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Nonylphenol exposure disrupts the fertility efficiency in adult male rats

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Abstract

The present research was aimed to investigate the effects of low doses of nonylphenol on male reproductive performance in adult rats. Thirty two male Wistar rats were randomly divided into four groups (weighing 160 ± 10 g in the beginning of the experiment). First group served as control and were injected with only DMSO, remaining second, third and fourth groups were administered with nonylphenol of 1, 10, 100 µg/kg Bw through intraperitonial injections for 55 days. To observe the fertility, control and experimental males were cohabited with sexually mature normal females. A significant ($p<0.05$) decrease was observed in the body weight gain of experimental males. Significant ($p<0.05$) changes were not observed in the mating index. There was a significant ($p<0.05$) decrease in fertility rate, measured by counting live fetuses in the uterus of normal females mated with males exposed to nonylphenol. Four female rats were sacrificed on 8th day of pregnancy, ovaries were examined for the numbers of corpora lutea. Remaining four female rats were sacrificed on 18th day of pregnancy and uteri were dissected and examined for determination of numbers of implantations, dead and live fetuses, and resorptions. Females mated with 100 µg/kg Bw nonylphenol exposed males showed resorptions and a significant ($p<0.05$) decrease in number of live fetuses per rat. The present results revealed significant decrease in fertilization capacity of adult male rats exposed to nonylphenol.

Key words: Xenoestrogens, Nonylphenol, Fertility, Implantation loss, Rat

1. Introduction

Over the past few decades numerous studies suggested that there is a gradual decrease in the male reproductive health such as male sexual development, reproductive capacity and fertility potential, semen quality and quantity (1-3). Several factors such as lifestyle factors, occupational agents, drugs, radiation, and environmental pollutants have been attributed as reasons for suppressed male fertility (4). Many scientific investigations reported that exposure to substances in environment which target male reproductive machinery with their diverse chemical properties affects male fertility (1, 4, 5-7). In general, pollutants target the male reproductive system either by mimicking or inhibiting the estrogenic or androgenic actions. Exposure to the environmental contaminants with estrogenic and/or androgenic properties leads to alterations in the functions of entire male reproductive system that impairs male fertility (8, 9) and these are categorically known as endocrine disruptors or xenoestrogens. Endocrine disrupting compounds are either natural or synthetic exogenous compounds that interfere with the physiology of normal endocrine-regulated events such as reproduction and growth (10). These environmental contaminants exhibit endocrine disruption by causing adverse health effects in reproductive development of an intact organism, or its progeny.

Nonylphenol is a non-ionic surfactant widely used in the preparation of many house hold products (11) and as the starting material for the production of

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number of derivatives synthesized in almost all the plastic producing industries (12,13). Nonylphenol is reported to accumulate in aquatic environment of sewage and house hold effluent (14). In addition, nonylphenol is also reported to have accumulated in soil and biota (15-18). Consequently, nonylphenol has been ubiquitous in environment across the globe that can inevitably induce the reproductive health of human and wildlife within the domain of its indispensible exposure. Several earlier studies reported that exposure to nonylphenol can induce abnormalities in male reproductive system such as reduced sperm production, decreased reproductive organ weights, testicular abnormalities, and decrease in reproductive hormone levels (19-23). Nonylphenol exposure also causes an increase in oxidative stress and histopathological changes in testes (24). Fetal, neonatal and juvenile exposure to nonylphenol showed disruption of reproductive developments in rats (19, 25-29).

Though several studies reported the exposure to high concentrations of nonylphenol produced various negative effects on male reproductive performance but literature on exposure to low doses of nonylphenol is quite inadequate on fertility performance. Generally, endocrine disrupting chemicals occurs at low concentrations in the environment. Thus, the present study was undertaken to evaluate the influence of different low doses of nonylphenol on male Wistar rats and assessed induced alterations in fertilization capacity.

2. Material and methods

Nonylphenol (CAS NO 46018, purity>98%) was purchased from Sigma-Aldrich Laborchemikalien, Seelze, Germany. All other reagents were of analytical grade and purchased from local commercial sources.

Adult male (n=32) (body weight 160 ± 10 g) and virgin female (n=32) (body weight 160 ± 10 g) Wistar rats were purchased from authorized vendor (M/S Raghavendra Enterprises, Bengaluru, India). The animals were fed on pellet diet (HLL Animal feed, Bengaluru, India) and water *ad libitum*, maintained under standard laboratory conditions (temperature, 22–25°C; light: dark cycle, 12:12 hr). The rats were acclimatized before the experimentation. Rats were randomly divided into four groups consisting of eight animals in each group. The animals of group I served as control and were injected with only DMSO while rats of group II, group III and group IV were injected with 1, 10, 100 µg/kg Bwt of nonylphenol with DMSO as vehicle. The body weight of the rats was recorded on day of initiation of treatment and also on the day of cohabitation.

After the treatment, each male rat was transferred to a mating cage and cohabited with untreated female rats in proestrus on a 1:1 basis in the home cage of the male. The maximum duration of pairing was 4 days. Female rats were checked for the presence of copulatory plugs, and vaginal washings were examined for the presence of spermatozoa each morning during cohabitation to determine whether copulation had occurred. The day on which presence of sperm in the vaginal plug observed during mating, was defined day zero of gestation. The conception time, the interval between the first day of cohabitation and the day of vaginal plug/or sperm in vaginal smear, was recorded for each female. The number of pregnant rats with each experimental group or the control group was recorded for determination of fertility index. Four pregnant confirmed females were euthanized on gestation day 8 and the remaining four rats were dissected on gestation day 18. Corpora lutea were recorded in ovaries of rats and the uterine tissue was examined for implantation sites. The numbers of live or dead fetuses were recorded. Fertility index and, pre- and post implantation loss was calculated.

The data were presented as mean ± standard deviation (S.D) and data were statistically analyzed using analysis of variance (one-way ANOVA) followed by Dunnet's test. Differences were considered to be significant at $p<0.05$.

3. Results

In the present study, the rats in control and experimental groups exhibited signs of sexual behavior. No significant changes in lacrimation, urination, respiration, vocalization, postural or gait abnormalities were observed in any of the control and nonylphenol treated rats. No mortalities were observed and none of the animals were excluded from the experiment. All the animals were in normal condition. In the present study conception time was increased in females mated with experimental males when compared to control rats. All male rats in 1, 10 and 100 µg nonylphenol groups were able to impregnate the normal control females. Whereas mating index was decreased in 100 µg nonylphenol treated rats (Table 1). Fertility index was lower in females mated with males exposed to 1, 10 and 100 µg nonylphenol when compared to control male rats (Table 1). Females mated with control males had normal implantations. A significant ($p< 0.05$) pre and post implantations losses and reduction in number of live fetuses were observed in females mated with nonylphenol treated males (Table 1, Figures 1and 2).

The mean body weight of the rats treated with 10 and 100 µg nonylphenol treated rats decreased significantly ($p<0.05$), whereas no significant changes were observed in 1µg treated rats when compared to controls (Figure 3).

Table 1. Effect of graded doses of nonylphenol on reproductive performance in male rats

Parameter	Controls	1 µg/kg BW	10 µg/kg BW	100µg/kgBW
Conception time (days)	1.55 [a] ± 0.25	2.01 [a] ± 0 .36 (29.67)	4.38 [ab] ± 0.97 (182.58)	6.67 [bc] ± 1.76 (330.32)
Mating index (%)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)
Fertility index (%)	100 (8/8)	100 (8/8)	100 (8/8)	100 (8/8)
No. of corpora lutea/rat	12.01 [a] ± 1.05	12.10 [a] ± 0.82 (0.74)	13.65 [b] ± 0.67 (13.65)	15.33 [c] ± 0.75 (27.64)
No. of implantations/rat	11.45 [a] ± 0.57	10.36 [ab] ± 0.77 (-9.51)	8.66 [b] ± 0.57 (-24.36)	7.66 [bc] ± 0.57 (-33.10)
Pre-implantation loss (%)	4.66	14.38	36.55	50.03
No. of live pups/rat	10.36 [a] ± 1.56	9.88 [a] ± 1.82 (-4.63)	8.13 [a] ±1.36 (-21.52)	5.81 [b] ± 1.78 (-43.91)
Post-implantation loss (%)	9.51	4.63	6.12	24.15

Values are mean ± S.D of 8 individuals.
Values in parentheses are percent change from that of control.
Mean values in a row that do not share the same superscript differ significantly at $p< 0.05$.

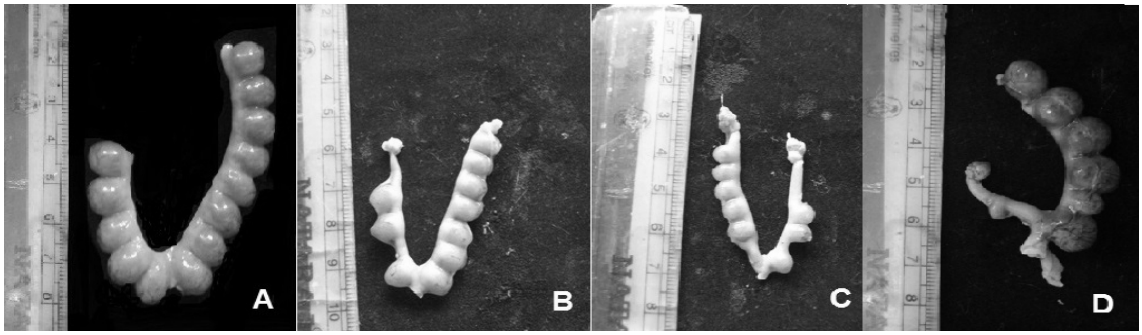

Figure 1. Uterus of females mated with control (A), 1 (B), 10 (C) and 100 µg (D) of nonylphenol/kg bw injected male rats showing implantations on 8th day of pregnancy.

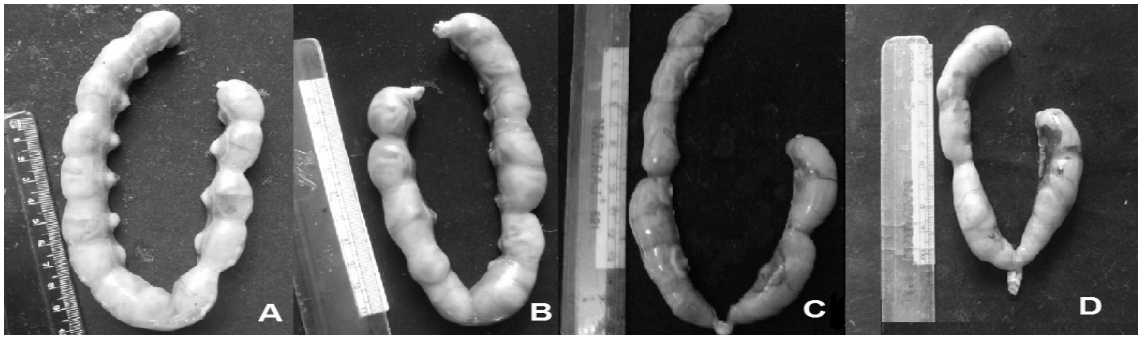

Figure 2. Uterus of females mated with control (A), 1 (B), 10 (C) and 100 µg (D) of nonylphenol/kg bw injected male rats showing implantations on 18th day of pregnancy.

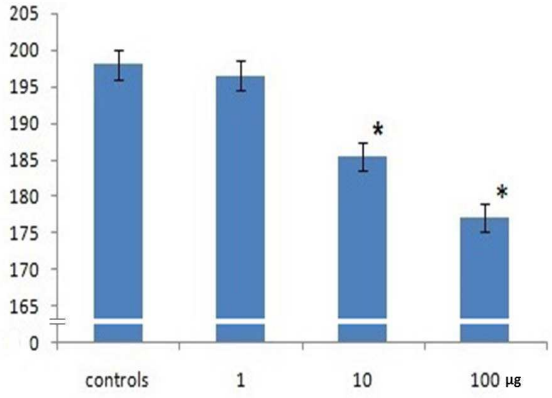

Figure 3. Effect of nonylphenol on body weights (g) of adult male rats.
Bars are mean ± S.D. of 8 individuals.
Bars with *differ significantly from control group at $p<0.05$.

4. Discussion

The reduced body weights in 10 and 100 µg nonylphenol treated animals, indicating that exposure to nonylphenol altered the whole body weights of animals. Similar results were observed when adult male rats exposure with octylphenol (30, 31) and aflatoxin B_1 (32). Adult male rats exposed with aflatoxin reduced body weight and leads to the changes of general metabolic condition of animals (32). The altered metabolic activity may result with reduction of weight of reproductive organs, testes and indicates the reduction of spermatogenesis in rat (data not shown). Sperm quality, quantity and hormones status are major factors for successful fertility. The maintenance of structural and functional integrity of testis and accessory sex organs towards proper spermatogenesis and effective fertility depends upon adequate bioavailability of testosterone (33). Earlier studies reported that administration of nonylphenol resulted in decreased spermatogenesis, decrease in sperm concentration, sperm quality and decrease in testosterone levels (19-21). Disturbed sperm production in rats due to nonylphenol exposure is associated with reduced sperm count, decreased sperm viability and motility, HOS tail coiled sperm, and also decreased serum testosterone levels (authors' unpublished data), those of which are inturn liable for fertilization dysfunction.

It is well known that the production and maturation of healthy spermatozoa is in turn regulated by testosterone. The levels of serum testosterone were significantly decreased in nonylphenol treated rats in a dose dependent manner as compared to the control rats (authors' unpublished data) (23, 34) which leads to disrupted spermatogenesis. Implantation rates were decreased in females mated with experimental males. Pre- implantation loss was significantly increased in a dose-dependent manner in females mated with nonylphenol treated male indicating the toxic effect of nonylphenol on spermatids and spermatogonia. Increased pre- and post-implantation loss in females mated with 100 µg nonylphenol treated males suggests compromised sperm fertility. Nonylphenol affects testicular steroidogenesis and thereby spermatogenesis and subsequently sub-fertility.

Male rats exposed to nonylphenol were able to impregnate the unexposed female and the number of live pups/rat was observed to be comparatively in lower number as compared to control male rat. This is well supported that infertility occurs when the sperm count falls significantly below normal. It was also reported earlier that the fertilization capacity of male also depends on the sperm quality such as motility, viability and morphology (35, 36). Females mated with control males had higher implantation. The fetal loss may occur both before and after implantation. The pre-implantation loss (45.37%) induced by nonylphenol treatment of males is due to a toxic effect on spermatids and spermatogonia and a significant post-implantation loss were observed in females mated with 100 µg nonylphenol treated males. The cause of this manifestation is probably due to fertilization of oocytes with damaged spermatozoa. Similar results were reported in other studies of pubertal exposure with different environmental contaminants such as aflatoxin B_1 (32), heavy metal (34, 37) and phytoestrogens (36) wherein observed suppressed reproductive output in adult male rats. The above studies revealed that reduction of spermatogenesis affect the fertility by decrease in mating and fertility index and increase in pre and post implantation losses in rats.

The present results clearly indicated that exposure to nonylphenol at low doses exhibited adverse effects in adult rats (Authors unpublished data) and their progeny which is characterized by decreased number of fetuses. Exposure to environmental contaminants at low concentrations might exhibit toxic effects in male reproductive performance.

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