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Possible occurrence of apoptosis in Endosulfan induced testicular toxicity in mice

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Abstract : *Endosulfan an organochlorine pesticide is known to cause deleterious effect on the reproductive organs of humans. In the present investigation, Endosulfan at 3.0 mg/kg b.w was administered to Swiss albino mice for 4 weeks. Animals were sacrificed and their testicular tissues were fixed for Transmission Electron microscopic study. The Electron microscopic study reveals the degenerative changes in testis at subcellular level. The plasma membrane, nuclear membrane, chromatin material and mitochondria, etc. show degenerative changes. It was concluded that Endosulfan causes damage to spermatozoa in Swiss albino mice.*

Key Words: Endosulfan, Swiss albino mice, Testis, Spermatozoa.

Introduction

The greatest challenge to the mankind of new millenium is the growing population. So, the pressure on agricultural land is mounting many folds. To augment the yield of crops pesticides such as endosulfan is very liberally used. Endosulfan is classified in India as an "Extremely Hazardous" pesticide (ITRC, 1989), Moderately Hazardous chemical by (WHO-class II), highly toxic substance (ATSDR, 1993; EXTOXNET, 1998) and moderately hazardous pesticide after taking LD_{50} value.

Cases of infertility in both male and female, abnormal child birth were reported in the Kassargode district of Kerala due to Endosulfan spray in cashewnut trees (Singh and Pandey, 1989). Finally it was banned in the state due to its health hazards. Endosulfan on animal model also has caused deleterious effect at cellular and subcellular levels (Sinha, *et al.,* 2004; Nath *et al.,* 2005; Nath, 2007).

So, in the present study an approach has been made to understand the effect of endosulfan when administered 3 mg/Kg body weight (bw).

Materials and Methods

Chemicals : Endosulfan was obtained from (Excel India Pvt. Ltd. Mumbai with EC 35%). The pesticide was prepared to 3 mg/Kg b.w with corn oil as vehicle and was administered orally to mice daily for 4 weeks.

Animals: Swiss albino mice were bred at the animal house, Mahavir Cancer Institute & Research Centre, Patna, India (CPCSEA Regd. no. 1129/bc/07/CPCSEA, dated 13/02/2008) and was duly approved by the IAEC. The animals had free access to water and feed pellets.

Experimental procedure : The age group of mice was 12 weeks old with an average body weight 30 $\pm$ 2 gm. Control animals (n=6) received distilled water and experimental group-I (n=12) received endosulfan 3 mg/kg b.w daily by gavage for 4 weeks. After 4 weeks mice were anesthetized with diethyl ether and their testis were removed,washed three times in isotonic saline and fixed in the fixative -2.5% gluteraldehyde for Transmission Electron Microscopy (TEM) study.

For TEM, samples were fixed in 2.5% gluteraldehyde at 4^{0}C for 24 hours, then washed 3-4 times with PBS (pH=7.4). They were post fixed in 1% Osmium tetraoxide (OsO_4) for 1 hr., dehydrated through graded series of ethanol,

infiltrated in propylene oxide and embedded in araldite. Semithin sections were cut at approximately 1 µm in thickness, stained with 1% toluidine blue and observed under light microscope. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and viewed under Morgagini – 268 D (SEI Co.) Transmission Electron Microscope at SIF-EM facility Unit (Sophisticated Instruments Facility at All India Institute of Medical Sciences (AIIMS), New Delhi). The TEM microphotography of testis of control mice were compared with endosulfan treated mice.

Results and Discussion

The control group of mice showed normal structure of spermatozoa. with nuclear membrane, chromatin, while plasma membrane present throughout the length of spermatozoa covering the acrosomal apical region, plasma membrane (PM), acrosome (AC) and head cap (HC) (1 µm) (Fig.1). Electron micrograph of testis of 3 mg/kg. b.w Endosulfan treated for

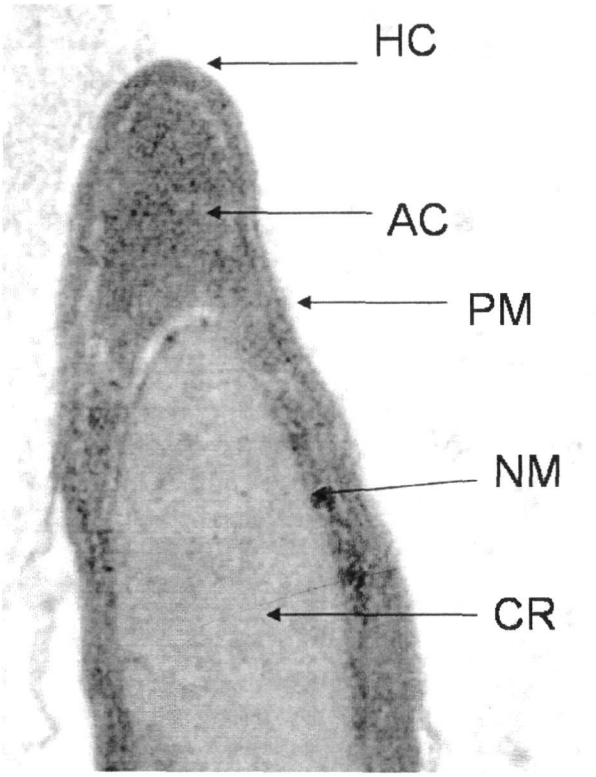

Fig. 1. Electron micrograph of testis from a control mice.

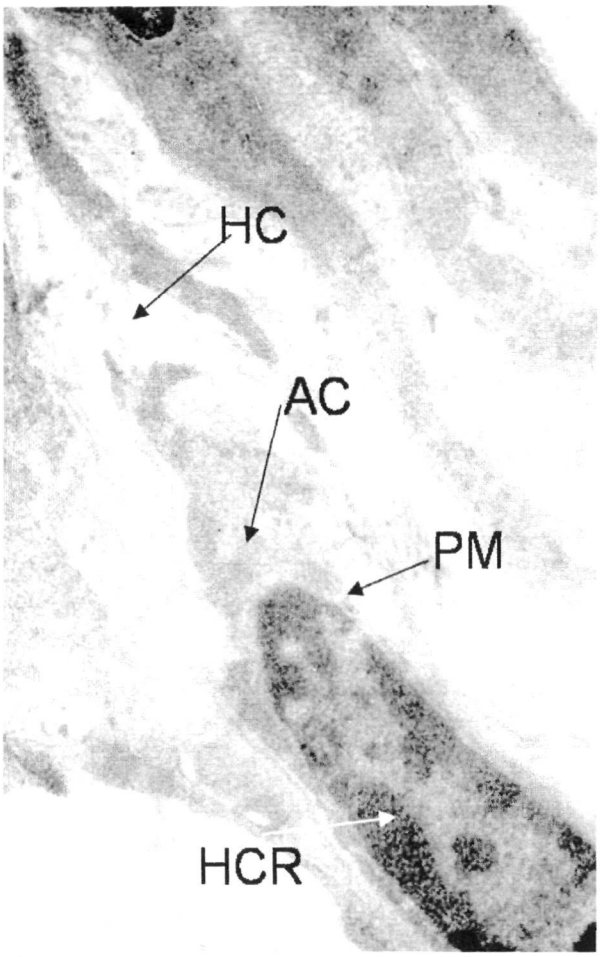

Fig. 2. Electron micrograph of testis of 3 mg/kg. b.w Endosulfan treated for 4 weeks

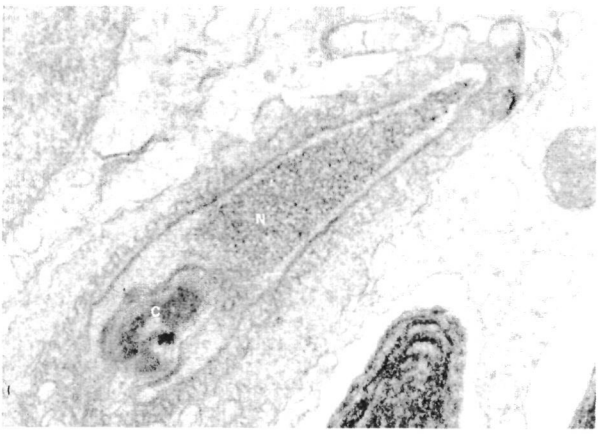

Fig. 3. Electron micrograph of testis of 3 mg/kg. b.w Endosulfan treated for 4 weeks.

showed degeneration in apical acrosome region (AC) with vacuolation in the cytoplasm. Degenerated head cap (HC) and heterochromatization (HCR) were clearly

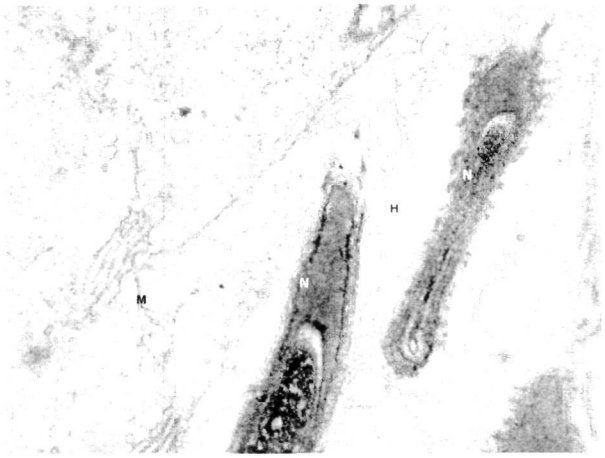

Fig. 4. Electron micrograph of testis of 3 mg/kg. b.w Endosulfan treated for 4 weeks

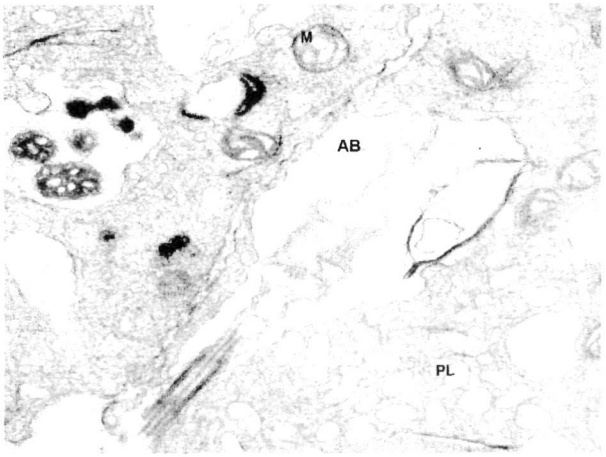

Fig. 5. Electron micrograph of testis of 3 mg/kg. b.w Endosulfan treated for 4 weeks

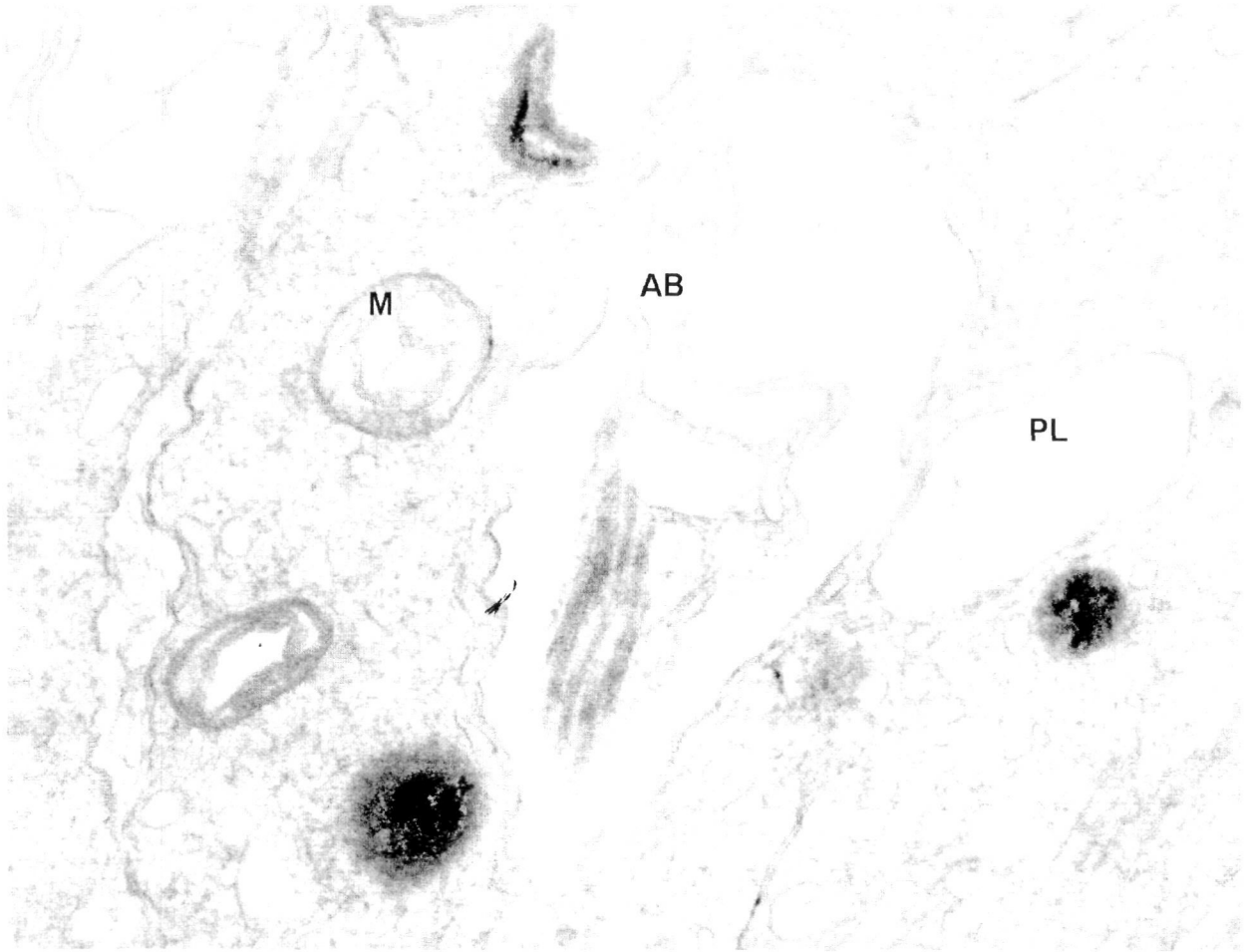

Fig. 6. Electron micrograph of testis of 3 mg/kg. b.w Endosulfan treated for 4 weeks

observed (Fig.2). Development of asymmetrical centrioles with in the Horse shoe shaped nucleus (C) was apparent and plasma membrane (PM) showed wavy and serrated margin (A) around the spermatozoa head. Dissolved mitochondrial (M) cristae signified the chronic level of toxicity

and degeneration in the cell (Fig.3). In 4 weeks endosulfan treated group some sections showed formation of apoptotic sequence during the spermiogenesis phase signifiying the onset of apoptosis. Formation of blebbing on plasma membrane on upper spermatozoa and halo (H) between plasma membrane and nuclear membrane (N) in the lower spermatozoa could be observed. Beginning of condensation of chromatin material and dissolved mitochondrial (M) cristae followed by dissolution of mitochondrial membrane suggests the onset of apoptosis (Fig-4). Final stage of apoptosis was visible in the spermatozoa as the formation of apoptotic bodies (AB) with increased phagolysosomal (PL) activity. The complete degeneration of mitochondria (M) was also observed (Fig. 5 and 6).

A large number of natural and synthetic chemicals, many of them found in the environment are disrupting the endocrine systems in vertebrate and invertebrate species (Crisp *et al.,* 1998). Pesticides are one of the major endocrine disruptors (Colborn *et al.* 1993; Kavlock and Ankley,1996). So, chemical interference with hormone actions, particularly during neuro development and consequent effects on cognitive behaviour are a current concern (Scantz and Widholm, 2001; Weiss, 2002). The appropriateness of toxicological screening for detection of endocrine active compound are well reported (Nilsson, 2000) and O' Conner *et al.,* 2002).

In the present investigation through Transmission Electron Microscopic study, deleterious effect of Endosulfan on the spermatozoa of mice was observed. It caused degeneration in them (Sinha *et al.,*1995; Sinha *et al.,* 1997; Suwalsky *et al.,* 2000; Nath, 2007). Furthermore, due to Endosulfan toxicity, a series of apoptotic bodies with degenerated mitochondrial cristae indicated the occurrence of apoptosis process. Degeneration of mitochondrial cristae and inner membrane, condensation of nuclear material are further related to the apoptotic phenomena in the spermatozoa. This has also been shown by Abou- Donia *et al.,* (2003) with other chemicals. It is possible that due to Endosulfan toxicity, the spermatozoa may trigger activation of proteolytic cascade, caspain and caspases. This also may give signal to endonucleases which in turn affects microtubules followed by cleavage of actin formation, blebbing on plasma membrane and non adherence of cellular activity. Thus, it may cause sequence of apoptotic nuclear events beginning with chromatin condensation, breaking down of nuclear membrane, DNA fragmentation, finally frill like appearance of microtubules from the nuclear membrane and formation of clear halo between nuclear material and plasma membrane. This process of apoptosis in spermatozoa with other chemicals has been well studied (Jackbson *et al.,* 1997, Kim *et al.,* 2001; Shen and White, 2001).

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