Indian J Physiol Pharmacol 1995; 39(1) : 80-82

INHIBITORY EFFECTS OF FORMALDEHYDE ON THE REPRODUCTIVE SYSTEM OF MALE RATS

PRADIP K. MAJUMDER AND VIJAY L. KUMAR*

Department of Pharmacology,
All India Institute of Medical Sciences,
Ansari Nagar, New Delhi - 110 029

(Received on February 15, 1994)

Abstract: Formaldehyde, when administered to rats at a dose of 10 mg/kg body weight/day for a period of 30 days, resulted in a significant fall in sperm motility, viability and count. In addition, the DNA content was significantly lower in testis and prostate while the tissue protein content of prostate and epididymis had decreased in the treated rats. *In vitro* exposure to formaldehyde also inhibited the sperm motility and viability.

Key words: formaldehyde male reproductive system accessory sex organs spermatozoa

INTRODUCTION

Chronic exposure of formaldehyde produces carcinogenic and mutagenic effects (1, 2). Besides inhibiting spermatogenesis, formaldehyde causes degeneration of testicular tissue and impairment of structure and functions of Leydig cells resulting in inhibition of steroidogenesis (3, 4). However, the direct effect of formaldehyde on sperm functions has not been reported earlier. The present study was therefore designed to elucidate and compare the *in vivo* and *in vitro* effect of formaldehyde on sperm motility and viability. The effect of formaldehyde on DNA and protein content of accessory sex organs was also studied.

METHODS

Adult male albino rats of Wistar strain (weighing 130-160 gm) were treated with formaldehyde at a dose of 10 mg/kg body weight given intraperitoneally over a period of 30 days (Group I). Whereas the control group received only distilled water intraperitoneally as vehicle (Group II). On 31st day, the animals were sacrificed by cervical dislocation and testis, prostate, seminal vesicles, epididymis were cleanly removed and weighed.

The tissue was homogenized in 1 ml 10 mM Tris-HCl buffer (pH 8.0). The concentration of protein was determined using Bio-Rad protein assay kit. DNA was estimated according to the method of Burton (5).

Cauda portions of epididymis of both the group I and group II rats were minced separately in 1 ml Kreb's solution to obtain sperm suspension. Sperm count, motility and viability were determined by the methods described by World Health Organization (6).

To study the *in vitro* effect of formaldehyde on sperm parameters, equal volumes of the sperm suspension of normal rats and different concentrations of formaldehyde were mixed and incubated at ambient temperature for different time intervals.

*Corresponding Author

Data were evaluated statistically using student's 't'-test and expressed as mean ± SEM.

RESULTS AND DISCUSSION

In the present study a fall in the tissue protein content was observed in prostate and epididymis while it was not affected in testis and seminal vesicles. On the other hand, the DNA content had significantly decreased only in testis and prostate of treated rats compared to the control rats (Table I). The sperm count had decreased by 50% in treated rats. The sperm viability was also significantly affected and only 30% of viable sperms in the treated group were motile as compared to 86% in the control group (Table II).

TABLE I : Effect of formaldehyde treatment on the male sex and accesory reproductive organs.

Organ	*Protein content µg/mg tissue*	*DNA content µg/mg tissue*
Testis		
Control	63.0 ± 4.53	9.8 ± 1.01
Treated	63.7 ± 7.84	4.6 ±0.37*
Prostate		
Control	95.7 ± 13.17	6.1 ± 1.39
Treated	80.3. ± 9.70	1.2 ± 0.49**
Seminal vesicle		
Control	60.7 ± 2.71	5.7 ± 0.53
Treated	62.9 ± [illegible]	5.0 ± 0.38
Epididymis		
Control	74.7 [illegible]	3.9 ± 0.51
Treated	70.5 [illegible]	3.7 ± 0.22

Values are expressed as mean ± SEM
n = 6 for the control group and n = 8 [illegible] treated group;
*Treated v/s control (P<0.0001); **Treated v/s control (P<0.001).

TABLE II : *In vivo* effect of formaldehyde on spermatozoa.

Parameters	*Control (n = 10)*	*Treated (n = 8)*
Sperm count (million/ml)	46.30± 5.01	20.40 ± 2.01*
Sperm viability (in percentage)	87.10 ± 0.83	72.60 ± 2.32*
Sperm motility (in percentage)	75.00 ± 10.90	22.00 ± 6.40*

Values are expressed as mean ± SEM ;
*V/s control (P < 0.0001).

In *in vitro* study, 80% sperms were viable over a period of 1 h in the control group. At a concentration of 5 ng/ml only 50% spermatozoa were viable for 30 min. At 500 ng/ml concentration 50% spermatozoa were viable for 6 min and at 2.5 µg/ml, the effect was profound and instantaneous, and the sperm viability dropped to zero within 10 min (Fig.1). Sperm motility was more sensitive to the presence of trace amounts of formaldehyde. At a concentration of 125 pg/ml, less than 10% sperms were motile for 10 min while at 5 ng/ml, all the spermatozoa were rendered immotile within this period (Fig.2).

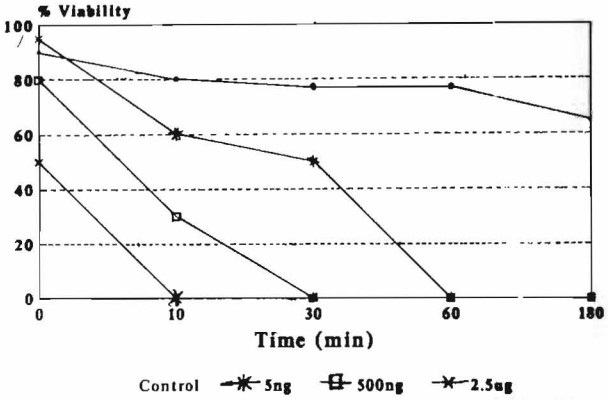

Fig. 1 : *In vitro* effect of formaldehyde on sperm viability. Sperm suspension was prepared from the cauda epididymis of normal adult rats as described in methods. 50 µl of this suspension was mixed with equal volume of different dilutions of formaldehyde to achieve the final concentrations as indicated in the figure. Sperms (viable and dead) were counted at different time intervals.

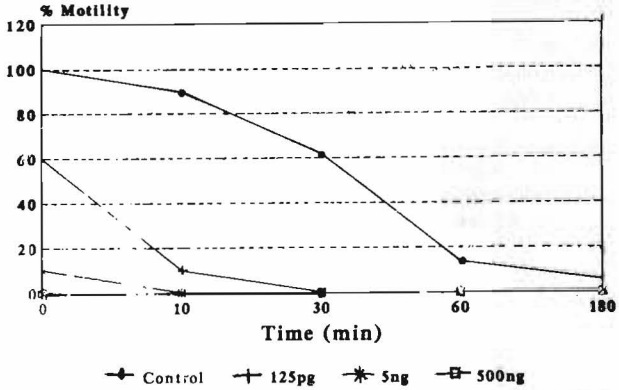

Fig. 2 : *In vitro* effect of formaldehyde on sperm motility. Sperm suspension was prepared from the cauda epididymis of normal adult rats as described in methods. 50 µl of this suspension was mixed with equal volume of different dilutions of formaldehyde to achieve the final concentrations as indicated in the figure. Sperm motility was determined at indicated time intervals.

Chronic exposure of formaldehyde has been shown to impair Leydig cell function and inhibit steroidogenesis resulting in a decline in the serum testosterone level (4). Prostate being one of the main target organs for testosterone is affected the most as shown by a significant decrease in the levels of tissue DNA and protein. A marginal effect on these parameters was also obtained in other accessory organs.

Though the weight and protein content of testis did not change, it was interesting to note a significant lowering in its DNA content. This effect could be attributed to the decrease in Leydig cell population, inhibition of spermatogenesis, degeneration and calcification of testicular tissue (7). These changes are reflected in our study in the form of low sperm count in rats treated with formaldehyde as compared to the control group. In addition, the functional parameters of spermatozoa like, viability and motility, were also affected to a significant extent by formaldehyde treatment.

Our *in vitro* results show that the sperm motility was more sensitive to the inhibitory effects of formaldehyde than their viability, and was first to be affected. The inhibitory effect on sperm functions may be a resultant of the suppression of mitochondrial respiration through inhibition of an energy transfer pathway between the respiratory chain and the site of ATP synthesis (8). At molecular level, formaldehyde reacts readily with various functional groups of biological macromolecules, like proteins, glycoproteins, nucleic acids and polysaccharides in a cross linking fashion thereby altering the physical characteristics of tissue (9). The cumulative toxic effect of intraperitoneal administration of formaldehyde on the male rat reproductive system suggest that a long-term occupational exposure to formaldehyde may lead to male sterility.

REFERENCES

1. Soffritti M, Maltoni C, Maffei F, Biagi R. Formaldehyde : an experimental multipotential carcinogen. *Toxicol Ind Health* 1989; 5 : 699-730.
2. Auerbach C, Moutschen D, Moutschen J. Genetic and cytogenetical effects of formaldehyde and related compounds. *Mutat Res* 1977; 39 : 317-361.
3. Shah BM, Vachharajani KD, Chinoy NJ, Roy Chowdhary A. Formaldehyde-induced changes in testicular tissues of rats. *J Reprod Biol Comp Endocrinol* 1987;7:42-52.
4. Roy Chowdhury A, Gautam AK, Patel KG, Trivedi HS. Steroidogenic inhibition in testicular tissue of formaldehyde exposed rats. *Indian J Physiol Pharmacol* 1992; 36:162-168.
5. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956;62:315-323.
6. WHO laboratory manual for the examination of human semen cervical mucus interaction, WHO, Cambridge, 1987.
7. Sharma SN, Kamboj VP, Kar AB. Calcification of the rat testis after local administration of formaldehyde. *Exp Pathol (Jena)* 1970; 4:309-316.
8. Tyler DD. The inhibition of phosphate entry into rat liver mitochondria by organic mercurials and by formaldehyde. *Biochem J* 1968; 107:121-123.
9. Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde fixation. *J Histochem Cytochem* 1985; 33:845-853.