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Effect of Formaldehyde Injection in Mice on Testis Function

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Abstract: The medical use of formaldehyde has focused especially on laboratory use. Harmful effects of formaldehyde injection, such as testicular tissue, are quite well documented. However, detailed studies of the effects of formaldehyde on testis functions are quite limited. For this study a total number of 150 mice (60 male and 90 female) were used. Five groups of male mice ($n = 6$) were subjected to intra peritoneal treatment of formaldehyde daily at doses of 0, 2.5, 5, 7.5 and 10 mg kg⁻¹ body weight in a period of 40 days. From each group 3 male mice were chosen for fertility rate. The results showed that the formaldehyde could exert a significant effect on body weight, gonado-somatic-index, fertility, motility and viability of sperm in all treated groups, but no significant decline of serum testosterone concentration, daily sperm production and testis weight were observed in the same groups. The data suggest that the formaldehyde could affect some of the testis function.

Key words: Formaldehyde, testis, mice function

INTRODUCTION

Formaldehyde is used in chemical synthesis as an intermediate in the production of such consumer goods as detergents, soaps, shampoos and as a sterilizing agent in pharmacology and medicine (Korpan *et al.*, 2000). Also formaldehyde is widely used in industrial and domestic environments e.g., paint, plywood, fabrics, cosmetics, heating and cooking emissions and so on (Sari *et al.*, 2004). Humans are exposed to FA from both direct environmental sources (Gurel *et al.*, 2005). However, many studies (Wong *et al.*, 2006; Yu *et al.*, 2005) showed that the levels of formaldehyde in occupational environment were far higher than this limit. In the previous studies, a number of studies have focused on the harmful effects of formaldehyde on respiratory system and hematological system (Ye *et al.*, 2005; Collins, 2004). In the previous studies, a number of studies have found gradual diminution in body and testicular weight and leydig cell impairment. Significant decline of serum testosterone was also observed (Chowdhury *et al.*, 1992). The frequency of dominant lethal mutations in female rats sired by males exposed to formaldehyde was significantly higher than the control group. There was also a reduction of fertile mating in females mated 1-7 day after treatment of males

with formaldehyde (Odeigan, 1997). Formalin feeding in male Japanese quail was associated with decreased weight of testis (Anwar *et al.*, 2001). To date, however, the reports concerning the effects of FA on male reproduction are still scarce and insufficient. So, the present study was designed to further investigate the effects of the administration of the formaldehyde on testis sperm motility, sperm viability, daily sperm production, serum testosterone concentrations, body and testicular weight, gonado-somatic-index and fertility in the male mice.

MATERIALS AND METHODS

Fifty male and 60 female mice at 3 weeks were obtained from Experimental Animal Center of Hesarak Institute in 2005. In this study formaldehyde was injected Intra-Peritoneally (IP). The male mice were divided into 5 groups: (1 control and 4 experimental). The control group received normal saline injections for a period of 40 days and experimental groups exposed to formaldehyde by IP for 40 days. Formaldehyde doses were, 2.5, 5, 7.5 and 10 mg kg⁻¹. From each group six mice were selected and after anesthetizing animals, architectomy was performed. After putting the left testicles into normal saline and using

homogenizer, one drop of the solution was put on the Neobar slide. The sperms were counted by using light microscope and the number of sperms in each cubic milliliter was calculated (Robb *et al.*, 1978).

Daily Sperm Production (DSP): For determining the number of sperms in each gram of the testicle, the number of the sperms was divided to the weight of the testicles and by dividing the result to 4.84, the rate of daily sperm production was calculated (Robb *et al.*, 1978).

Viability: For determining the percentage of viability of the sperms, the above solution was put on the lam and by adding eosine-nicrosine solution, the percentage of the viable sperms, which did not get colored, was calculated.

Motility: For determining the percentage of sperm motility, ductus deferens was extracted from the body and was incubated in normal saline solution in 37°C. Then the ductus deferens was fragmented to extract the sperms and one drop of the solution containing sperms was put on the Neobar lam by slow shaking. The sperms were counted in 10 different visual fields; the number of sperms moving forward was determined and the percentage of the moving sperms was calculated.

Testosterone concentration: From each group, 6 mice were selected and their blood was poured into lab tubes and kept in -40°C for 24 h. Then the blood of the mice was centrifuged and taken to the laboratory for determining the serum concentration of testosterone.

Body weight, testis weight and Gonado-Somatic-Index (GSI): From each group six mice were selected. To determine the changes in body weight, (at the beginning of the experiment) each of the mice was weighed before being anesthetized. After washing the testicles, their weight was measured. The ratio of the weight of both testicles to the body weight was calculated and the percentage was determined and recorded as GSI.

Fertility: From each group three male rats were chosen and each of them was kept with three female mice in a separate cage. After 10 days, the female mice were taken out, anesthetized and the embryos in their uterus were counted. The ovaries were then extracted and washed in normal saline (sodium chloride solution 0.9%). The number of corpus luteum was counted and the fertility rate was determined by dividing the number of embryos to the corpus luteum (Oberlander *et al.*, 1994). In the cases that no embryos were found, the above experiment was repeated for three times.

Statistical analysis: One-way analysis of variance plus Complementary Duncan test.

RESULTS AND DISCUSSION

Significant decline of motility, viability and fertility was observed in all treated groups. Formaldehyde injection at up to 5 mg kg⁻¹ was associated with decreased weight of body and gonado-somatic-index. Daily sperm production and serum testosterone concentration and testis weight of all treated mice did not differ significantly compared to the control group of animals (Table 1).

In this study, injection administration of HCHO for 40 days to adult male mice brought about significant changes in body weight and Gonado-Somatic-Index (GSI), but no significant differences in testis weight was observed. The other researches showed gradual diminution in body and testicular weight in rats were subjected to intra peritoneal treatment of formaldehyde daily at doses 5, 10 and 15 mg kg⁻¹ body weight over a period of 30 days (Chowdhury *et al.*, 1992). Also formalin feeding in male Japanese quail at up to 5 mL kg⁻¹ was associated with decreased weight of testis (Anwar *et al.*, 2001). In this study, the reduction in motility and viability of sperm by formaldehyde were observed. Suppression of sperm motility by the other toxins has been reported by Rao (1997) and Mohamed *et al.* (1986) and growth and

Table 1: Effect of control and experimental groups of among different parameters male's rats exposed to formaldehyde injection

Parameters	Doses mg kg ⁻¹				
	0	2.5	5	7.5	10
Sperm motility (%)	75.0000±2.887 ^a	64.33000±2.171 ^{ab}	48.33000±5.575 ^{bc}	55.0000±4.472 ^b	31.17000±8.565 ^c
Sperm viability (%)	78.33000±6.489 ^a	52.00000±7.528 ^{ac}	54.83000±4.578 ^{ab}	61.20000±6.829 ^b	50.50000±9.976 ^c
Daily sperm production (%)	55.67000±4.096 ^a	40.67000±8.574 ^a	39.33000±5.264 ^a	42.83000±4.826 ^a	48.00000±6.094 ^a
Testosterone concentration (ng mL ⁻¹)	0.30000±0.1000 ^a	2.01300±1.6487 ^a	3.51700±1.0390 ^a	1.28300±0.9027 ^a	1.90000±1.2277 ^a
Body weight differences (g)	13.83000±0.441 ^{ab}	12.17000±2.509 ^{ab}	8.50000±2.206 ^{bc}	5.92000±0.821 ^c	14.58000±0.768 ^c
Testis weight (g)	0.10390±0.01093 ^a	0.09175±0.00698 ^a	0.10618±0.01085 ^a	0.10123±0.00311 ^a	0.09780±0.00803 ^a
Gonado-somatic-index (g)	0.62635±0.05498 ^{ab}	0.59754±0.04166 ^{ab}	0.69660±0.02235 ^a	0.72321±0.03699 ^a	0.56390±0.04576 ^b
Fertility rate (%)	70.46000±18.421 ^a	58.16000±15.014 ^{ab}	59.44000±9.341 ^{ab}	25.26000±12.670 ^b	40.58000±11.745 ^{ab}

Mean±SE, N = 6, unequal letter(s) in each data indicate significant difference at the level of p≤0.05

viability of neonates from mice given oral formaldehyde with doses of 540 mg kg⁻¹ of body weight per day were unaffected (Seidenberg *et al.*, 1987). In this study no significant difference was observed in the daily sperm production of control group and experimental groups. Also no effects on sperm count were seen from formaldehyde after occupational exposure, or in mice following a high acute exposure (Seidenberg *et al.*, 1987). Previous studies have shown that the daily sperm production significantly decreases by the other toxins (Thakur *et al.*, 2003; Oishi, 2002). Another parameter assessed in this study was the effect of formaldehyde on serum testosterone concentration. There were no significant differences between control and formaldehyde treated groups. Although, the other researchers observed a significant decline of serum testosterone through intra peritoneal injection of 10 and 15 mg kg⁻¹ doses in rat (Chowdhury *et al.*, 1992), but marihuana usage dose not affect human male testosterone concentration significantly (Abel, 1981). Other studies suggest that Feeding entophyte-infected fescue seed to ram lambs was associated with potential decreased fertility and increased serum concentration of testosterone (Burke *et al.*, 2006). Also testosterone increases in men after a low doses of alcohol has reported by Seidenberg *et al.* (1987). In this study, it was observed that in female mice despite more presence of corpus luteum, fertility percentage was obviously decreased. This reduction in the fertility of female mice might be due to the adverse effects of formaldehyde on male sexual organs. The other researchers have shown that the frequency of dominant lethal mutations in female rats sired by males exposed to formaldehyde was significantly higher than the control group. There was also a reduction of fertile mating in females mated 1-7 days after treatment of males with formaldehyde (Odeigan, 1997). The above mentioned results are the same as induction of reversible infertility in male rat by ornidazol (Oberlander *et al.*, 1994).

Thus increasing infertility is due to the decrease in motility and viability of sperm and not lowering the sperm production. Results from this study suggest that the formaldehyde might increase infertility in men.

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