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CYTOTOXIC POTENTIAL OF ENDOSULFAN ON OOCYTES OF FEMALE MICE: AN ELECTRON MICROSCOPIC STUDY

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ABSTRACT

Indiscriminate use of pesticides has increased many folds in the recent times. For the better yield of crops, the farmers are widely utilizing the pesticides. These pesticides have deleterious effect on humans causing health related issues in the population which has also led to hormonal imbalance in females leading to infertility. The present research work on animal is harmful impact of endosulfan on reproductive toxicity in female mice. Endosulfan at the dose of 3.0mg/Kg body weight/day was administered by Gavage method to female mice for1 week, 2 weeks, 3 weeks & 4 weeks. At the end of experimental period, decreases of body weight were observed. The study reveals that after the exposure of endosulfan, there was significant damage at the cellular level in ovarian cells of mice. Thus, it is concluded that endosulfan toxicity effect on cellular level of the ovarian oocytes thereby affecting the process of oogenesis and foetal development leading to infertility in female mice.

KEYWORDS: *Electron Microscopy; Endosulfan; Oocytes; Mice.*

INTRODUCTION

India is an agriculture-based country with a production of the crops at very large scale. But, unfortunately, due to the pests, there is significant damage in crop pro-duction. The pest burden is increasing every year due to the appearance of new pests and diseases.[1,2] Among all types of pesticides present, organochlorines have proven more mortality of pests or are the best to be used in the pest management.[3,4,5]

One of the broad-spectrum insecticide, Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6, 9-methano-2,4,3-benzodioxathiepin-3-oxide) is an organo-chlorine insecticide and acaricide.Endosulfan is used to control agriculture insects and mite pests on a variety of fields, fruits, and vegetables. It causes acute toxicity in animal and human beings due to over exposure.

Endosulfan is absorbed by the human via stomach, lungs, and through skin. The previous studies performed elucidate the stereoselective metabolism of endosulfan in different organs and characterized the cytochrome p450 enzyme that is involved in metabolism of endosulfan. The CYP3A enzymes are major enzymes contributing to stereoselective disposition of endosulfan. CNS is the main target of endosulfantoxicity.[6] Endosulfan and its metabolites have been found in both tissue and serum samples. Endosulfan affects kidney, liver, immune system, ovary, and testes. Endosulfan acts on the testes, causing problems in spermatogenesis and spermiogenesis. Exposure to sublethal doses of endosulfan and its metabolites induce DNA damage and Mutation. Spermiogenesis is the third phase of spermatogenesis, in this phase the spermatocyte undergoes structural changes. First the acrosome is formed, then the tail develops and additional a majority of the super flows cytoplasm is removed and which taken up by Sertoli cells. Finally, mature sperm is developed.[7]

During the cap phase the acrosomal undergo structural changes and gets its final shape the maturation phases.[8] Endosulfan affects shaping of sperm head; acrosome and nuclear condensation the shape of a sperm head is species-specific and, in the sickle, shaped.[9] The acrosome is a bag of enzymes which sits at the anterior pole of the sperm head. The acrosome contains enzymes required for the sperm to penetrate the surrounding layers of the two sites. The formation of acrosome begins with the production of proacrosomal granules from the golgi apparatus.[10] The re-shapping of the head and acrosome nuclear condensation occur in parallel. Due to endosulfan during spermiogenesis, the size of the spermatids had decreased to ~5% of a somatic cell nucleous. The campation occur through dramatic changes in the way the DNA is packed and falls under the broad banner of epigenetic changes in chromatid structure that affect transcription.

Endosulfan causes a disturbance in spermiogenesis, leadingto low sperm count, production of abnormal

sperm, deshaped acrosomal head, tails of elongated spermatids and decrease in the quality of serum, impairment of sperm motility, reduction of fertilization ability. Endosulfan can directly injure the testes. A testicular toxin and various derived compounds were shown to induce severe damage to the spermatogenic epithelium in mice model.[11] The effect of endosulfan on the testes appears to be manifested mainly in the Sertoli cells, presenting more morphological changes under scanning electron microscopy.Endosulfan can also interfere with the normal functioning of mitochondrial enzymes. The endosulfan alters the activity of some marker enzymes such as-glucose- 6-phosphate dehydrogenase, lactate dehydrogenase, y-glutamyl transpeptides and alkaline phosphate, that decrease mitochondrial energy production in swiss albino mice.[12] Endosulfan exposure cause over-production of reactive oxygen species (ROS), resulting in a decline of sperm count and infertility in wildlife and human.[13] Oral administration of endosulfan disturbs and alters the process of oogenesis that leads to female infertility.

Hence, the aim of the present study was to investigate some cellular alteration was observed after the administration of organochlorine pesticide endosulfan in female swiss albino mice.

MATERIALS AND METHODS

Animals: Adult Swiss albino mice were used in the experiment and their weight ranges from 30-35 g. The age of mice for the experiments was 12 weeks old. They were house in the different polypropylene cages containing sterile paddy husk as the bedding material. They were maintained under a well-regulated light and dark (12h:12h) schedule at 24°± 3° and were allowed free access to laboratory food and tap water. The mice were grouped at the ratio of 1:2 with female.

Test chemical

Pesticide endosulfan, manufactured by Excel India Pvt. Ltd., Mumbai with EC 35% was utilized for the experiment.

Study protocol

Mice were divided into five groups (n=10 per group) untreated control, and endosulfan treated (1-week, 2-weeks, 3-weeks & 4-weeks) @ 3.0 mg/kg b.w./day. Endosulfan were administered orally because major available residue in the environment enter the non-target animal is by orally. The vehicle and test drug were administered to the respective group for 28 days. On 29th day the animal was sacrificed by cervical dislocation after the scheduled treatment. The ovary from all the animals were removed and cut into pieces with the help of a sharp and sterilized blade.The treated mice and control group were sacrificed on targeted day of treatment. Ovary 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 % glutaraldehyde for overnight and washed with 0.I M phosphate buffer at 40C each. Post Fixation was done in 1 % Osmic acid (OsO4) in 0.1 M in chilled phosphate buffer and again washed with 0.I M phosphate buffer at 40C. 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 its semi thin (of the order of 1-2 μ) and ultra-thin sections of silver gray colour were cut on ultra-microtome. Then grids were prepared after final staining. The ultra-thin sections were observed under Transmission electron microscope.

RESULTS

Transmission Electron Micrographs of control ovary of mice showed double membrane of nucleus with normal chromatin material. Mitochondria as well as the ribosomes were distinct while mitochondrial cristae and lipid droplets were clearly visible with normal endoplasmic reticulum (Figure -1&2). Ovary of mice treated with Endosulfan @ 3.0mg/kg b.w./day for 1 week showed nucleus with intact and irregular nuclear membrane increased heterochromatin are seen (figure-3). Ovary of mice treated with Endosulfan @ 3.0mg/kg b.w./day for 2-week Dissolved plasma membrane was clearly visible with vacuolated spaces and dilation in nuclear pore complex with degeneration in mitochondria. Oocytes of nuclear membrane are slightly degenerated (Figure 4).

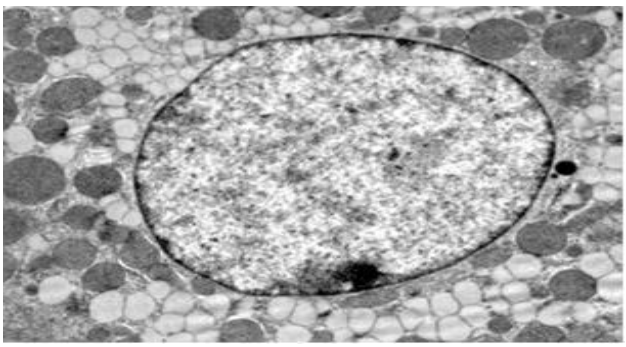

Figure -1.

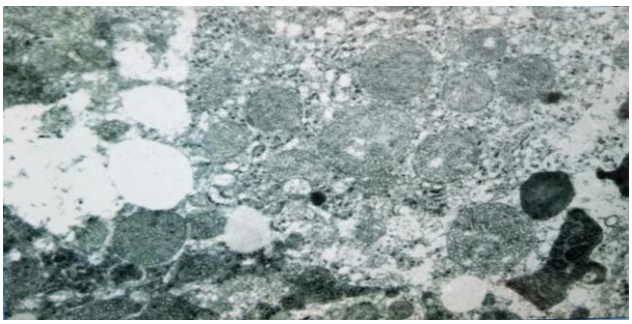

Figure- 2.

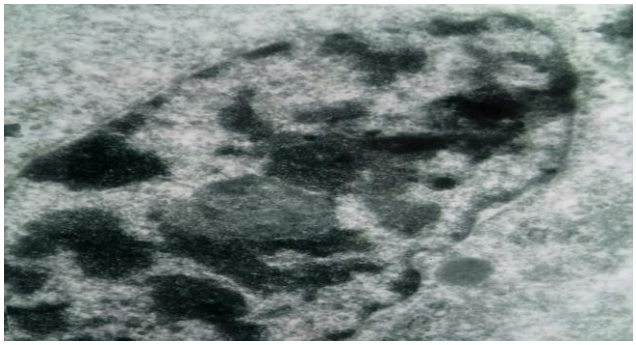

Figure-3.

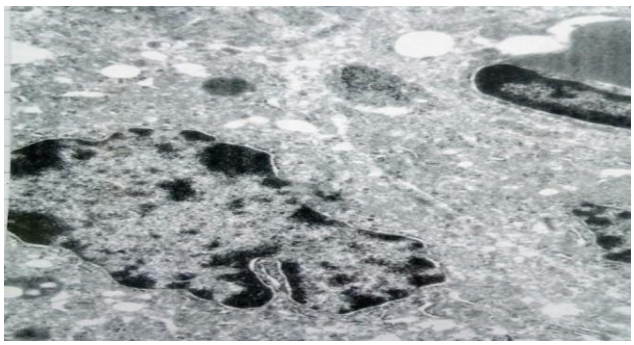

Figure-4.

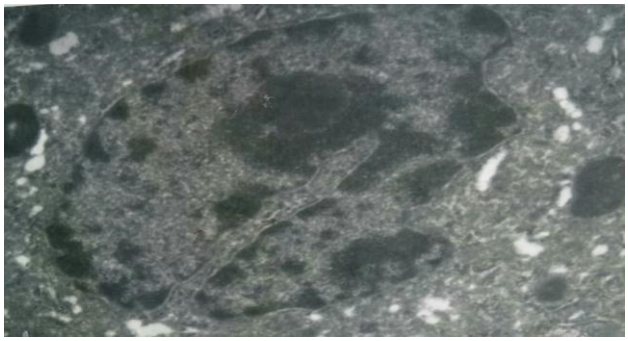

Figure-5.

Figure-6.

Figure 1&2: Transmission Electron Micrographs of Control ovary of mice showing normal architecture of double membrane of nucleus, chromatin material. Mitochondria as well as the ribosomes are very distinct. Mitochondrial cristae with lipid droplets are clearly visible (x 14,000).

Figure 3: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 1 week showing nucleus with invagination. Dilated nuclear pore complex were observed with increased heterochromatisation. Dissolved plasma membrane is clearly visible with vacuolated spaces. Degeneration in mitochondria are clearly visible (x 14,000).

Figure 4: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 2 weeks showing degenerated and deshaped nucleus. Mitochondrial membrane was fragmented. Wavy nuclear membrane was clearly visible. Heterochromatinised elongated nucleus with patch like nucleolus and dilated nuclear pore complex are clearly observed. RER are in highly degenerated condition (x 14,000).

Figure 5: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 3 weeks showing degenerated and deshaped nucleus and nuclear membrane ruptured at many places. Nucleolus is not distinct. Mitochondrial membrane was highly degenerated in mitochondria (x 14,000).

Figure 6: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 4 weeks showing nucleus in degenerated condition. Nuclear pore complex is highly dilated. Nucleolus is not prominent. Lipid droplets are increased in the cytoplasmic region. Rudimentry plasma membrane are also observed nucleus are becomes disintegrate, (x 14,000).

Ovary of mice treated with Endosulfan for 3 weeks showed degenerated and deshaped nucleus and nuclear membrane ruptured at many places. Nucleolus is not distinct. Mitochondrial membrane was highly degenerated in mitochondria. Wavy nuclear membrane with heterochromatinised elongated nucleus was observed. The nuclear pore complex was dilated at many places. Rough endoplasmic reticulum and mitochondria were in highly degenerated condition (Figure 5). Endosulfan 4 weeks treated ovary showed nucleus in highly degenerated condition. Oocytes of nucleus are becoming disintegrate. Nuclear pore complex were highly dilated while nucleolus were not prominently observed. Lipid droplets were highly increased in the cytoplasmic region while rudimentary plasma membrane was observed (Figure 6).

DISCUSSION

Endosulfan is an organochlorine insecticide used on a variety of field. As in the cases of most other pesticides, Endosulfan can cause acute toxicity in animals and human beings due to overexposure.[14] Several studies have been reported that Endosulfan has adverse effects on health.[15,16] In a previous study, the adverse effect of Endosulfan was also reported on follicular development of BALB/c mice.[17] Hiremath and Kaliwal[18] observed that Endosulfan treatment caused a significant decrease in compensatory ovarian hypertrophy, an increase in the

number of atretic follicles and disruption of the estrous cycle.

The results of the present study suggested that Endosulfan cause ovarian damage and it indicates toxicity reached at cellular level, which affects the follicular functions and ultimately affect the reproductive function and fertility. The relationship between female fertility and ovarian follicle development is well recognized.[19] Pathak et al.[20] suggested that higher levels of some of the organochlorine pesticide like Endosulfan may be associated with preterm delivery and increased oxidative stress. In the present experiment, Endosulfan treated mice showed reduction in the body weight during all days of observation, as compared to the control group.

Endosulfan treatment inpubertal rate inhibits testicular functions.[21] Endosulfan administered mice showed degeneration of germinal epithelium to the greater extent. Large vacuolated spaces were also observed in mature graffian follicle. Degeneration in corpus luteum was also evident. Serrated double membrane of nucleus was observed in ovary. Vacuolization in mitochondria was observed. Polyribosome was also observed. Degenerated nuclear membrane was evident. Nuclear fragmentation was also observed with degenerated mitochondria.

CONCLUSION

Therefore, from the entire study it can be concluded that endosulfan caused a deleterious effect on the ovarian oocytes of Swiss albino mice at the cellular level leading to infertility.

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