats. Since vitamin is easily available and cheap, it can be employed in to reduce oxidative stress in the body produced by phthalates.

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