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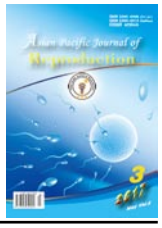

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Dose and duration dependent cytotoxicity of aroclor 1254 in the testis of mice

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ABSTRACT

Objective: To evaluate the dose and duration dependent cytotoxicity of aroclor 1254 on mice testis. **Methods:** The study tests the hypothesis that in vivo exposure of very low dose aroclor 1254, comparable to that of possible human exposure from different environmental sources, will provoke dose and duration dependent histological damage in the mice testis. Male mice were orally administered with the two doses 0.1 and 1.0 mg/kg b.w /d. of aroclor 1254 for 7, 14, 21 and 28 d. **Results:** Results showed the degenerative changes in the testis of mice, namely, atrophied seminiferous tubules, expanded space in interstitial and necrosis in the germinal epithelial cells of seminiferous tubules, of the seminiferous tubules and deceleration of spermatogenesis. **Conclusions:** Therefore, the results of the present study suggested that the sub-acute exposure of very low doses of aroclor 1254, can subsequently mediate the cytoskeleton dysfunction in the testis of mice.

1. Introduction

Polychlorinated biphenyls (PCBs) are highly lipophilic and stable compound. Aroclor 1254 commercial mixture of PCBs, which are usually given a four-digit number, of which the first two digits that refer to the number of carbon atoms attached to the biphenyl ring and the last two digits indicate the percentage of chlorine atoms[1]. Polychlorinated biphenyls are well known persistent organic pollutants, that present in the environmental and disrupting endocrine and reproductive functions in wild animals, experimental animals, and as well as in humans reproductive system still occur widely[2,3]. PCBs exposure induce the production of reactive oxygen species affects the lipid metabolism, endocrine function including reproductive dysregulation, immune system damage, nervous system disorders, skin disorders such as chloracne, and an array of sensory defects in aquatic organisms at many tropic levels as well as in both humans and wildlife[4-8]. In male reproductive dysregulation, it reduce weight of testis and accessory sex organs, decrease the cellular contents in the seminiferous tubules, disturbs the spermatogenesis, reduce the sperm counts in mammals[9-11]. In the human, the levels of PCBs have been inversely correlated to the increased testicular sperm counts that may possibly be associated with Sertoli cell changes[12]. Chronic PCBs exposure has also been associated with the histological alterations which are undoubtedly responsible for the depressed fertility in mice[13,14]. Therefore, the present study was to evaluate the low concentration, similar to the environmentally available concentration in the PCB polluted areas, and sub-acute dose and exposure duration dependent cytotoxicity of aroclor 1254 on the mouse testis.

2. Materials and methods

A total of 36 healthy 4 mo old male Swiss Albino mice of about 30-40 g body weight were considered for the experimental purposes. The animals were acclimatization for a period of 2-3 d prior to the experiment and grouped before the experiments. The experiments were conducted according to the ethical norms approved by the CPCSEA (R. No. 757/03/a/CPCSEA, 06-05-2003). The toxicant, aroclor 1254 (CAS No. 11097-69-1) was procured from Sigma

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Chemical Company Inc., India. For each experiment four groups of animals were used. Each group contained three animals. Each experiment was repeated at least three times. The doses selected were very low concentrations of aroclor 1254, comparable to that of a possible human exposure from different environmental sources. Oral administration of aroclor 1254 different doses (0.1 and 1.0 mg/kg body weight /d) dissolved in corn oil (vehicle) for four different exposure durations of 7, 14, 21 and 28 d. After exposure of desired doses and duration, the testis were removed, fixed in Bouin's Fluid, cleaned, dehydrated in graded series of alcohol and embedded in paraffin wax[15]. Ten micrometer thick sections were cut and stained with hematoxylin and eosin (H & E). For observational purpose few slides were considered. Each slide contained two sections and ten field areas of each section were examined for the histopathological changes. The examination was done using Carl Zeiss Axioscop 2 compound microscope fitted with 3CCD color vision camera (DONPISHA XC-003).

3. Results

In the present investigation compared to control, significant histopathological changes in the testis exposed to different sub-lethal concentrations of aroclor 1254 were evident. In the control group,

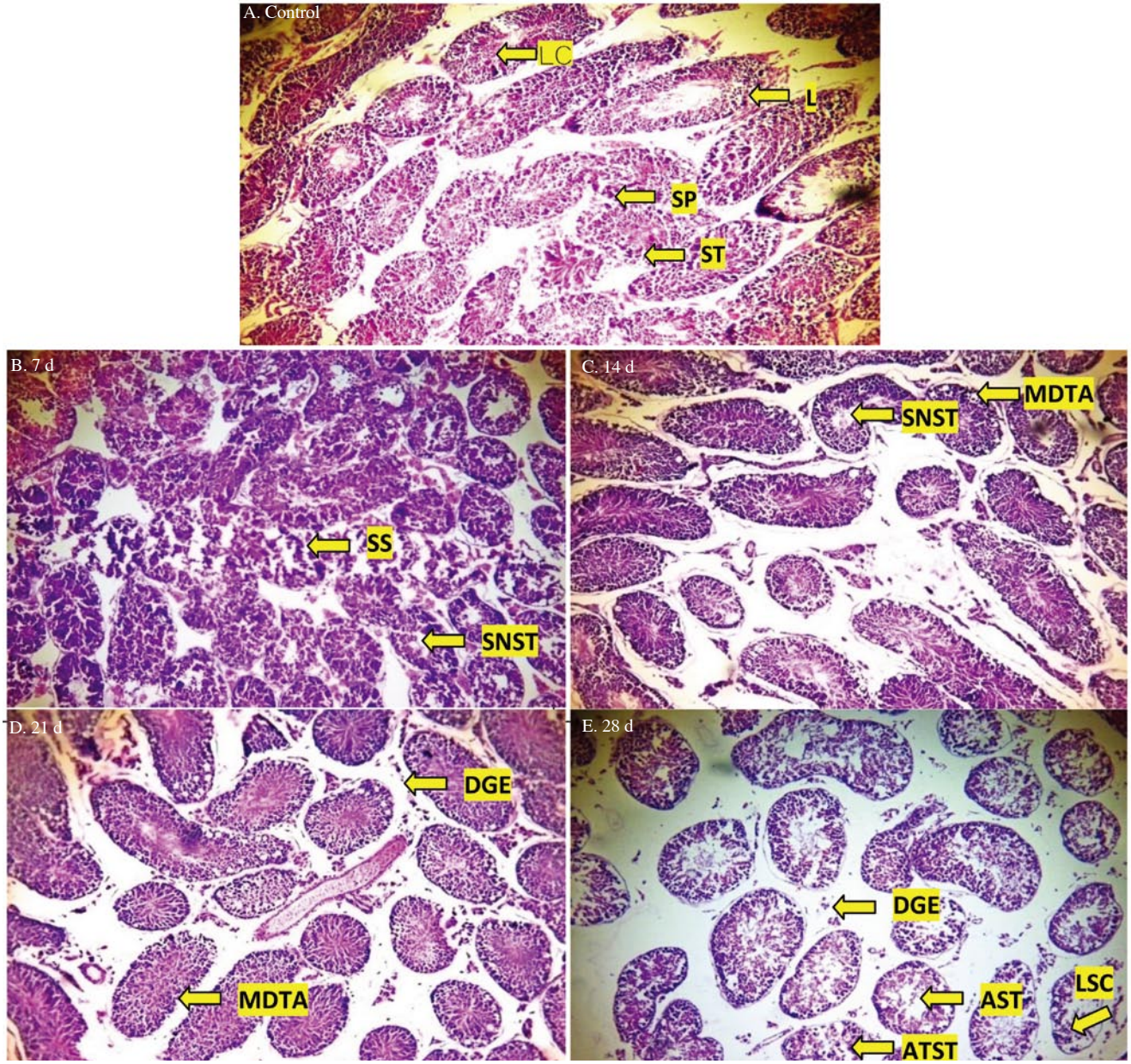

Figure 1. Photomicrographs showing the histopathological alterations in the testis of mice after the exposure of 0.1 mg/kg/day dose of aroclor 1254 for 7, 14, 21 and 28 d as compared control (H & E, 100 ×).

(A) Control revealed the normal testis with Leydig cells (LC), spermatozoa (SP), seminiferous tubules (ST), lumen (L). (B) After 7 d exposure: Severe necrosis in SNST, SS. (C) After 14 d exposure: MDTA, severe necrosis in SNST. (D) After 21 d exposure: MDTA, disorganization of DGE. (E) After 28 d exposure: AST, LSC, and DGE, ATST.

normal regular features of the seminiferous tubules, compactly organized germinal epithelium were observed (Figure 1A & 2A). However, histopathological examinations revealed considerable tissue damage in the aroclor 1254 exposed animals. Several types of lesions were observed in the testis exposed to all the doses and exposure durations. After the exposure of 0.1 mg/kg b.w. /d dose of aroclor 1254 for 7 d, marked changes like severe necrosis in seminiferous tubules (SNST) and scattered spermatids (SS) was observed (Figure 1B). In day 14 exposure duration, more SNST and mild degenerative change in tunica albuginea (MDTA) were observed (Figure 1C). After 21 d exposure, MDTA and disorganization of germinal epithelium (DGE) were also observed (Figure 1D). whereas, in the highest exposure duration of 28 d, atrophy in the seminiferous tubules (AST), loss of spermatogenic cells (LSC), DGE and Atrophied seminiferous tubules (ATST) were observed (Figure 1E). On the other hand, after the exposure of 1 mg/kg b.w./day of aroclor 1254 for 7 d, marked variation in MDTA and DGE were observed while, in case of 14 d exposure disturbances in the AST and ATST was also evident in some cases (Figure 2A, 2B & 2C). However, AST, SS, some vacuoles (V) and MDTA were observed in the testis tissue of the mice exposed for 21 d in the high dose (1 mg/kg b.w. /day) (Figure 2D). On the other hand, after exposed for the longest duration (28 d) in this dose, the testis tissue

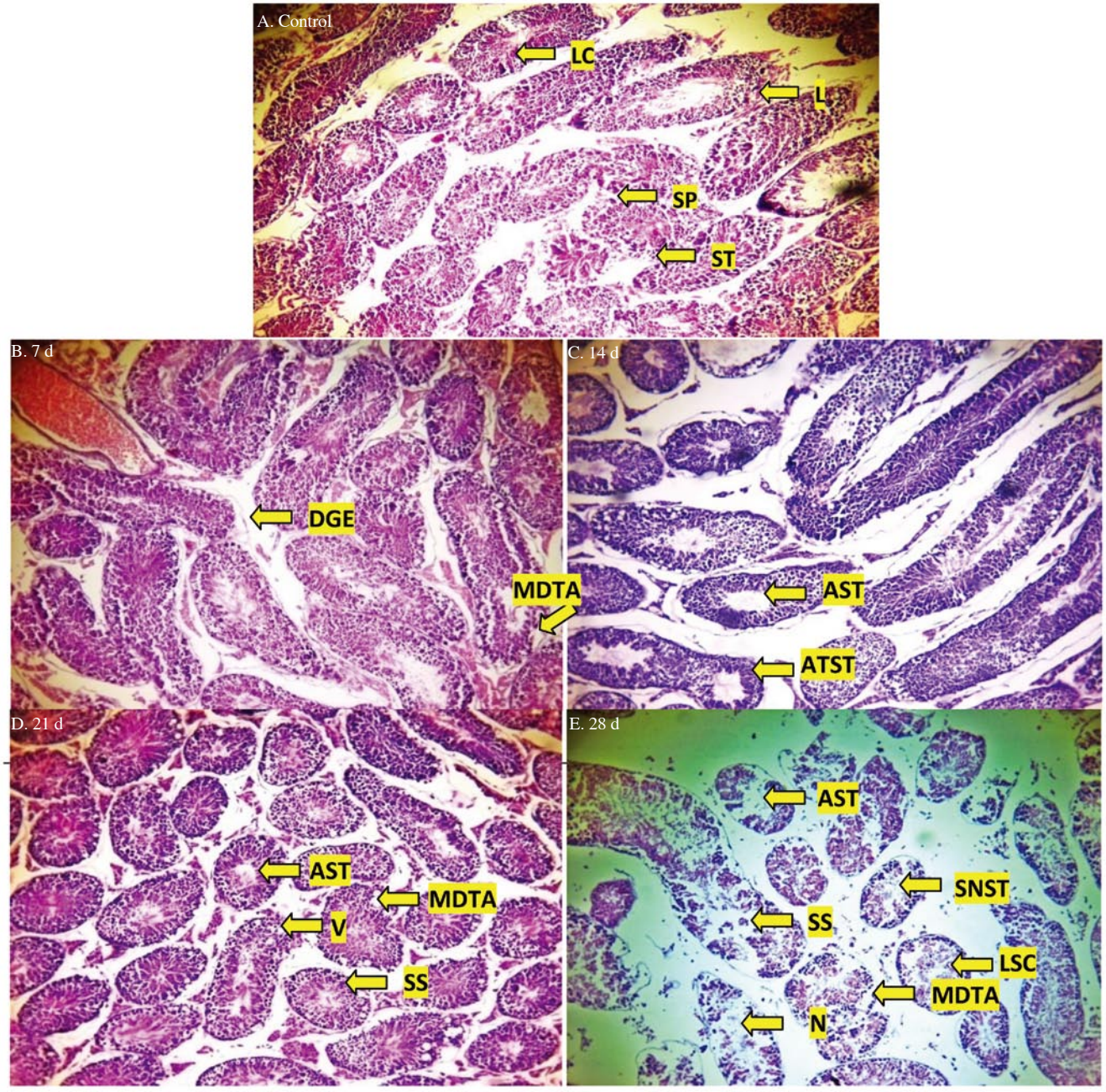

Figure 2. Photomicrographs showing the histopathological alterations in the testis of mice after the exposure of 1 mg/kg/day dose of aroclor 1254 for 7, 14, 21 and 28 d as compared control (H & E, 100 ×).

(A) Control revealed the normal testis with Leydig cells (LC), spermatozoa (SP), seminiferous tubules (ST), lumen (L). (B) After 7 d exposure: MDTA, DGE. (C) After 14 d exposure: AST, ATST. (D) After 21 d exposure: AST, MDTA, SS, vacuoles (V). (E) After 28 d exposure: Atrophy in the seminiferous tubules (AST), loss of spermatogenic cells (LSC), SS, MDTA, necrosis (N), SNST.

showed marked nacrosis with high degree of AST, LSC, SS, MDTA, SNST (Figure 2E).

4. Discussion

Exposure of hazardous xenobiotics like PCBs to humans is still a major public health concern. In fact, the exposure of some POPs, like PCBs have the antagonistic, additive, and/or synergistic effects on target organs and tissues may occur. The observed result showed the cyto-architectural changes in seminiferous tubules, necrotic changes in spermatids and spermatozoa present in lumen of seminiferous tubules. Previous studies on mice, rats and rabbits reported the alterations in the testis histology and dysfunction, namely, structural defects in spermatids and sperms, formation of vacuoles in seminiferous tubules, decrease in spermatogenic cells and degeneration of leydig cells in albino mice after the exposure of PCBs and pyrethroids[16]. The exposure to aroclor 1254 showed the irregular seminiferous tubules with spermatogenesis arrest and disorders in the germinal cell shape. In addition, deceleration of spermatogenesis, degeneration of germ cells, thinning of boundary of the seminiferous tubules with multiple breaks at different locations and widening of interstitial spaces between the tubules were also observed. Alston *et al.*[13] evidenced the degenerative alterations in seminiferous tubules, appearance of pyknotic nuclei, subluminal nuclei as part of spermatozoa heads and lacked tails which is indicating that the exposure of PCBs interfered with spermiogenesis. In fact, an ameliorating effect induced by PCBs and pyrethroids on pathological changes in the rat and testis was reported[17-21]. The present study clearly indicated the dose and exposure duration dependent toxic effects of the aroclor 1254 on the histology of the mice testis. However, the alterations in the histology of mice testis were clearly indicative of direct and organ-specific effects of PCB.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

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References

[1] Nessel CS, Gallo MA. Dioxins and related compounds. In: Lippmann M, eidtor. *Environmental toxicants: Human exposure and their health effects.* New York: Van Nostrand Reinhold; 1994, p.163-182.

[2] Bruss SP, Schuppe HC, Schill WB. The male reproductive system and its susceptibility to endocrine disrupting chemicals. *Andro* 2004; **36**: 337-345.

[3] Vidaeff AC, Sever LE. In utero exposure to environmental estrogens and male reproductive health: A systematic review of biological and epidemiologic evidence. *Repro Tox* 2005; **20**: 5-20.

[4] Kelly KA, Havrilla CM, Brady TC, Abramo KH, Levin ED. Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. *Env Hea Pers* 1998; **106**: 375-384.

[5] Livingstone DR. Cytochrome P-450 and oxidative metabolism in invertebrates. *Biochem Society Trans* 1990; **18**: 15-19.

[6] Livingstone DR. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar Poll Bull* 2001; **42**: 656-666.

[7] Kuratsune M. Yusho. *A human disaster caused by pcbs and related compounds*. Fukuoka: Kyushu University Press; 1996.

[8] Shimizu K, Ogawa F, Watanabe M, Kondo T, Katayama I. Serum antioxidant levels in Yusho victims over 30 years after the accidental poisoning of polychlorinated biphenyls in Nagasaki, Japan. *Tox & Indus Hea* 2003; **19**: 37-39.

[9] Gray LE, Ostby J, Marshalln R, Andrew J. Reproductive and thyroid effects of low level polychlorinated biphenyl (Aroclor 1254) exposure. *Funda App Tox* 1993; **20**: 288-294.

[10] Murugesan P, Senthil Kumar J, Balasubramanian K, Aruldhas MM, Arunakaran J. Impact of polychlorinated biphenyl Aroclor 1254 on testicular antioxidant system in adult rats. *Hum Exp Tox* 2005; **24**(2): 61-66.

[11] Ahmad SU, Tariq S, Jalali S, Ahmad MM. Environmental pollutant Aroclor 1242 (PCB) disrupts reproduction in adult male rhesus monkeys (*Macaca mulatta*). *Env Res* 2003; **93**: 272-278.

[12] Rozati R, Reddy PP, Reddanna P, Mujtaba R. Role of environmental estrogens in the deterioration of male factor fertility. *Fer Ster* 2002; **78**:1187-1194.

[13] Alston DA, Tandler B, Gentler B, Smith EE. Testicular histopathology in deer mice (*Peromyscus maniculatus*) following exposure to polychlorinated biphenyls. *Chemosphere* 2003; **52**(1): 283-285.

[14] Mazen NF, Zidan RA. Histological study on the effect of Aroclor 1254 on the epididymis of adult rats and the role of L-NAME administration. *Ultrastruct Pathol* 2017; **41**(2): 154-167.

[15] Michael JD. *The toxicologist's pocket handbook*. 2nd ed. New York: Informa Healthcare; 2008, p. 44.

[16] Sakr SA, Azab AE. Effect of pyrethroid inhalation on the testis of albino rat. *Pak J of Bio Sci* 2001; **4**(4): 498-500.

[17] Aydin M, Turk G, Yuksel M, Cevik A, Apaydin A, Yilmaz S. Effect of electromagnetic field on the sperm characteristics and histopathological status of testis in rats. *Med Weter* 2007; **63**(2): 178-183.

[18] Khaki AA, Choudhry R, Kaul JK, Minaii B, Baybordi A, Oskuii G, et al. The effect of an electromagnetic field on the boundary tissue of the seminiferous tubules of the rat: A light and transmission electron microscope study. *Folia Morpho* 2006; **65**(3): 188-194.

[19] Lee JS, Ahn SS, Jung KC, Kim YW, Lee SK. Effects of 60-Hz electromagnetic field exposure on testicular germ cell apoptosis in mice. *Asi J of And* 2004; **6**: 29-34.

[20] Ongel K, Gumral N, Ozguner F. The potential effects of electromagnetic field: A review. *Cell Mem Free Rad Res* 2009; **1** (3): 85-89.

[21] Manna S, Bhattacharyya AD, Basak DK, Mandal TK. Single oral dose toxicity study of α -cypermethrin in rats. *Ind J Pharma* 2004; **36**(1): 25-28.