Research Article

Transmission Electron Microscopic Study of Endosulfan Induced Architecture of Spermatozoa in Mice

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ABSTRACT

Endosulfan is a pesticide of organochlorine group. The lipophilic nature of endosulfan suggests that endosulfan initially accumulates in fatty tissues and that relatively high amounts can be found in the liver and kidneys after exposure. In the present investigation spermatozoa architecture after endosulfan treatment in mice have been evaluated. Hence, the dose of 3 mg/Kg b.w of Endosulfan was continuously administered to male mice for 35 days. The mice were sacrificed on 35th day to observe the architecture of spermatozoa through Transmission Electron Microscope (TEM). Electron micrograph of spermatozoa of 3mg/kg Endosulfan treated for 35 days showed degeneration in apical acrosome region with degenerative changes in plasma membrane and nuclear membrane of head region of spermatozoa. The degeneration were also observed in 9+2 arrangement of microtubule and mitochondrial sheath of mid piece of spermatozoa while principal piece showed without plasma membrane which denotes complete degeneration of plasma membrane. Outer dense fibers were fused with microtubules. The divergent arms were degenerated and not visible clearly. Thus marked degenerative changes were observed in the architecture of spermatozoa (Head Piece, Mid Piece & Principal Piece) after exposure of endosulfan.

Keywords: Endosulfan, Spermatozoa, mice, Transmission Electron Microscope.

INTRODUCTION

Endosulfan is an organochlorine pesticide used primarily to kill insects and mites on crops, vegetables and grains. Endosulfan acts like xenoestrogen may impair the normal embryonic development and disrupt normal reproductive functions in adulthood. Altered spermatogenesis was also reported in male mice treated by gavage with 3 mg technical endosulfan/kg/day for 35 days [1] . A dose-related decrease in testicular testosterone, plasma testosterone, LH, and FSH in groups of male Wistar rats administered endosulfan at 0, 7.5, or 10 mg/kg/day for 15 or 30 days [2]

Endosulfan belongs to group of endocrine disrupting chemicals and acts like xenoestrogen. Endocrine disrupting chemicals (EDCs) are a structurally diverse group of compounds that may adversely affect the health of humans, wildlife and fisheries or their progenies, by interaction with the endocrine system [3]. It has been suggested that EDCs pose a potential risk and can alter the hormone balance in humans and wildlife [4]. Neurological disorders (epilepsy, cerebral palsy and mental retardation), Congenital malformations , reproductive disorders and cancers of various organs reported from Kasargode district of north Kerala (India) have been linked with **endosulfan**, an organochlorine pesticide. Government of Kerala (India) banned this pesticide due to its hazardous effect on reproductive health in cashewnut workers of Kassargod district. Incident of Kasargode district of north Kerala has inspired me to undertake work on Endosulfan (Pesticide) therefore the present investigation is aimed to observe the adverse effect of endosulfan on sperm morphology with level of Electron Microscope.

MATERIALS AND METHODS

In the present investigation, experiments were performed on 10-12 weeks old healthy male Swiss albino mice, *Mus musculus*. For the optimal growth and reproduction the mice were kept in ideal condition under a well regulated light and dark (12h:12h) schedule at 23±1°C in 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. Experiment was approved by Institutional Animal Ethics Committee (IAEC)

Animals were given food and water *ad libitum*

The oral LD_{50} value of endosulfan for mice was estimated by standard interpolation method, which was 7 mg / kg b.w. The standard data reference for LD_{50} of endosulfan for mice is 7.36 mg/ b.w. [5]: Endosulfan manufactured by Excel Industries Mumbai (E.C - 35%) was dissolved in distilled water to prepare sublethal dose of 3 mg/kg b.w was administered for 35 days by gavage method. A vehicle of control

group of mice was established and served with equal volume of distilled water by gavage method. The treated mice and control group were sacrificed after 35th day of treatment. Cauda epididymis were excised and fixed in 2.5% glutaraldehyde for Transmission Electron Microscopy study.

Tissue Processing For Transmission Electron Microscopy

Small pieces of tissues were fixed in 2.5% gluteraldehyde for overnight. Washed with 0. IM phosphate buffer at 4 ^{0}C each. Post Fixation was done in 1% Osmic acid (OsO_4) in 0.1 M in chilled phosphate buffer again washed with 0.I M phosphate buffer at 4 ^{0}C. Tissues were dehydrated in graded series of alcohol. Clearing of tissues were done in toluene, infiltration of tissues were carried out in toluene plus araldite mixture. Then tissues were brought to pure araldite and tissues were embedded in plastic moulds in embedding medium, and the blocks are withdrawn out of the moulds. Blocks were trimmed then it's semi thin (of the order of 1-2 µ) and ultra thin sections (of silver gray colour) are cut on ultramicrotome. The semi thin sections were stained and observed under light microscope for marking of section area. Then grids were prepared after final staining. The ultra thin sections were observed under Transmission Electron Microscope.

RESULTS

The control group of mice showed normal structure of spermatozoa (Head region). Head region showed normal acrosome (AC), head cap (HC) covering the acrosomal material, condensation of chromatin material (Cr) and plasma membrane (PM) & nuclear membrane (NM) were observed almost normal.(Fig. 1).

Electron micrograph of spermatozoa of 3mg/kg Endosulfan treated for 35 days showed degeneration in apical acrosome (AC) region with degenerated head cap (HC). Degenerative changes were clearly observed in Plasma membrane (PM) and Nuclear membrane (NM) of head region of spermatozoa consequently reduced condensation of chromatin material observed (Fig. 2 & 3).

Mid piece of spermatozoa of control mice showed intact plasma membrane(PM) with distinct mitochondrial sheath which contained mitochondria (M). Outer dense fibers (ODF) were clearly observed. 9+2 arrangement of microtubules (Mt) were present with normal structure. (Fig.4). Electron micrograph of spermatozoa of 3mg/kg Endosulfan treated for 35 days showed disorientation of middle piece (MP) from neck region, plasma membrane of middle piece got ruptured, mitochondrial sheath were degenerated and dissolved mitochondria signified the level of toxicity and degeneration in the cell, massive degeneration were also observed in th9+2 sarrangement of microtubule.(Fig. 5)

Electron microphotograph of Principal piece from control mice showed normal structure of plasma membrane intact over principal piece, fibrous sheath, 9+2 arrangement of microtubules and divergent arm were also observed normal . (Fig. 6), where as treated with Endosulfan @3mg/kg b.w. for 35 days showed principal piece without plasma membrane which denotes complete degeneration of plasma membrane. Outer dense fibers were fused with microtubules. The divergent arms were degenerated and not visible clearly (Fig. 7).

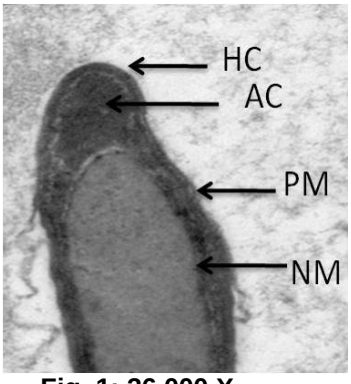


Fig. 1: 36,000 X
(Electron micrograph of spermatozoa (Head region) from a control mice

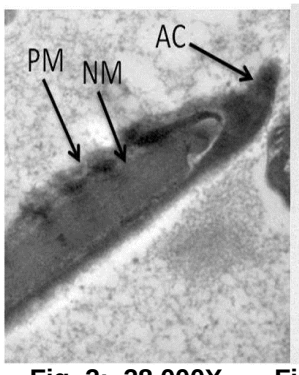


Fig. 2: 28,000X

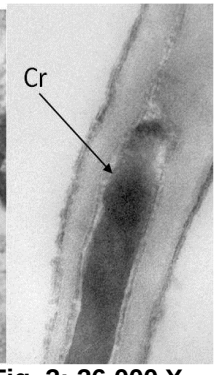


Fig. 3: 26,000 X
Electron micrograph of spermatozoa (Head region) treated with Endosulfan @3mg/kg b.w. for 35 day

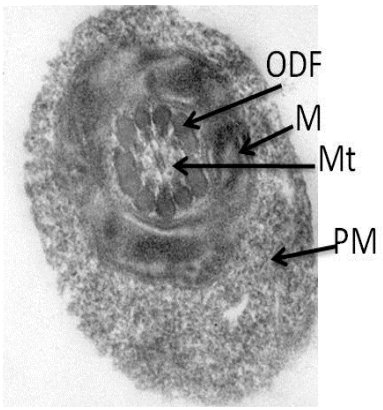
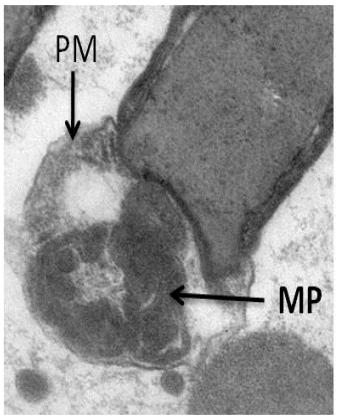


Fig. 4: 54,000 X.
Electron micrograph of spermatozoa (Mid Piece) from a control mice.



(Fig. 5). 40,000 X
Electron micrograph of spermatozoa (Mid Piece) treated with Endosulfan @3mg/kgb.w.for 35 days.

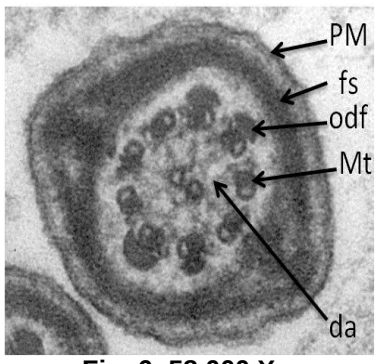


Fig. 6: 58,000 X
Electron micrograph of spermatozoa (Principal Piece) from a control mice.

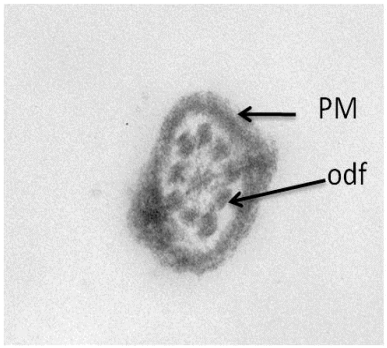


Fig. 7: 42,000 X
Electron micrograph of spermatozoa (Principal Piece) treated with Endosulfan @3mg/kg b.w. for 35 day.

DISCUSSION

The present study was aimed to determine the toxic effect of Endosulfan on male reproductive system. In the present study reproductive potential of sperm architecture of male mice was observed using Transmission Electron Microscope (TEM). The Endosulfan dose selected in the present study showed toxic symptoms in mice by deleterious changes in architecture of spermatozoa.

Exposure to pesticides could cause male infertility by causing a significant decrease in sperm quality and quantity [6]. Anomalies in sperm and hormonal imbalance (Testosterone) of Mus musculus due to Endosulfan exposure were studied in detailed [7].

Endosulfan was classified by the WHO in the category of technical products that are moderately hazardous and it has been shown that endosulfan has estrogenic property [8] and male rats are more sensitive to the effect of endosulfan than female rats [9].

It has been observed that after endosulfan treatment changes are hazardous and specially claims abnormality in spermatozoa. Endosulfan is readily absorbed by the stomach, by the lungs and through skin meaning that all routes of exposure can pose a hazard. As a result of its world wide use, it can be a potential environmental contaminant and may cause a public health hazard [10]. Khan & Sinha reported that the endosulfan, phosphamidon, and maneozeb induced various types of structural alterations in chromosomes, pairing impairment among homologous chromosome and division disruptive changes in the primary spermatocytes of mice[1]. Low level of endosulfan can inhibit the human sperm acrosome reaction initiated by progesterone and lycine [11]. Similar study observed in the present investigation that endosulfan

treatment to mice @ 3mg/kgb.w. for 35 days causes leakage at the tip of acrosome and acrosome became flattered. Blunt shape of acrosome is found because of loss of acrosomal hook. Degenerative changes were clearly observed in Plasma membrane (PM) and Nuclear membrane (NM) of head region of spermatozoa consequently reduced condensation of chromatin material observed which is in support of work of Auger [12] that Sperm chromatin condensation is another valuable index of sperm quality that is essential for the capacity of the sperm to fertilize the ovum. This suggests that a negative effect on chromatin condensation of the sperm could be another mechanism by which endosulfan exposure leads to reproductive toxicity.

It has been reported that endosulfan causes degradation of testicular cells in laboratory animals [1,13]. Endosulfan have shown a negative effect on reproductive health in men residing in the Kassargode district of Kerala[14]. Endosulfan causes spermatozoa degeneration observed by Nath, A. And R. Kumar.[15] Altered testicular enzyme activities, indicating spermatogenesis, were reported in mature rats treated by gavage with 2.5 mg technical endosulfan / kg/ day for 70 days [16]. Similar results had been observed in my investigation that mid piece dissociated from neck region, mitochondrial sheath was degenerated and plasma membrane of middle piece get ruptured, 9+2 arrangement of microtubules were not visible. Principal piece showed without plasma membrane which denotes complete degeneration of plasma membrane. Outer dense fibers were fused with microtubules. The divergent arms were degenerated and not visible clearly. This shows that non motility of sperm activity, hence sperm cells are unable to reach to the ovum for fertilization. Similar observation have been observed by Nath & Chand [17] in fish & amphibia.

Thus in the present investigation it has been observed that plasma membrane degenerated from head piece, middle piece and principal piece of sperm cells. This shows that plasma membrane of sperm cells are highly sensitive with oxidative stress produced by toxicants like endosulfan, which favors with the work of Lenzi[18] that Plasma membranes of the sperms have a high content of polyunsaturated fatty acid; hence, they are highly sensitive to oxidative stress and lipid peroxidation.

CONCLUSION

After 35 days administration of endosulfan causes severe effect not only on architecture of spermatozoa but chromatin material in the nucleus were also reduced, leaving only residue of chromatin material in the middle of nucleus. Loss of plasma membrane from entire region of sperm cells was also observed.

Thus from the above study it can be concluded that Endosulfan exposure leading to male reproductive system by damaging the architecture of sperm cells..

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