Journal of Environmental Chemistry and Ecotoxicology Vol. 4(14), pp. 250-253, December 2012
Available online at http://www.academicjournals.org/JECE
DOI: 10.5897/JECE10.068
ISSN-2141-226X ©2012 Academic Journals

Full Length Research Paper

Impairment of testis in rats after exposure to orthochlorobenzylidene malononitrile (CS): An experimental study

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Accepted 6 November, 2012

The acute toxic effects of orthochlorobenzylidene malononitrile (CS) on the rat testis were analyzed in terms of body weight, serum testosterone and histopathology of testis. The animal experiments were designed by intra-peritoneal injections of CS at 25 and 50% of lethal dose at 50% (LD_{50}) for 10 days. In three groups of 10 each Charles Foster rats, olive oil (0.5 ml), 10 and 20 mg/kg (in 0.5 ml olive oil) intra-peritoneal (ip) was daily administered, respectively (Groups A, B, and C) for 10 days. The rats were sacrificed on the 11th day by cervical dislocation and blood and testes were collected for analysis. The results showed loss in body weight in Group C (20 mg/kg CS). A decline in testes weight of Groups B and C was noted. There is degeneration of testis and high cholesterol with low serum testosterone which suggested block of testosterone biosynthesis at Leydig cells in Group C. Group B showed partial testicular degeneration and no significant changes in serum testosterone levels. The low serum testosterone in Group C shows inhibition of spermatogenesis. This study is a first report on the acute toxicity of CS for rat testis by low testosterone level leading to inhibition of spermatogenesis.

Key words: Orthochlorobenzilidene malononitrile (CS), rat, testis.

INTRODUCTION

Orthochlorobenzylidene malononitrile (CS) was synthesized in 1928 by Corson and Stoughton (Ballantyne and Callaway, 1972) for use in warfares (Punte et al., 1963) as it causes mucous membrane and skin irritation (Ballantyne and Swanston, 1978), which is due to reduction of chlorine atoms to hydrochloric acid (Hu et al., 1989). In 1958, CS replaced chloraceto-phenone (CN) in riot control operations to incapacitate persons (due to unpleasant symptoms involving eyes, skin, mouth, nose, and respiratory tract) without an injury or illness (Anderson et al., 1996) and for the reason that, it is effective and safer than CN when used in open air (Beswick, 1983). Intensive CS used during short time at focal points to disperse violent public indicated ill effects (Chapman and White, 1978). CS was shown to cause structural and functional impairment of rat respiratory tract (Debarre et al., 1999) due to its direct effect on nerve endings (Brimblecombe et al., 1972) adrenal and thyroid (Roy-Chowdhury et al., 1978a). The present first hand report was an outcome that occupationally exposed workers at the production site of CS to infertility and loss of libido (Roy-Chowdhury and Venkatakrishna-Bhatt, 1999) which prompted us to carry out a biological evaluation in order to confirm the suspicions of people about the cumulative action of CS leading to infertility.

MATERIALS AND METHODS

Thirty male adult Charles Foster inbred rats weighing 180 ± 5 g age of 6 to 8 weeks were equally divided into 3 groups (Group A, B, and C) and held a week before testing. They were kept at 12:12 h light-dark cycle in a climate control room (humidity 50.90% and temperature 24°C) with free access to staple diet [wheat flour 70%, bengal gram (a cereal of Bengal (India) origin) 29%, fish-meal 5%,

Table 1. Effect of CS on body weight, testicular and epididymal weight, serum testosterone and morphology.

Group	Body weight* (gms)	Testis weight (gms)	Epididymis weight (mg/100 g b.w.)	Seminal vesicle weight (mg/100 b.w.)	Serum testosterone (ng/ml)	Microscopic observations (20 slides/group)
Control (Group A)	205.10 ± 5.53	1.74 ± 0.6	341 ± 2.1	378.1 ± 3.1	484.32 ± 78.5	Active spermato-genesis
Group B. (10 mg/kg CS)	194.89 ± 5.53	1.58 ± 0.8	26 ± 2.2[b]	345.7 ± 2.7	480.00 ± 72.82[NS]	Partial changes in gametogenic cells complete
Group C (20 mg/kg CS)	174.25 ± 4.81[a]	1.34 ± 0.4[b]	280 ± 0.8[a]	305.2 ± 5.42[a]	215.10 ± 27.70[a]	Degeneration of gametogenic cell

NS, Not significant; p < 0.001; b = p < 0.05; *, mean body weight obtained before sacrifice. Values are Mean ± SE; n = 10 in each group.

dried yeast powder 4%, shark-liver oil 1%] and *ad-libitum* water. Since the study is for a long period, the mice were reared with special attention regarding cleanliness of cage, replacement of paddy husk every day, easy amenities of drinking water without spillage (lick-delivery open nostled bottles (6" × 3" × 2" tapering rubber bearing blunt open delivering glass tube of 5 mm diameter) and sufficient maneuvering space by limiting 6 animals/Perspex transparent cage (2' × 2') with a perforated cover for free air-circulation (Nigam and Venkatakrishna-Bhatt, 2011). The protocols for animal experiments were approved by the Institutional Ethical Committee.

Group A served as control (n = 10) which received 0.5 ml intra-peritoneal (ip) olive oil daily for 10 days. Groups B (n = 10) and C (n = 10) were treated with ip CS in olive oil at 10 and 20 mg/kg in a volume of 0.5 ml similar to the control. The CS doses were 25 and 50% of the lethal dose at 50% (LD_{50}) (40 mg/kg ip) for the male rat (Roy-Chowdhury et al., 1979).

After 10 days of treatment on the 11th day, blood was collected from overnight fasting rats from retro-orbital venous plexus for the estimation of serum testosterone by radioimmunoassay (Jeffcoate, 1971) where the anti-human serum testosterone as the anti-sera and testosterone$^{(Isotope\ 125)}$ has the labeled isotope which was used as RI[1] of the kit. The animals were then sacrificed by cervical dislocation and testes were dissected out, weighed, half-paired, and fixed in Bouin's fluid. 5 µm paraffin sections were cut and stained with haematoxylin and eosin for microscopical examination (Roy-Chowdhury and Venkatakrishna-Bhatt, 1983). Half-paired portion from each group were used for the estimation of testicular cholesterol (Sperry and Webb, 1950). Statistical analysis was performed by student's 't' test (Campbell and Machin, 1999).

RESULTS

Table 1 shows the effect of CS on the body weight, testicular weight and cholesterol, serum testosterone and morphology of control and test groups. Significant decline in body weight was noted in Group C, which received CS 20 mg/kg ip daily for 10 days and in general, a dose-response effect was observed. The testicular weight decreased significantly (p < 0.05) in the test groups. The testis cholesterol is enhanced at the high CS dose commensurate to the decreased serum testosterone (that is, 330 ± 0.9 against 410 ± 0.6 mg/100 g in control versus Group C), respectively. Figure 1 demonstrates cross section of testis of control (Group A, n = 10) and CS treated groups (Group B, n = 10), and Group C, n = 10). The results showed apparent alterations with definitive degenerative changes of testicular tissue along with highly significant testicular cholesterol and decreased serum testosterone in Group C. In Group B, rat testis showed partial degeneration of testicular tissue along with non-significant levels of testicular cholesterol and serum testosterone in comparison with normal spermatogenesis and Leydig cells of the control (Group A). There has been significant (p < 0.01) exponential decrease in body and testis weights after exposure to CS, whereas changes were significant (p < 0.05) in epididymis, seminal vesicle weight, and serum testosterone by a diminution with the high CS dose being commensurate with the partial changes in the gametogenic cells and complete degeneration of the gametogenic cells, respectively.

DISCUSSION

CS "tear gas" is a lacrimating riot control agent causing eye irritation, excessive lacrimation, and blepharospasm. CS is manufactured and used as a tear gas primarily for peace keeping operations involving crowds or only one or two isolated violent individuals (Bernard et al., 2005). The necessity to stop dangerous and highly agitated persons leads law enforcement forces to search for strong incapacitating properties of chemicals. The incapacitating properties are the result of the chemical action on nerve endings (Brimblecombe et al., 1972). The effects and signs observed concern eyes, skin, nose, and respiratory tract (Punte et al., 1962) besides sensory irritation (Alarie et al., 1973). These observed effects need not be at the operational site but also at the manufacturing site unless safety measures are adopted. Inspire of safety measures, workers in these chemical plants, do complain of impotency and the literature in this area is not available

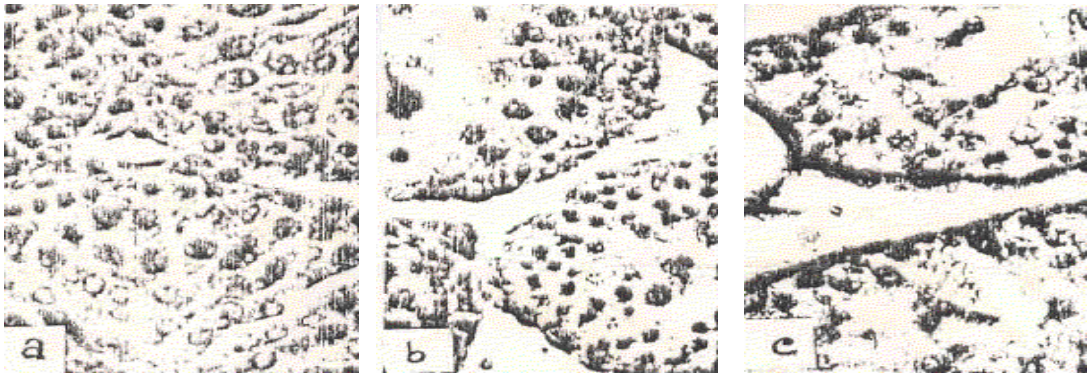

Figure 1. a) Testicular cells from control rat showing normal cellular organization in tubules and Leydig cells 400×; b) testis from a rat at a dose of 10 mg/kg (ip) CS showing intertubular space, degenerative changes in spermatogonic cells and Leydig cells 400×; c) testis from a rat at a dose of 20 mg/kg (ip) CS showing complete degeneration of seminiferous tubules and Leydig cells 400×.

or forthcoming. As a follow up of an earlier study of CS effects on adrenal and thyroid (Roy-Chowdhury et al., 1978a), CS effects on rat testis has been reported in this pilot study. We have examined the ip acute and subacute CS effects on the rat testis over a period of 10 days. CS solvent olive oil did not interfere with body weight nor any of the parameters studied though known to elevate the oxidative status in respect of plasma inorganic phosphorus (Patel and Venkatakrishna-Bhatt, 1999) potassium in cerebral hemispheres and lower potassium in plasma, red blood corpuscles, cerebellum, liver when compared to saline (0.9% NaCl) fed control rats exposed over a period of 30 days (Patel and Venkatakrishna-Bhatt, 2000). It decreased sodium in the target organs except in the red blood corpuscles. During acute exposure, olive oil had no effect in respect of studies on the rat thyroid (Roy-Chowdhury et al., 1978b) and adrenals (Roy-Chowdhury et al., 1979). The doses of CS were selected as 10 and 20% of LD_{50} (that is, 40 mg/kg/ip/rat; Roy-Chowdhury et al., 1979) only to find the initial formative changes of the testis abnormality in the rat since effects produced by these potent tear gases are strongly dependent on the concentration of the compound and the duration of exposure. Thus, the screening of riot control agents for safety includes conventional, acute and sub-acute chronic toxicity in order to identify any specifically vulnerable vital organs (Upshall, 1973). The ip route is specifically chosen to ensure a total effect of CS and to precipitate its effect on the targeted organ at mild and moderate dose levels in a short period as a pilot and confirmative study.

The gross CS effects on different animal models were well documented in literature (Marrs et al., 1983). Primarily data in this study shows a dose dependent effect in the changes observed in test groups against the control group. Significant decline in body weight was noted in Group C receiving 20 mg/kg/ip body weight. Loss in body weight reflects various metabolic changes that could be a primary effect of CS. Diminution of testicular weight was also observed in both the experimental groups (Table 1). A definite degeneration of the testicular tissue along with significantly high testicular cholesterol level and low serum testosterone suggests the inhibition of testosterone biosynthesis by the Leydig cells (Roosen-Runge, 1962). Inhibition of spermatogenesis in CS treated rats is a result of degenerative changes and less number of spermatogonial cells along with few sperms in the lumen of seminiferous tubules. Further changes like interstitial oedema, rupture of seminiferous tubules, basement membrane thickening, and spermatogenesis suppression at different stages were observed. In Group B, partial degeneration in testicular tissues along with non-significant level of testicular cholesterol and serum testosterone were observed and this may possibly be due to less toxic dose in comparison to Group C. The partial degeneration by low CS with no change testosterone levels suggests a process of recovery whereas low testosterone definitely suggests testicular damage at a higher dose of CS. This inadequate level of testosterone in Group C may lead to inhibition of spermatogenesis (Roy-Chowdhury, 1987). The diminution of serum testosterone levels and degeneration in Leydig cells indicated a definite alteration in the process of stereoidogenesis. During carbon disulphide toxicity similar observations were made, where marked degenerative changes in Leydig cells with less number of spermatozoa were found (Tepe and Zenick, 1984). Testicular and epididymal weight change need not coincide with serum testosterone decline (Patel et al., 1999). However, the inhibition of spermatogenesis is not always degeneration of testicular tissue. Further, degeneration of testis and its high cholesterol matched with low serum testosterone suggested block of testosterone biosynthesis at Leydig cells in Group C. Group B showed partial testicular degeneration and no significant changes in serum testosterone levels. The low

serum testosterone in Group C shows inhibition of spermatogenesis. This study is the first report on the acute toxicity of CS for rat testis by low testosterone level leading to inhibition of spermatogenesis.

Pure inhibitory effect of spermatogenesis may lead to a type of spermatocytic arrest or testicular hypoplasia. Degeneration is not an inhibition of cell development but a process of damage to mature cells. Further thickening of peri-tubular membrane, degeneration and disorganization of spermatogonial cells, and less number or absence of sperms in the lumen was observed in CS 50% LD_{50} treated groups. In a few tubules, lumen was filled with cellular debris. The present study is a preliminary report and further elucidation of seminal vesicle, prostate, Leydig cells, vas deferens, sertoli, and spermatogonia cell architecture under exposure to CS would give more insight about the CS male reproductive toxicity.

ACKNOWLEDGEMENTS

Thanks are due to the late Dr. A. Roy Choudhury who assisted during the study and in drafting the paper (Regional Occupational Health Centre (E), Block DP, Sector V, Vidhan Nagar (Salt Lake City), Calcutta 700 091, West Bengal, India), the Director, DRDE, Gwalior and the Director, NIOH, Ahmedabad for the laboratory facilities. We also thank the Editor and the Publishers of this paper.

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