The effect of pure phenol on sperm parameters and fertility rate in male mice

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Abstract:

BACKGROUND: Phenol is an estrogenic and toxic compound and people are widely exposed to it in different ways. OBJECTIVES: The aim of this study was to determine the negative effects of phenol on the fertility of male mice, by investigating sperm parameters including viability, motility, fertility rates and daily sperm production (DSP). METHODS: A total of 106 adult NIH mice were divided into four groups, one control and three experimental groups (n=13). The treatment groups were given daily dose of phenol for 35 days through gavage method (30, 75 and 100 mg/kg) while the control group received only normal saline. At day 36, six mice were sacrificed from each group. Gonadosomatic index (GSI), viability, motility and daily sperm production were determined carefully. The remaining 7 mice from each group were used to mate with 2 female mice. On GD10, the female mice were sacrificed and the fertility was verified. RESULTS: Daily sperm production in treatment groups decreased significantly compared to the control group (p≤0.05). Body weight, sperm motility, viability and fertility percentage were significantly decreased in 75 and 100 mg/kg groups in comparison with the control group (p≤0.05). While the testes showed no significant changes in weight in any groups. Gonadosomatic index in the treatment groups compared to the control group, was significantly decreased (p≤0.05). CONCLUSIONS: Pure phenol could reduce fertility rate through decreasing motility, daily sperm production and sperm viability.

Key words: fertility, mice, phenol, sperm

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Introduction

Phenol is one of the most common representatives of toxic organic compounds and most people are widely exposed to it in different ways. Most industrial effluents with high phenol levels come from petroleum refineries, phenolic resin production, plastic and coke oven industries (Louei et al., 2012) and from other natural sources. This makes the chemical to be an important environmental and occupational hazard (Bruce et al., 1987). Phenol has anesthetic and disinfectant properties and is widely used in pharmaceutical products such as ointments, ear and nose drops, sprays, and antiseptic lotions (Finkelstein et al., 2007). The
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Abstract

Detrimental health effects attributed to phenol toxicity in the humans or animals include renal toxicity (Tootian et al., 2012), hematotoxicity (Louei and Salati, 2012; Baj et al., 1994), immunotoxicity (Hsieh et al., 1988), and neurological disorders (Windus-Podehl et al., 1983). The cytotoxicity of phenol was attributed to phenoxy-type radical production from phenol and their ability to impair epithelial cell membrane integrity (Tootian et al., 2012). There are several reports regarding the adverse effects of phenol derivatives including Bisphenol A (Vom Saal et al., 1998; Salian et al., 2009), Nonylphenol (El-Dakdoky and Helal, 2007), Octylphenol (Bian et al., 2006) on male reproductive system during prenatal and neonatal periods.

As far as we know, there is little information on the effects of phenol on sperm parameters and fertility rate in mice. Therefore, this research was done to determine the adverse effects of pure phenol on sperm parameters including motility, viability and daily sperm production and also fertility rate in mature male mice.

Materials and Methods

Chemicals: At first, solid crystals of pure phenol (Merck KgaA 64271 Darmstadt, Germany. C6H5OH; mw 94.11 purity 95%) were weighed, as much as 1 g and dissolved in 100 ml normal saline and 10000 ppm solution was made.

Animals and treatments: A total of 52 male adult mice with 30-40 g weight and 54 female adult mice with 20-30 g weight (NIH strain) were purchased from Razi Institute (Kardj, Iran) and transfered to the Embryological laboratory, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran-Iran. The animals were kept under a 12:12 light cycle with a room temperature of 22-24°C and relative humidity of 50-65%. The animals were acclimated to the laboratory for one week prior to the commencement of the experiment and were fed ad libitum with food and water throughout the experiment. Male mice were divided into 3 treatment groups and one control group randomly (n=13).

The treatment groups received 30, 75 and 100 mg/kg of phenol solution daily by gavage method, using animal feeding intubation needle (Popper and Sons, New York). The control group received only normal saline. Animals were treated for 35 consecutive days. This treatment period is determined to coincide with the period during which one cycle of mice’s spermatogenesis occurs (Rees, 1993). All procedures were carried out in accordance with the institutional guidelines for animal care and use.

Daily sperm production: In each group, 6 randomly selected mice were subjected to necropsy and the left testis as well as vasodeferen duct were removed. After weighing the right testis, its tunica albuginae was separated precisely and weighed. Then testis was homogenized in 5 ml PBS using a manual glass homogenizer. One droplet of this homogenate solution was placed on the hemocytometer and unbroken nuclei of elongated spermatids were counted. All counts from the six (6) hemocytometer chambers were averaged. DSP and its efficiency (DSP/g testis) were determined by dividing the number of spermatids per testis and the number of spermatids per gram of testis with specific factor 4.84 (days during steps 14-16 spermatids for mice) (Takahashi and Oishi, 2003).

Assessment of sperm motility: To determine the sperm’s motility, the left vasoderafer duct was placed in a 35-mm plastic Petri dish containing 2 mL PBS and minced with a scissors. Following incubation at 37°C for 15 min, the tissue was removed and sperm suspension was collected. The percentage of sperms which had progressive movement was determined (Cosentino et al., 1990). Sperm motility was determined by using microscopic meth-
Sperms which had progressive movement were considered.

Evaluation of sperm viability: For determining the percentage of viability of the sperms, the above suspension was put on the slide and by adding eosin-nigrosin solution, the percentage of live sperms, which were not stained, was calculated (Blom, 1950).

Evaluation of fertility rate: After the treatment period, each of the remaining 7 male mice from each group, were kept in an individual cage with two virgin untreated female mice for 10 days. Then the female mice were sacrificed and the embryos were verified and counted. The ovaries were removed, then after trimming of fat, they were washed with normal saline (sodium chloride solution 0.9%). The corpora lutea were counted and the fertility rate was computed by division of the number of embryos by the number of corpora lutea (Oberländer et al., 1994; Fazeli-pour et al., 2010).

Gonadosomatic index: In male mice, in order to determine DSP, the weight of the right and left testes of each mice were divided by the secondary weight of the animal and the percentage of gonadosomatic index was calculated (Fessehaye et al., 2007).

Statistical analysis: Data were expressed in means ± standard deviation (SD). The SPSS software version 16 and One-way analysis of variance (ANOVA), followed by Tukey’s test was used for statistical analysis. Differences at p≤0.05 were considered as statistically significant.

Results

Effects of phenol on daily sperm production: There was a significant reduction in DSP in all treatment groups in comparison with the control group (p≤0.05). Also, there was a significant decrease in DSP between the groups which were exposed to 30 and 75 mg/kg of phenol (p=0.05). However, the difference between groups having mice treated with 75 and 100 mg/kg was not significant (Table 1).

Effects of phenol on sperm motility: When mice were treated with 75 and 100 mg/kg of phenol, significant reduction in sperm motility was found in comparison with the control group and mice treated with 30 mg/kg of phenol (p≤0.05). However, there was no significant difference between mice treated with 75 and 100 mg/kg (Table 1).

Effects of phenol on sperm viability: The percentage of viable sperm reduced significantly in all treated groups in comparison with the control group (p≤0.05). In the treated groups, there was a significant decrease between mice treated with 30 mg/kg and mice treated with the highest dose (p≤0.05; Table 1).

Effects of phenol on fertility rate: The fertility rate reduced significantly (p≤0.05) at the highest dose (100 mg/kg) in comparison with the control group. Also, there was a significant difference between mice treated with 30 and 100 mg/kg (Table 2).

Effects of phenol on Gonadosomatic index (GSI): There was a significant reduction in the Gonadosomatic index of treatment groups compared with the control group (p≤0.05), but the difference between treatment groups was not significant (Table 1).

Effects of phenol on weight of testes and body weight gain: The reduction of testis weight in treatment groups was not significant. Body weight gains in the 75 and 100 mg/kg groups, significantly decreased in comparison with the control group (Table 1).

Discussion

The results of this study show that oral exposure to phenol, can decrease the gonadosomatic index (GSI) in all used doses and body weight gain at the highest dose, but causes no adverse effects on the weight of the testis. In some studies, intraperitoneal administration of nonylphenol in a similar period (35 days), led
to a significant decrease in testicular weight at the highest dose (42.5 mg/kg) (El-Dakdoky and Helal, 2007). Reduction in epididymal weight and sperm density were attributed to the dysfunction of sertoli cells and apoptosis of sertoli and germ cells (Qiu et al., 2005; Han et al., 2004). Another research revealed that administration of bisphenol A (BPA) in the diet of Crj: CD-1 (ICR) mice at the concentration of 0.25%, increased the weight of testes, but no absolute or relative weights of testes were affected in the C75BL/6 CrSlc mice. However, subcutaneously administered bisphenol A at a dose of 200 mg/kg in male jcl: Wistar rats, decreased body weight gain and weight of testis significantly. It was concluded that dietary BPA is not too toxic and estimated that the minimum toxic/maximum non-toxic dose is about 200-300 mg/kg/day. Regarding the toxicity of male reproductive organs, subcutaneous BPA is more toxic than dietary BPA and the route-dependency of BPA toxicity may be due to the differences in toxicokinetices (Takahashi and Oishi, 2003). It has been reported that oral exposure of bisphenol A dimethacrylate (Bis-DMA) at doses of 25 and 100 µg/kg for 4 weeks in mice, could decrease body and testicular weight significantly (Darmani and Al-Hiyasat, 2004). The result of these studies reveals that differences in administration route and duration, dose levels, species and strain could cause different effects on body and testicular weight.

The testicular sperm head count and daily sperm production are considered useful indicators, in detecting the adverse effects on spermatogenesis quantitatively (Ban et al., 1995). In this study, daily sperm production reduced significantly in the treatment groups, in comparison with the control group. Also, the difference between the 30 and 75 mg/kg groups was significant. In other research, it has been shown that oral exposure of bisphenol A dimethacrylate (Bis-DMA) for 4 weeks in mice, can also reduce daily sperm production and sperm count significantly (Darmani and Al-Hiyasat, 2004). Such reduction may be attributed to the direct effects of Bis-DMA on testicular Leydig and sertoli cells, causing a reduction in testosterone production. Sertoli cells, act as so-called nurse cells, providing the structural and metabolic support required by developing

### Table 1. Effects of phenol on testis weight and body weight gain in treatment and control groups. Different lowercase letters indicated significant differences with control group as p≤0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>30 mg/kg</th>
<th>75 mg/kg</th>
<th>100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis weight (g)</td>
<td>0.097±0.01a</td>
<td>0.1±0.01a</td>
<td>0.1±0.01a</td>
<td>0.01±0.01a</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>10.16±3.97c</td>
<td>5.9±4.65b</td>
<td>2.02±4.09a</td>
<td>2.33±3.55b</td>
</tr>
<tr>
<td>Gonadosomatic index (GSI)</td>
<td>0.089±0.0084c</td>
<td>0.57±0.13b</td>
<td>0.56±0.08b</td>
<td>0.53±0.08b</td>
</tr>
</tbody>
</table>

### Table 2. Effects of phenol on daily sperm production, motility, viability and fertility rates in treatment and control groups. Different lowercase letters indicated significant differences with control group as p≤0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>30 mg/kg</th>
<th>75 mg/kg</th>
<th>100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily sperm production (DSP)</td>
<td>11.14±1.58</td>
<td>7.7±0.52</td>
<td>5.45±0.75</td>
<td>4.11±0.89</td>
</tr>
<tr>
<td>Motility</td>
<td>90.67±4.18</td>
<td>83.33±8.76</td>
<td>45±10</td>
<td>58.33±12.11</td>
</tr>
<tr>
<td>Viability</td>
<td>100±0.00</td>
<td>65.75±3.79</td>
<td>54.94±8.24</td>
<td>55±8.67</td>
</tr>
<tr>
<td>Fertility rate</td>
<td>96.3±4.45</td>
<td>95.48±5.12</td>
<td>70.78±26</td>
<td>73.09±33.33</td>
</tr>
</tbody>
</table>

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germ cells. Many factors which are essential for germ cell development are synthesized by sertoli cells (Griswold, 1995; Meehan et al., 2000) and the quantity of daily spermatozoa production is governed by the number of sertoli cells in the seminiferous tubules (Amann, 1970). Any agent that impairs the viability and function of sertoli cells, may have profound effect on spermatogenesis. So, the dysfunction of sertoli cells and apoptosis of sertoli and germ cells may induce the reduction in sperm production (Bian et al., 2006). It is clear that not only the highest dose of phenol can reduce DSP, but also a low dose at 30 mg/kg causes significant reduction in DSP. This revealed that doses lower than 30 mg/kg would also have adverse effects on DSP.

Sperm motility can be used as an important indicator of toxic effect, reflecting weakness of ability on male reproduction. The present study showed that the highest dose of phenol could significantly decrease sperm motility. When rats were treated with 450 mg/kg of octylphenol for 30 days, significant reduction in sperm motility was observed in comparison with the control group (Bian et al., 2006). In addition to testicular factors, epididymal dysfunction may be involved in abnormal sperm motility. It is possible that the effect of octylphenol on sperm motility parameters resulted from its effect on the epididymis (Hess et al., 2001). During their passage through the epididymis, spermatozoa undergoes a maturational process which confers the ability to fertilize an ovum. In addition, spermatozoa acquires the ability for forward progression (McGeady et al., 2006). Thus, significant reduction in sperm motility may be caused as a result of the adverse effects of phenol on the cytoskeleton structure of the flagellum during spermiogenesis and sperm maturation in the epididymis. It has been reported that sperm motility parameters were altered obviously by some toxicants, at the dose not affecting other reproductive endpoints (Toth et al., 1992).

Sperm motility and viability in mice treated with 100 mg/kg of phenol recorded an insignificant decrease in comparison with the 75 mg/kg group, due to the body resistance of mice that received the highest dose.

Ultimately, daily sperm production, motility and viability, reflect in fertility. In these results, the fertility rate recorded a dose-dependent reduction, parallel to the decreases in DSP, viability and motility in the treatment groups. However, the reduction was only significant in the 100 mg/kg group. In spite of the significant reduction in daily sperm production and viability in the 30 mg/kg group, fertility rate and motility did not significantly reduce when compared with the control group. On the other hand, a significant decrease in fertility rate of the 100 mg/kg group, seems to be related to the severe reduction in DSP, but not to reduction in motility and viability.

In conclusion, the results of this study suggest that oral exposure of phenol for 35 days, the completion period of spermatogenesis, had adverse effects on sperm parameters including fertility rate. Although, changes in DSP, motility and viability of sperm in the treatment groups were significant, the highest dose of phenol resulted in the worst effect on the fertility of male mice.

Acknowledgments

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اثر فنل خالص بر پارامترهای اسپرم و میزان باروری در موش سوری نر

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زمینه مطالعه: فنل ترکیبی استروژنیک و سمی است که اغلب افراد به طرق مختلف و به میزان وسیعی در معرض آن هستند.

هدف: هدف از این تحقیق تعیین اثرات منفی فنل بر باروری موش سوری نر از طریق بررسی پارامترهای تحرك، قدرت زنده ماندن و تولید اسپرم است.

روش کار: نیومن (NH) موش سوری نژاد 1064.10锅ازانی در گروه 1 و 18 گروه 2 و 3 به سه گروه تیمار و یک گروه کنترل تقسیم شدند.

نتایج: موش باقیمانده از هر گروه، هر 60 روزی میانه یک ماه تحت تاثیر قرار گرفتند. در روز دهم آبستی موش ها به همراه و میزان باروری تعیین شد.

نتیجه گیری نهایی: فنل خالص با کاهش تحرك و تولید اسپرم و قدرت زنده ماندن اسپرم می تواند میزان باروری را کاهش دهد.

واژه های کلیدی: باروری، فنل، موش سوری، اسپرم

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