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Effects of vitamin E on sperm parameters and reproductive hormones in developing rats treated with para-nonylphenol

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Abstract

Background: para-nonylphenol (p-NP) is able to induce malformations in male reproductive system.

Objective: The aim of this study was to investigate the preventing role of vitamin E (Vit.E) on sperm parameters and reproductive hormones in developing rats.

Materials and Methods: Pregnant rats were divided into 4 groups: control, p-NP, Vit.E and p-NP+Vit.E. Treatments were performed on day 7 of gestation and continued during weaning. The male pups were then divided into the same groups as the mothers and were treated till 90 days of age. Finally, body and left testis weight were recorded and left epididymis was cut in Ham's F10. Released sperm were used to analyze number, motility and viability of the sperm. Blood serum was used to assess follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and testosterone.

Results: In p-NP-treated rats, a significant decrease was found in body and testis weight, sperm number and sperm motility compared to control and p-NP+Vit.E groups. A significant increase was also found in sperm viability in Vit.E group (83.3±7.6) compared to both p-NP (59.5±7.5) and control (66.3±9.7) groups. Rats treated with p-NP showed a significant decrease in FSH level and a significant increase in estrogen level. However, testosterone and LH level remained constant. In p-NP+Vit.E group, the change of estrogen level but not FSH was significantly reversed compared to p-NP group.

Conclusion: Vit.E not only is able to compensate the toxic effects of p-NP on testis weight, sperm number, sperm motility and estrogen level, but also increases sperm viability in developing rat.

Key words: *Para-nonylphenol, Rat, Reproductive hormones, Sperm parameters, Vitamin E.*

Introduction

Male infertility could be one of tragic realities in industrial area. It is now evident that several aspects of male reproductive health have changed to trigger infertility in males. Problems in the production, maturation and fertilizing ability of sperm might be the most common reasons for male infertility. Male reproductive abnormalities may be as a result of exposure to environmental toxicants such as alkylphenols (1). Alkylphenols ethoxylathes (APEs) are a class of non-ionic surfactants and para-nonylphenol (p-NP) is a final degradation product of APEs which widely used in the preparation of detergents, paints, phenolic resins, herbicides (2) and also being used in the preparation of plastic containers for food packing (3). Human can be exposed to p-NP not only via dermal absorption or inhalation but also via contaminated foods and drinking water (4). P-NP shows weakly estrogenic properties (5, 6) and can therefore mimic the effects of estrogen in body by binding to estrogenic receptors during fetal and

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neonatal development as well as adulthood to induce abnormalities in male reproductive system (7). In this concern, several studies have suggested that developmental exposure of p-NP may affect male reproductive system in rat. These effects included a reduction in sperm production, cryptorchidism, reduced reproductive organ weight and testicular abnormalities (8-10).

In addition, adult male rats exposed to 250 mg/kg p-NP showed alterations in the level of hormones involved in male reproductive system including, testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (11).

p-NP like some other environmental toxicants is able to induce oxidative stress in reproductive system. Chitra and co-workers (1) for instance, have recently reported that the p-NP causes oxidative stress in epididymal sperm of adult rats. It has been now documented that vitamin E (Vit.E) is a potent scavenger of free radicals and is able to prevent the membrane damage mediated by free radicals (12). In addition, the antioxidant's role of this vitamin has been reported in reducing testicular oxidative stress (13).

Previous studies have reported the adverse effects of p-NP on adult and developing male reproductive tract. To our knowledge, however, no study has examined the effect of Vit.E on p-NP-mediated toxicity in epididymal sperm and hormonal levels of developing rat. The present study was performed to evaluate the effect of Vit.E on epididymal sperm parameters and the level of FSH, LH, testosterone and estrogen in p-NP-treated developing rats.

Materials and methods

Animals and treatments

Adult albino male and female Wistar rats (250±20 gr) were purchased from Pasture's Institute, Iran. The animals were housed in plastic cages at 12-h light/dark cycle, 24±2 $^{\circ}$C and fed with standard commercial laboratory chew and water. For copulation, a female was paired with single male in individual cage. Mating was confirmed by vaginal plug detection and the presence of sperm in vaginal smears.

This day was regarded as 0 day of gestation. Pregnant females (n=24) were then transferred into individual cages and divided into four groups: control which received corn oil, p-NP (250 mg/kg/day, Acros Organics Company, New Jersey, USA), Vit.E (100 mg/kg/day, Sigma, USA) and p-NP+VE. p-NP was dissolved into a measured amount of corn oil and the reagents were orally given to the rats by gavage. The treatments were performed on day seven of gestation, to avoid p-NP interference with blastocyte implantation and initial embryonic growth (9), and continued during weaning. After lactation, male offspring (n=24) were divided into the same groups as the mothers and oral treatments were continued until 90 days of age. At the end, the rats were weighed, anesthetized by the injection of pentobarbital (60 mg/kg) and sacrificed. Left testis and cauda epididymis of the animals were dissected. The testis was cleared from fat tissue and its weight was recorded.

Sperm count

The dissected epididymis of each animal was transferred into 10 ml Ham's F10 medium and cut to small slices, in order to swim out the sperm into the medium. After 10 min of diffusion, 1 ml of the solution was diluted with 9 ml formaldehyde fixative.

The diluted solution was transferred into each chamber of Neubauer hemocytometer and sperm heads was manually counted under a microscope. Sperm count was performed according to WHO guidelines (14) and data were expressed as the number of sperm per ml.

Sperm motility

Assessment of sperm motility was done according to WHO protocol (14). In brief, 10 µl of the sperm suspension was placed on a microscopic slide and coversliped. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 sperm for each animal. The percentage of sperm motility was analyzed for following motion parameters: percentage of progressively motile sperm (PMS), non-progressively motile sperm (NPMS) and non-motile sperm (NMS).

Sperm viability test

Eosin-nigrosin staining was used to asses sperm viability according to WHO protocol (14). Briefly, eosin (1%, Merck, Germany) and nigrosin (10%, Merck, Germany) was prepared in distilled water. One volume of sperm suspension was mixed with two volume of 1% eosin. After 30 second, an equal volume of nigrosin was added to this mixture. Thin smears were then prepared and observed under a light microscope at 100X magnification. Viable sperm remained colorless while nonviable sperm stained red.

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Serum hormonal analysis

The anesthetized animals were immediately used for collecting blood samples from heart. The blood was drained into glass tubes, coagulated at 37°C , centrifuged at 1700 ×g for 10 min and serum was then stored at -80 °C till the measurement of hormones. The concentration of testosterone and estrogen was measured by DRG Diagnostics kit, Germany, while FSH and LH concentration were analyzed using Monobind CA kit, USA. In all cases, serum hormone analysis was done according to the manufacture's protocol.

Statistical analysis

Results are expressed as mean±SD for six animals per group. One-way analysis of variance (ANOVA) was used to assess the statistical significance of the data. $p<0.05$ was considered significant.

Results

Body and testis weight

For each animal, body weight was recorded at the end of weaning and after termination period. No significant difference was found in the mean weight of all groups after weaning (data not shown). After termination, there was a significant decrease ($p<0.05$) in body weight and absolute testis weight in rats treated with p-NP, compared to control group (Table I). Co-administration of p-NP and Vit.E (p-NP+Vit.E group) showed a significant increase ($p<0.05$) in testis weight when compared with p-NP group (Table I).

Sperm count

Results showed a highly significant decrease ($p<0.001$) in epididymal sperm number in p-NP group compared to control group (Table II). p-NP+Vit.E group showed a highly significant increase ($p<0.001$) in sperm number as compared with p-NP group (Table II).

Sperm motility

The treatment of animals with p-NP significantly decreased ($p<0.001$) the percentage of PMS and increased ($p<0.001$) the percentage of NMS when compared with control group (Table II), but no significant difference was observed in the percentage of NPMS. In animals treated with p-NP+Vit.E, a significant increase ($p<0.001$) and decrease ($p<0.001$) was found in the percentage of PMS and NMS respectively as compared with p-NP group (Table II).

Sperm viability

The percentage of viable sperm in p-NP group appeared lower than control group, but there was no significant difference. However, a significant increase ($p<0.01$) was observed in sperm viability in rats exposed to p-NP+Vit.E compared to both p-NP and control groups (Table II). Rats treated with Vit.E alone also showed a significant increase ($p<0.01$) in sperm viability when compared with control group (Table II).

Hormonal levels

Rats treated with p-NP showed a significant decrease ($p<0.001$) in the level of FSH and a significant elevation ($p<0.001$) in estrogen concentration, while the level of other hormones remained constant when compared with control group (Table III). In p-NP + Vit.E group, the change of estrogen level but not FSH was significantly ($p<0.001$) reversed as compared with p-NP group (Table III).

Table I. Body and testis weight.

	Control group	Vitamin E group	p-NP group	p-NP+ vitamin E group
Body Weight (g)	305± 19	307± 23	206± 29*a	246± 32
Testis Weight (g)	1.4± 0.1	1.4± 0.06	1.1±0.1*a	1.4± 0.1*b

* $p<0.05$, a: compared to control. b: compared to para-nonylphenol (p-NP). Mean ± SD, n=6.

Table II. Epididymal sperm number, sperm motility and sperm viability.

	Control group	Vitamin E group	p-NP group	p-NP+ vitamin E group
Sperm number (10^6)	14.8± 0.2	16.4±0.1	10±0.1	12.4±0.8**b
PMS%	67.83± 7	73.67±5	54.50± 5**a	70.17±6**b
NMS%	12.67± 3	8.83±1	20.00±4**a	10.17±2**b
NPMS%	21.67±3	19.00±2	24.83±7	19.67±5
Sperm viability%	66.3± 9.7	83.3±7.6*a	59.5±7.5	76.7±9.7*b

PMS: Progressively motile sperm, NMS: Non-motile sperm, NPMS: Non-progressively motile sperm. * $p<0.01$, ** $p<0.001$, a: compared to control. b: compared to para-nonylphenol (p-NP). Mean ± SD, n=6.

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Table III. The level of follicle stimulating hormone (FSH), mIU/ml; luteinizing hormone (LH), mIU/ml; testosterone, ng/ml and estrogen, pg/ml in different groups of rats.

Group	FSH	LH	Testosterone	Estrogen
Control	0.68± 0.19	0.37±0.29	3.7±0.46	6.7±0.37
Vitamin E	0.60± 0.19	0.42±0.25	3.9±1.4	5.0±1.4
p-NP	0.13± 0.04	0.45±0.25	2.3±0.95	21.5±8.3*a
p-NP+ vitamin E	011±0.03	0.09±0.04	2.3±0.69	8.9±2.8*b

*p<0.001, a: compared to control, b: compared to para-nonylphenol (p-NP). Mean ± SD, n=6.

Discussion

This study examined the adverse effect of p-NP on body and testis weight, epididymal sperm number and sperm motility as well as FSH and estrogen levels in developing rats. In addition, Vit.E showed to reverse the toxic effect of p-NP on testis weight, sperm number, sperm motility and estrogen level.

Our study demonstrated that p-NP affected body and testicular weight in rats exposed with this toxicant. Decrease in body weight might be due to decrease in organs weight (15) or food consumption (16, 17). Since testicular and body weight are largely independent variables (9), in this study absolute but not relative testicular weight was considered. In agreement with pervious study (1), our results also showed a significant decrease in absolute testicular weight in rats treated with p-NP. This effect might be as a result of a decrease in diameter of testicular lumen, seminiferous tubule diameter, epithelial thickness and increase in interatubular spaces, resulting to testicular atrophy (5).

In p-NP-treated animals, a significant decrease in the total sperm number was observed. It is now evident that p-NP by inducing oxidative stress exerts toxic effects on organs such as testis (18). We therefore hypothetized that toxic effect of p-NP on the reduction of sperm number could be as a result of p-NP-induced stress oxidative. This was based on Chitra and co-workers report (1), showing that graded doses of p-NP elicits the depletion of antioxidant defense system in rat sperm. If our hypothesis was true, Vit.E, a well known antioxidant (12, 19), should have reversed hazardous effect of p-NP on sperm number. Interestingly, we showed that in p-NP+Vit.E group, Vit.E significantly ameliorated p-NP-mediated decrease in sperm number.

Motility and viability of sperm appear to be the most important parameters for the assessment of sperm fertilization capacity and the integrity of sperm membrane may play an important role on these parameters. Sperm plasma membrane has a high content of polyunsaturated fatty acids which is easily susceptible to lipid peroxidation caused by oxidative stress (20). The change in sperm motility pattern induced by p-NP could be due to the ability of this toxicant in the induction of oxidative stress by lipid peroxidation. To support this idea, we showed that Vit.E significantly reversed motility pattern in p-NP+Vit.E group compared to p-NP group. In according with this, a water-soluble vit.E analogue (Trolox) showed to improve boar sperm motility and mitochondrial membrane integrity (21). The role of this vitamin has also been reported to improve sperm quality and fertility in human sperm (22).

Although sperm viability in developing rats exposed with 250 mg/kg/day of p-NP showed no significant difference, deceased sperm viability should be noted. It is likely, that the effect of p-NP on sperm viability might be dose dependent. An interesting finding in sperm viability assay was that Vit.E alone increased sperm viability compared to control group. Traditionally, Vit.E is called as anti-sterility vitamin (23) and its association with normal function of male reproductive system has been well established (24).

Since the application of antioxidants including Vit.E has been shown to enhance sperm viability (25), it is reasonable to suggest that Vit.E supports sperm antioxidant system to improve sperm viability. This effect may also explain increased sperm viability in p-NP+Vit.E group compared to p-NP group.

p-NP as an endocrine disruptor is an estrogen-like component which mimics the effect of estrogen to induce hazardous effects on male reproductive axis (8). Several lines of studies have shown that the exposure of neonatal and adulthood animals by estrogen can impair sperm production and maturation (26, 27). Our results showed that p-NP could significantly increase estrogen and decrease FSH level. One possibility for this dysregulation might be that p-NP with its estrogenic property suppressed FSH. The reason for this hypothesis is the role of estrogen as a negative feed back regulator for FSH secretion (28).

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Estrogen has been shown to increase the expression of inhibin (29), which is an important mediator of FSH secretion in male by Sertoli cells (30). Therefore, another possibility for the effect of p-NP in the decrease of FSH might be mediated by the ability of p-NP in changing the production of inhibin. The decrease of FSH mediated by p-NP might also explain why p-NP was able to decrease sperm number.

Conclusion

Our results indicate that p-NP has a negative influence on body and testis weight, sperm number, sperm motility, FSH and estrogen in developing rats exposed with p-NP. In addition, Vit.E not only is able to compensate the adverse effects of p-NP on testis weight, sperm number, sperm motility and estrogen level, but also increases sperm viability in such animals.

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References

1. Chitra KC, Latchoumycandane C, Mathur PP. Effect of nonylphenol on the antioxidant system in epididymal sperm of rats. *Arch Toxicol* 2002; 76: 545-551.
2. White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 1994; 135: 175-182.
3. Junk GA, Svec HJ, Vick RD, Avery MJ. Contamination of water by synthetic polymer tubes. *Environ Sci Technol* 1974; 8: 1100-1106.
4. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJJr, et al. Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 1996; 104: 741-803.
5. de Jager C, Bornman MS, van der Horst G. The effect of p-nonylphenol, an environmental toxicant with oestrogenic properties, on fertility potential in adult male rats. *Andrologia* 1999; 31: 99-106.
6. Watanabe H, Suzuki A, Goto M, Lubahn DB, Handa H, Iguchi T. Tissue-specific estrogenic and non-estrogenic effects of a xenoestrogen, nonylphenol. *J Mol Endocrinol* 2004; 33: 243-252.
7. Sikka SC, Wang R. Endocrine disruptors and estrogenic effects on male reproductive axis. *Asian J Androl* 2008; 10: 134-145.
8. Lee PC, Arndt P, Nickels KC. Testicular abnormalities in male rats after lactational exposure to nonylphenols. *Endocrine* 1999; 11: 61-68.
9. de Jager C, Bornman MS, Oosthuizen JM. The effect of p-nonylphenol on the fertility potential of male rats after gestational, lactational and direct exposure. *Andrologia* 1999; 31: 107-113.
10. Nagao T, Wada K, Marumo H, Yoshimura S, Ono H. Reproductive effects of nonylphenol in rats after gavage administration: a two-generation study. *Reprod Toxicol* 2001; 15: 293-315.
11. Han XD, Tu ZG, Gong Y, Shen SN, Wang XY, Kang LN, et al. The toxic effects of nonylphenol on the reproductive system of male rats. *Reprod Toxicol* 2004; 19: 215-221.
12. Gurel A, Coskun O, Armutcu F, Kanter M, Ozen OA. Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. *J Chem Neuroanat* 2005; 29: 173-178.
13. Kutlubay R, Oguz EO, Can B, Guven MC, Sinik Z, Tuncay OL. Vitamin E protection from testicular damage caused by intraperitoneal aluminium. *Int J Toxicol* 2007; 26: 297-306.
14. World Health Organization (WHO) laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 1999, 4th ed. Cambridge university press.
15. Kyselova V, Peknicova J, Buckiova D, Boubelik M. Effects of p-nonylphenol and resveratrol on body and organ weight and in vivo fertility of outbred CD-1 mice. *Reprod Biol Endocrinol* 2003; 1: 30.
16. Ferguson SA, Flynn KM, Delclos KB, Newbold RR. Maternal and offspring toxicity but few sexually dimorphic behavioral alterations result from nonylphenol exposure. *Neurotoxicol Teratol* 2000; 22: 583-591.
17. Cunny HC, Mayes BA, Rosica KA, Trutter JA, Van Miller JP. Subchronic toxicity (90-day) study with para-nonylphenol in rats. *Regul Toxicol Pharmacol* 1997; 26: 172-178.
18. Chitra KC, Mathur PP. Vitamin E prevents nonylphenol-induced oxidative stress in testis of rats. *Indian J Exp Biol* 2004; 42: 220-223.
19. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med* 2007; 43: 4-15.
20. Agarwal A, Prabakaran SA, Said TM. Prevention of oxidative stress injury to sperm. *J Androl* 2005; 26: 654-660.
21. Pena FJ, Johannisson A, Wallgren M, Rodriguez Martinez H. Antioxidant supplementation in vitro improves boar sperm motility and mitochondrial membrane potential after cryopreservation of different fractions of the ejaculate. *Anim Reprod Sci* 2003; 78: 85-98.
22. Nouri M, Ghasemzadeh A, Farzadi L, Shahnazi V, Ghaffari Novin M. Vitamin C, E and lipid peroxidation levels in sperm and seminal plasma of asthenoteratozoospermic and normozoospermic men. *Iranian Journal of Reproductive Medicine* 2008; 6: 1-5.
23. Uzunhisarcikli M, Kalender Y, Dirican K, Kalender S, Ogutcu A, Buyukkomurcu F. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. *Pesticide Biochemistry and Physiology* 2007; 87: 115-122.
24. Chen H, Liu J, Luo L, Baig MU, Kim JM, Zirkin BR. Vitamin E, aging and Leydig cell steroidogenesis. *Exp Gerontol* 2005; 40: 728-736.
25. Verma A, Kanwar KC. Effect of vitamin E on human sperm motility and lipid peroxidation in vitro. *Asian J Androl* 1999; 1: 151-154.
26. Guarducci E, Nuti F, Becherini L, Rotondi M, Balercia G, Forti G, et al. Estrogen receptor alpha promoter polymorphism: stronger estrogen action is coupled with lower sperm count. *Hum Reprod* 2006; 21: 994-1001.

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27. Tong MH, Song WC. Estrogen sulfotransferase: discrete and androgen-dependent expression in the male reproductive tract and demonstration of an in vivo function in the mouse epididymis. *Endocrinology* 2009; 143: 3144-3151.
28. O'Donnell L, Robertson KM, Jones ME, Simpson ER. Estrogen and spermatogenesis. *Endocr Rev* 2001; 22: 289-318.
29. Charpentier AH, Bednarek AK, Daniel RL, Hawkins KA, Laflin KJ, Gaddis S, et al. Effects of estrogen on global gene expression: identification of novel targets of estrogen action. *Cancer Res* 2000; 60: 5977-5983.
30. Burger HG, Robertson DM. Editorial: inhibin in the male--progress at last. *Endocrinology* 1997; 138: 1361-1362.

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