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Short Communication

Assessment of reproductive toxicity in male rats following sub chronic exposure to benzene

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ABSTRACT

Benzene is well established as a cause of cancer in humans. Widespread exposure to benzene has stimulated research into the possible existence of its toxic effects on the reproductive organs. The present study was therefore undertaken to assess the effects of benzene on male reproductive organs. Benzene at the dose levels of 50 mg and 100 mg/kg body weight/day was administered orally to male rats for 90 days to evaluate the toxic alterations in testicular biochemistry, sperm dynamics and histology. The body weight of the animals did not show any significant change; however, significant reduction in the weight of the testes was recorded. Benzene also brought about a marked reduction in epididymal and testicular sperm counts in treated males. Histology of the benzene-exposed testes showed mild to severe degenerative changes in the seminiferous tubules at low and high dose levels. Testicular glycogen and sialic acid levels were reduced significantly whereas the protein and cholesterol content was significantly elevated. All these toxic effects were moderate at low doses and severe at higher dose exposure. From the results of the present study it is concluded that benzene induces severe testicular damage and results in reduction in sperm count after sub chronic exposure and thus affects fertility. The present study indicates that benzene is a potent testicular toxicant.

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KEYWORDS: benzene, rat, testicular toxicity, histopathology, sperm count.

INTRODUCTION

Benzene (C_6H_6) is an organic hydrocarbon and one of the most widely used chemicals in the synthesis of various paints, polymers, resins and synthetic fibers [1, 2]. There is human exposure to a wide range of environmental volatile organic compounds, among which benzene has been known to have deleterious health effects and it is an identified carcinogen [3].

Exposure to benzene leads to multiple forms of cancer in rodents and humans. Benzene is metabolized in the liver by cytochrome P450 2E1 to various phenolic metabolites which accumulate in the bone marrow [4]. Enzymatic bioactivation of benzene produces reactive intermediates that results in the formation of reactive oxygen species (ROS) [5, 6]. Benzene-induced ROS production alters cell cycle of spermatogenesis, and increases cell death *via* damaging DNA and RNA [7, 8].

Exposure to benzene at work places (petrol pump workers, traffic police and workers at paint industries) hampers sperm production and change sperm characteristics and also reduces acrosin activity [9-11]. Testicular toxicity and antispermatogenic effects have been reported in rodents after exposure to benzene [7, 12, 13]. There is a paucity of research data on how the sub chronic toxicity of benzene resulted in the reduction in sperm count and enzyme activity. Therefore, the present study was conducted to

understand how benzene alters the histoarchitecture of testes and leads to infertility in male rats after 90 days of exposure.

MATERIALS AND METHODS

Sexually mature male Wistar rats (250 ± 20 gms) were used for the present experiment. The animals were housed in plastic cages, fed a standard laboratory diet and water *ad libitum*, exposed to a 12 hrs light/dark cycle, and maintained at a laboratory temperature of 20 ± 2 °C. All the protocols for experiments were approved by the Institutional Animal Ethical Committee (IAEC) of the Department of Zoology, The Maharaja Sayajirao University of Baroda according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Animals were divided into the following four groups, containing 15 rats in each group: group 1, control; group 2, low dose (LD, 50 mg/Kg body weight/ day); group 3, high dose (HD, 100 mg/Kg body weight/ day); and group 4, vehicle control (VC, corn oil).

At the end of the experimentation, the rats were weighed, sacrificed under light ether anesthesia. The male reproductive organs were removed, washed with PBS and processed for biochemical and histopathological studies.

Testicular sperm count

Epididymis of each rat was placed in 1 ml of phosphate buffer saline (PBS) immediately after dissection. Epididymis was mechanically minced in PBS. The tissue sample was aspirated several times to form a homogenous cell suspension. 20 µl of the suspension was placed on a "hemocytometer counting chamber" and the testicular sperm concentration was determined under microscope and expressed as million sperm cells per ml of suspension.

Sperm morphology

The sperm suspension from the epididymis was used for the analysis of sperm morphology. One drop of the suspension was smeared onto a glass slide and stained by eosin. In total, 2000 sperm on each slide were evaluated and the percentage of abnormal sperm on each slide was recorded. Abnormal heads and tails were evaluated using the criteria described by Mori *et al*. [14].

Biochemical parameters

Total protein [15], sialic acid [16], glycogen [17] and cholesterol [18] were estimated spectrophotometrically.

Histological analysis

For microscopic examination (histological studies), fresh tissue samples of the testes were fixed in 10% neutral buffered formalin solution. Tissues were sectioned at 5 µm and stained with hematoxylin and eosin. Histopathological alteration was assessed under a light microscope.

Statistical analysis

The present experimental data were subjected to statistical analysis and presented as the mean and standard error of the mean (SEM). The statistical significance of the differences between the mean values of control and experimental groups was evaluated through one-way analysis of variance (ANOVA) followed by Bonferroni's posthoc test. Statistical analysis was performed using GraphPad Prism 6 software.

RESULTS

During 90 days of benzene exposure, there was no mortality observed in any of the groups. Change in animal body weight is one of the indicators of onset of toxicity. Reduction in body weight in the benzene-treated groups was noted in comparison to the reference group (Table 1). In the control animals, body weight increments were seen throughout the experiment while in the toxicant-treated group loss of body weight was noted after 60 days. There was a statistically significant reduction in the absolute and relative weights of testes ($P \leq 0.05$) in treated rats when compared with control rats (Table 1). There was no observed change in the relative and absolute weights of the epididymis of treated male albino rats.

Sub chronic exposure to benzene caused a significant decrease in the epididymal sperm count compared to control (LD, $P \leq 0.05$; HD, $P \leq 0.01$). As shown in Table 2, the percentages of abnormality in sperm morphology increased significantly in LD and HD benzene-exposed rats compared to control. The significantly increased ($P \leq 0.05$) incidence of sperm with abnormal heads in HD rats was detected. Moreover, the number of

different types of sperm head defects was significantly higher in the LD treatment group ($P \leq 0.05$). The common head defects included detached head from the tail and sperm with knob-twisted flagellum.

Oral exposure to benzene significantly decreased testicular protein ($P \leq 0.01$) content whereas glycogen content was recorded to be significantly low ($P \leq 0.05$) compared to the reference group. Moreover, the content of cholesterol was also found to be significantly higher in both the doses group of rats ($P \leq 0.05$). There was significant depletion in sialic acid level in LD and HD group of testicular content (Table 3).

Figure 1 shows photomicrographs of the testes from the different experimental groups. Testes from the low-dose benzene group showed a slight change from the normal histological features with decreased number of sperm and irregular germinal epithelium (Figure 1A). The histological section of HD groups showed severe degenerative changes marked by damaged germinal epithelium, irregular and incomplete spermatogenesis, vacuolar degeneration, pyknotic nucleus and colloid-filled lumen devoid of sperm. The sertoli cell nuclei were unprotected without peritubular membrane. Pachytene nuclei were recorded with different degrees of degeneration.

Table 1. Animal body weight and organ weight values after 90 days of benzene treatment in male rats.

Groups	Animal weight		Testis		Epididymis	
	0 day (gm)	90th day (gm)	Absolute weight (gm)	Relative weight (g/100 gmbw)	Absolute weight (gm)	Relative weight (g/100 gmbw)
Control	256 ± 10.4	268 ± 11.3	12.9 ± 1.4	4.8 ± 0.4	5.2 ± 0.6	1.9 ± 0.3
VC	258 ± 9.8	270 ± 12.6	12.5 ± 1.3	4.6 ± 0.6	5.6 ± 0.6	2.0 ± 0.4
LD	262 ± 10.5	260 ± 11.8	10.1 ± 1.3*	3.86 ± 0.5 *	4.8 ± 0.7	1.8 ± 0.4
HD	258 ± 10.2	259 ± 10.9	9.8 ± 1.6*	3.78 ± 0.5*	4.6 ± 0.9	1.7 ± 0.5

Results are expressed as mean ± SE (n = 5). * = $P \leq 0.05$.

Table 2. Subchronic effect of benzene on sperm count and sperm morphology.

Parameter	Control	VC	LD	HD
Sperm count (million/ml)	28.2 ± 0.94	28.5 ± 0.94	25.8 ± 1.1*	24.7 ± 1.2**
Abnormal sperm morphology (%)	1.4 ± 0.3	1.4 ± 0.4	2.6 ± 0.8*	2.68 ± 0.9*

Results are expressed as mean ± SE (n = 5). * = $P \leq 0.05$; ** = $P \leq 0.01$.

Table 3. Biochemical modulation on oral administration of benzene in albino rat testes.

Group	Protein mg/gm of tissue	Cholesterol mg/gm of tissue	Glycogen mg/gm of tissue	Sialic acid nmol/mg protein
Control	252 ± 11.3	7.5 ± 0.9	2.0 ± 0.2	5.1 ± 0.3
VC	254 ± 11.8	7.8 ± 0.7	1.8 ± 0.5	5.1 ± 0.6
LD	310 ± 14.7**	10.8 ± 1.3*	1.1 ± 0.8	4.1 ± 0.4*
HD	314 ± 15.9**	11.2 ± 1.7*	0.9 ± 0.7*	4.0 ± 0.5*

Results are expressed as mean ± SE (n = 5). * = $P \leq 0.05$; ** = $P \leq 0.01$.

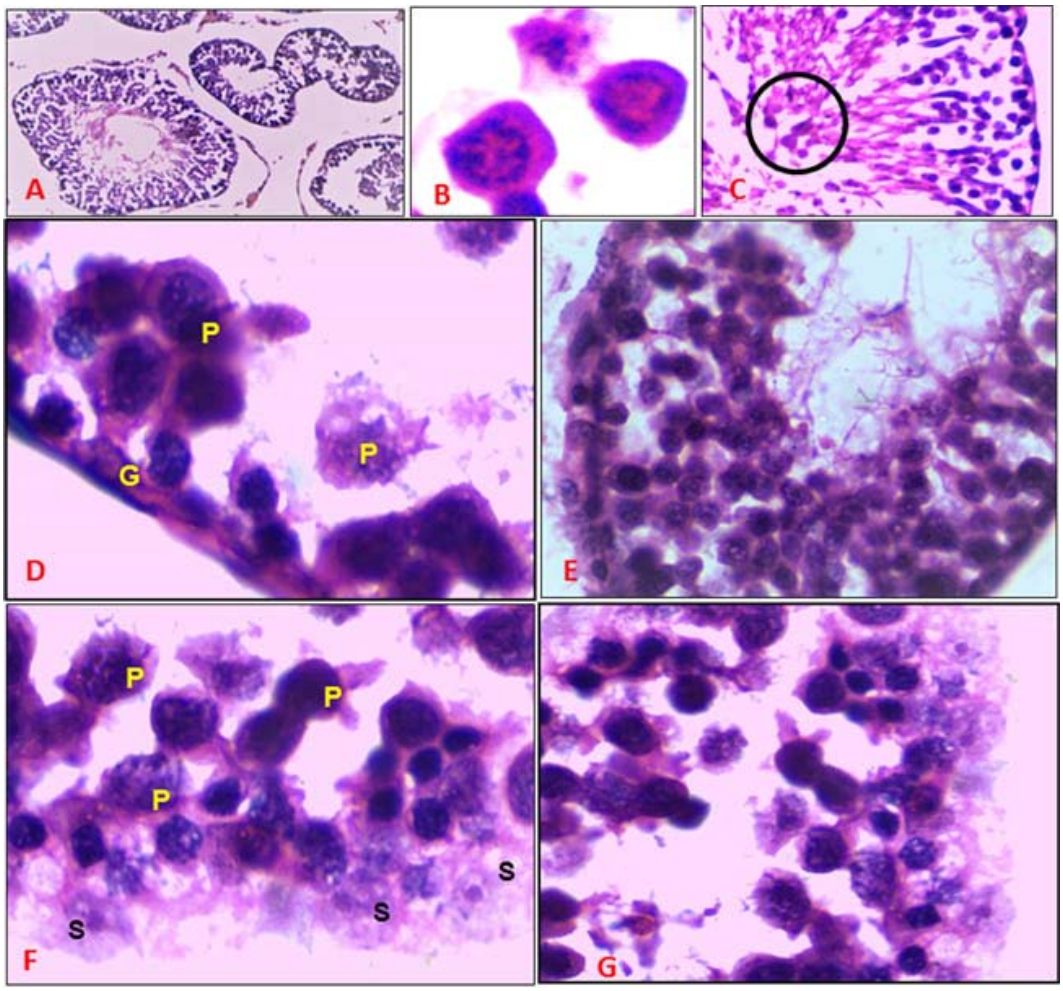

Figure 1. Histopathological alteration of testes after sub chronic exposure of rats to benzene. A) Interstitial damage, elongated tubules without sperm and seminiferous tubule degeneration with immature spermatid, B) Nuclear damage with disorganised chromatin fragments, C) Lumen of tubules filled with sloughed spermatogenic cells and cell debris, D) Extensive disintegration of pachytene (P), necrotic lesion in pachytenes, sertoli cell disintegration and absence of mature spermatogonia, E) Vacuolar degeneration and lumen with abnormal sperm, F) Peritubular membrane damage and unprotected sertoli cell, pachytene nuclei with different degrees of degeneration, G) Pyknotic nuclei and loss of cell adhesion.

DISCUSSION

After 90 days of benzene exposure, non significant reduction in the total weight of rats was observed as a result of oral irritation or buccal pain during food intake. Benzene exposure to male albino rats caused marked alteration in the weight of their testes. The significant reductions in the testicular weight of the treated animal suggest regressive changes in the seminiferous tubule [19, 20].

Benzene induces biochemical changes in the male reproductive organ. The level of glycogen was observed to be significantly low after sub chronic exposure to benzene. Inhibition of glycogen synthesis may be due to altered glucose metabolism that eventually decreased spermatogenesis [21, 22]. The level of protein concentration was observed to be significantly higher in the benzene-treated groups. The high content of protein may be due to overproduction of growth protein for spermatogenesis [23]. Benzene induces DNA mutation and mutational translation resulted in the accumulation of non-functional proteins [24].

Statistically significant reduction in the testicular sialic acid level due to benzene administration indicates inhibition of testosterone and the activity of gonadotrophins. The low level of sialic acid concentration in treated rats might be due to the loss of viability and fertilizing ability of spermatozoa [22, 25]. Due to sub chronic exposure to benzene, low level of testicular sialic acid content was observed. Depletion of sialic acid content in male rats, due to xenobiotic exposure was observed by other scientists [26-28]. In the present study, increased level of cholesterol was recorded and this suggests benzene exposure hampers the production of androgen.

Benzene exposure to rats causes development of spermatotoxicity including sperm deformities and reduction in sperm count. Administration of the mixture of an organic solvent with benzene caused reduction in total spermatozoa count and daily sperm production with deformities in sperm morphology [7, 29, 30]. Declined level of sialic acid resulted in a decrease in the rate of spermatogenesis [31]. Sialic acid content and low count of sperm noted in the current study is in correlation with the observed adverse effects of benzene. Vacuolar degeneration of spermatogenic germ cells of seminiferous tubules, pyknotic nuclei, desquamation and dissociation of germ cells in tubule lumen were commonly observed. Furthermore, benzene treatment caused decrease in testicular tubular diameters. Lumen of seminiferous tubules was filled with colloid or cellular debris without sperm. The present result is in accordance with previously reported studies that indicate the depletion of sperm count and the cytotoxic effects on testes after benzene exposure [7, 13, 32].

CONCLUSION

The current investigation demonstrates the testicular toxicity of benzene after sub chronic oral exposure in male albino rats. Sialic acid and glycogen content were found to be depleted whereas elevation in protein and cholesterol content was noted after 90 days of benzene treatment. Inhibition of testicular spermatogenesis as a result of benzene administration exhibits an evidence of depletion of sperm count and deformities. It is associated with decreased testicular weight and degeneration of seminiferous tubules.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Velasco-lezama, R., Barrera_Escorcia, E., Torres, A. M. and Aguilar, R. T. 2001, Toxicology, 162, 179.
2. Rushton, L., Schnatter, A. R., Tang, G. and Glass, D. C. 2014, Br. J. Cancer, 110, 783.
3. Karakisios, S., Papaloukas, C. and Kassomenos, P. 2007, Atmosph. Environ., 41, 9555.
4. McHale, C. M., Zhang, L. and Smith, M. T. 2012, Carcinogenesis, 33, 240.
5. Badham, H. J. and Winn, L. M. 2010, Toxicol. Sci., 113, 207.
6. El-Shakour, A. A., El-Ebiarie, A. S., Ibrahim, Y. H., Moneim, A. and El-Mekawy, A. M. 2015, J. Environ. Occup. Sci., 4, 33.
7. Singh, R. K. and Bansode, F. W. 2011, J. Environ. Biol., 32, 687.
8. Lavranos, G., Balla, M., Tzortzopoulou, A., Syriou, V. and Angelopoulou, R. 2012, Reprod. Toxicol., 34, 298.
9. Xiao, G., Pan, C., Cai, Y. Z., Lin, H. and Fu, Z. M. 2001, Industrial Health, 39, 206.
10. Mandani, P., Desai, K. and Highland, H. 2013, Toxicology, Article ID 397524.
11. Kamal, A. and Rashid, A. 2014, Int. J. Occup. Med. Environ. Health, 27, 830.
12. Sabourin, P. J., Chen, B. T. and Lucier, G. 1987, Toxicol. and Appl. Pharmacol., 87, 325.
13. Zhao, D., Zhu, C., Sun, S., Yu, H., Zhang, L., Pan, W., Zhang, X., Yu, H., Gu, J. and Cheng, S. 2007, Toxicol. Indust. Health, 23, 47.
14. Mori, K., Kaido, M., Fujishiro, K., Inoue, N., Koide, O., Hori, H. and Tanaka, I. 1991, Br. J. Ind. Med., 48, 270.
15. Lowry, O. H., Rosbrough, N. J., Farr, A. L. and Randall, R. J. 1951, J. Bio. Chem., 193, 265.

16. Warren, L. 1959, J. Biol. Chem., 234, 1971.
17. Seifter, S., Dayton, S. and Novic, E. 1950, Arch. Biochem., 25, 191.
18. Henly, A. A. 1957, Analyst, 82, 286.
19. Allan, C. M., Garcia, A., Spaliviero, J., Zhang, F., Jimenez, M., Huhtaniemi, I. and Handelsman, D. J. 2004, Endocrinology, 145, 1587.
20. O'Shaughnessy, P. J., Hu, L. and Baker, P. J. 2008, Reproduction, 135, 839.
21. Tizzard, I. R. 1996, Veterinary Immunology-An Introduction, WB Saunders Co., (5th Ed.), 43.
22. Joshi, S. C., Mathur, R. and Gulati, N. 2007, Toxicol. Indust. Health, 23, 439.
23. Huang, Q., Lianzhong, L., Ambreen, A., Jie, Z., Liangpo, L., Meiping, T., Syed, A. M., Akber, S. E. and Heqing, S. 2016, Sci Rep. 6, 32518.
24. Christopher, E. E. and Asuquo, E. A. 2017, Int. J. Occup. Med. Environ. Health, 30, 1.
25. Kumari, M. and Singh, P. 2013, Int. J. Fertil. Steril., 7, 225.
26. Bedwal, R. S., Edwards, M. S., Katoch, M., Bahuguna, A. and Dewan, R. 1994, Ind. J. Exp. Biol., 32, 243.
27. Kackar, R., Srivastava, M. K. and Raizada, R. B. 1997, Industrial Health, 35, 104.
28. Aiso, H., Arito, T., Nishizawa, K., Nagano, S. Y. and Matsushima, T. 2005, J. Occup. Health, 47, 249.
29. Kishta, O., Adeeko, A., Li, D., Luu, T., Brawer, J. R., Morales, C., Hermo, L., Robaire, B., Hales, B. F., Barthemy, J., Cyr, D. G. and Yrasler, J. M. 2007, Reprod. Toxicol., 23, 1.
30. Shi, Y., Song, Y., Wang, Y., Liang, X., Hu, Y., Guan, X., Cheng, J. and Yang, K. J. 2009, Biomed. Biotechnol., 1, 11.
31. Mann, T. 1964, Biochemistry of the Semen and the Male Reproductive Tract. John Wiley and Sons (Ed.), London, 391-392.
32. Gupta, R. S., Bhatnager, A. K., Joshi, Y. C. and. Sharma, M. C. 2005, Pharmacol., 75, 57.