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CYTOPROTECTIVE POTENTIAL OF WITHANIA SOMNIFERA AGAINST ENDOSULFAN ON OVARY OF FEMALE MICE

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ABSTRACT

In the recent times, indiscriminate use of pesticides has increased many folds. The farmers for the better yield of crops are utilizing the pesticides. Presently, these pesticides have caused health related problems in the population. It has led to hormonal imbalance in females leading to infertility. The present research work on animal deciphers the antidote effect of withania somnifera in endosulfan induced ovarian toxicity in female mice. Endosulfan at the dose of 3mg/Kg body weight was administered orally to female mice for respectively 1 week, 2 weeks, 3 weeks & 4 weeks. Thereafter withania somnifera at the dose of 1000mg/Kg body weight was administered for 4 weeks to observe the positive effect of it on ovarian cells. The study reveals that after the administration of endosulfan, there was significant damage at the sub cellular level in ovarian cells of mice but after administration of withania somnifera there was significant reversal at the sub cellular levels. The basic property of withania somnifera eliminates the deleterious toxicity of endosulfan denotes that it not only possesses antioxidant and rejuvenating property but also maintains the cellular integrity of the ovarian cells leading to normal functioning of it.

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

INTRODUCTION

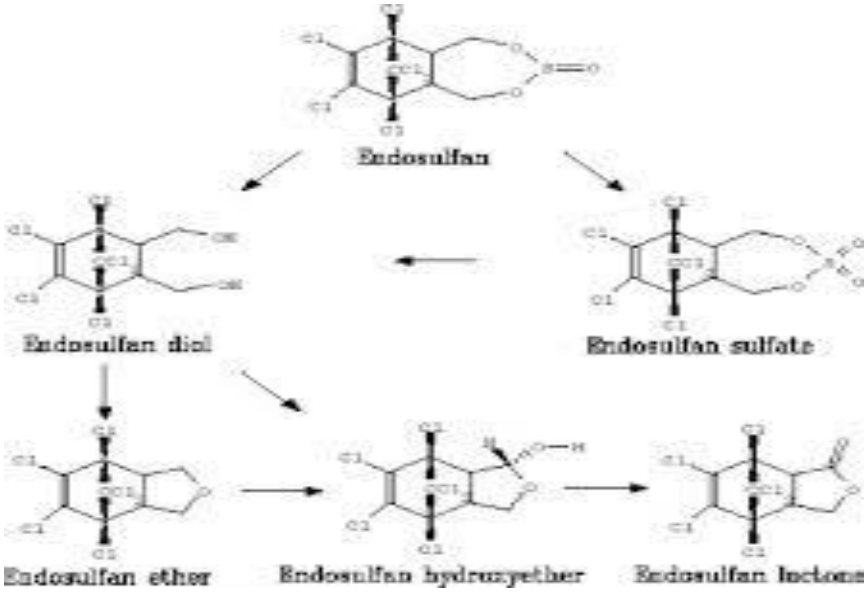
India is an agriculture-based country with a production of the crops at very large scale. But, unfortunately, due to the pests, there is significant damage in crop pro-duction. The pest burden is increasing every year due to the appearance of new pests and diseases.[1,2] Among

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 endosulfan toxicity.[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.

Ashwagandha (Withania somnifera, WS), belongs to the family Solanaceae, and is known to be an Ayurvedic herb worldwide for its numerous beneficial health activities since ancient times. It is widely used for the treatment of various diseases such as epilepsy, depression, arthritis, diabetes, and palliative effects such as analgesic, rejuvenating, regenerating, and growth promoting effects. It has a multifarious effect on vital organs of the body.[14-17] Hence,

the present work was aimed to study the protective role of W. somnifera against endosulfan induced ovarian toxicity in 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.

Calculation of LD50 and Maximum Permissible Dose (MPD) of Withania Somnifera (Ashwagandha) aqueous root extract

For calculating the LD50 value of Withania Somnifera for mice by standard method was reported by Balachandran and Govindrajan (2005), as 2500 mg/kg b.w. as LD50. At 600 mg/Kg & 1100 mg/Kg b.w. although there were no death reports but the no side effects were seen at 1100 mg/kg b.w. So,1000 mg/Kg b.w. was selected as Maximum Permissible Dose (MPD) for the experiment.

Study Protocol

Mice were divided into six 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 followed by 4 weeks administration of withania somnifera (1000 mg/Kg body weight /day). Endosulfan were administered orally because major available residue in the environment enter the non-target animal is by orally. Animal were sacrificed after the scheduled treatment by cervical dislocation. The ovary from all the animals were removed and cut into pieces with the help of a sharp and sterilized blade. Ovary were excised and fixed in 2.5 % glutaraldehyde for Transmission Electron Microscopy (TEM) 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).

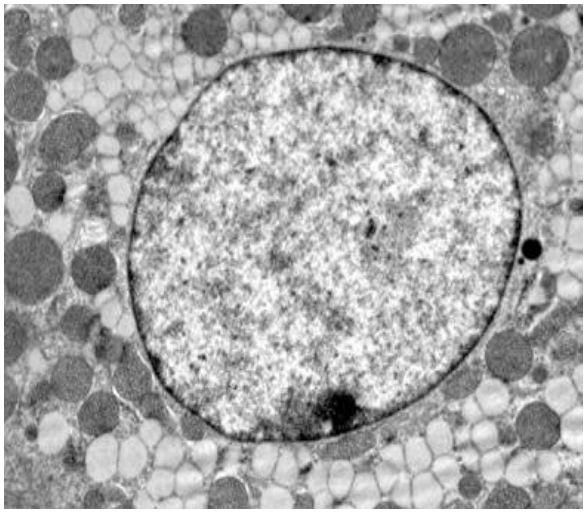


Figure -1

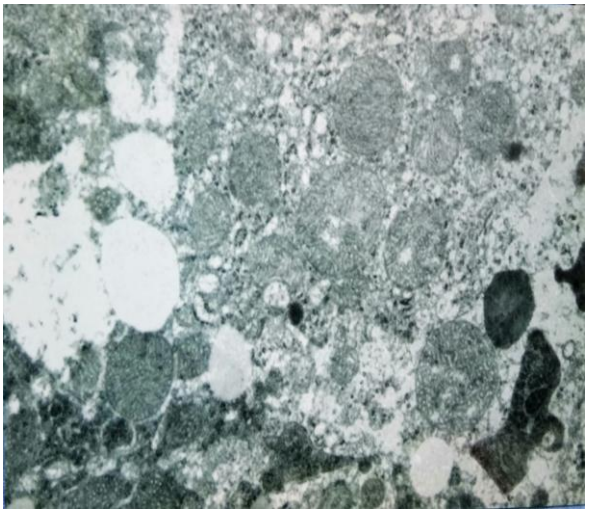


Figure- 2

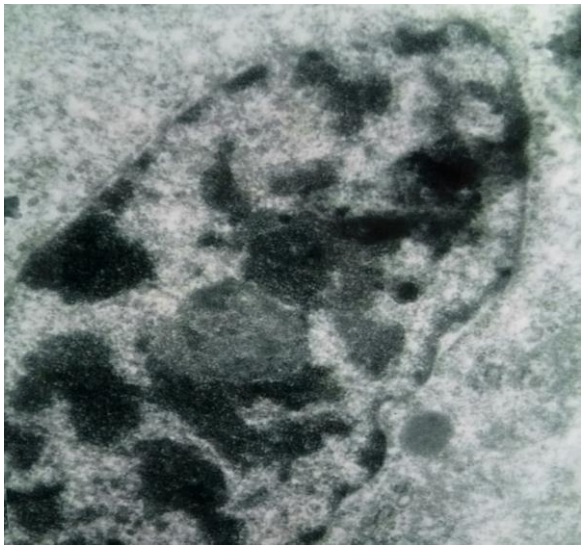


Figure-3

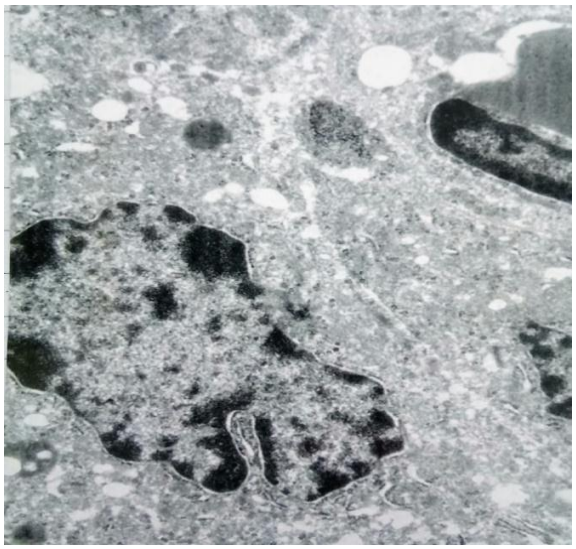
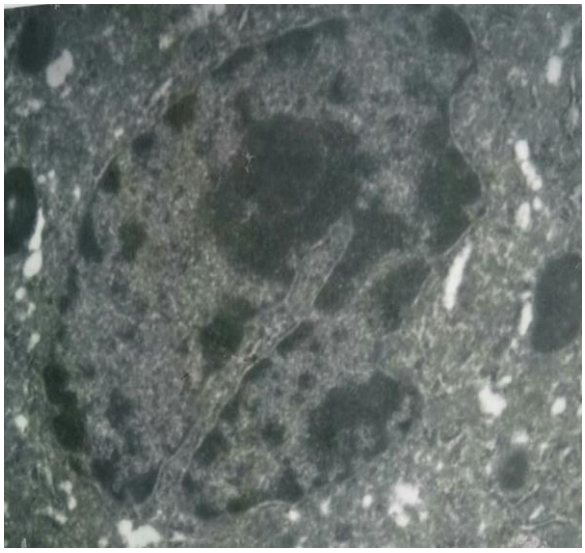


Figure-4



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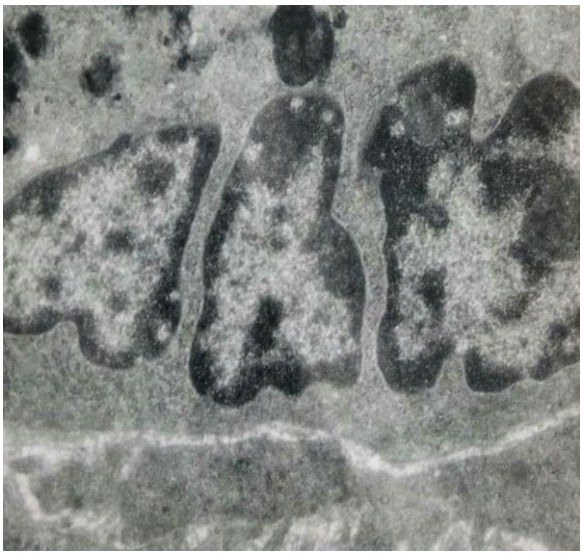

Figure-6

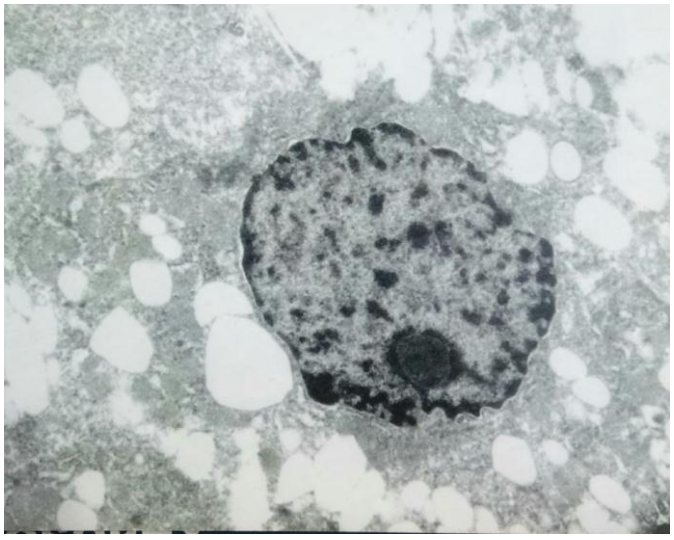

Figure- 7.

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 visible (x 14,000).

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

Figure 7: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 4 weeks followed by Withania somnifera administered for 4 weeks showing normal shape of nucleus with nucleolous with mitochondria. Double membrane of nucleus are recovered to a great extent.

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

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).

Withania somnifera administration for 4 weeks has played a promising role in combating the toxic effect of endosulfan. Withania somnifera administered for 4 weeks showed almost normal nucleus & nuclear membrane. Although the ameliorating impact was very slow, still cell organelles proved to be in better condition than those due to Endosulfan toxicity. Double membranes of nuclei are recovered to a great extent. Heterochromatin was more dense. Restoration was also seen in plasma membrane. Nucleolus tending towards normality. Lipid droplets were prominent in cytoplasm. Normal configuration of mitochondria were observed. (Figure 7)

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.[18] Several studies have been reported that Endosulfan has adverse effects on health.[19,20] In a previous study, the adverse effect of Endosulfan was also reported on follicular development of BALB/c mice.[21] Hiremath and Kaliwal[22] 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.[23] Pathak et al.[24] 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.[25] 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.

Withania somnifera is one of the herbal medicines widely used for the treatment of infertility and sexual dysfunction. This plant has been known to contain more than 80 types of phytochemicals such as steroidal and nonsteroidal alkaloids, steroidal lactones and saponins like isopelletierine, anaferin, anahygrine, hygrine, cuscohygrine, tropine, pseudotropine, withananine, ashwagandha, withaferins, withananinine, pseudowithanine, somnine, somniferine, somniferinine, 3-tropyltigloate, withanine, withasomine, visamine, mesoanaferine, sitoindoside, hentriacontane, amino acids such as aspartic acid, glycine, tryptophan, proline, alanine, tyrosine, hydroxyproline valine, cystine, glutamic acid, and cysteine, calcium, phosphorus, iron, flavonoids, starch, reducing sugars, proteolytic enzyme "chamase," glycosides, dulcitol, and volatile oil. Of all these components, withaferin A and sitoindosides had the key role in WS therapeutic effect.[26-32]

Based on the present study, it was shown that extracts of WS fruits, leaves, stems, and especially roots enhance sperm quality indices such as motility and count in men[33,34] and also decrease the effects of chemical toxins on gonads in both men and women. WS can increase gonadal weight in both sexes, enhance folliculogenesis and spermatogenesis, and improve LH, FSH, and testosterone balances.[35-40] Sexual behavior indices such as female sexual function index and female sexual distress index improve statistically significant after WS extract administration.[41]

CONCLUSION

Therefore, from the present study it can be concluded that *Withania somnifera* has a positive effect in the treatment of infertility at sub cellular level of ovary of mice against endosulfan induced toxicity. Due to the growing interest in using herbal medicine especially those which possess the antioxidative and reproductive system supporting properties, further studies are needed to be designed with higher population and more-structured methodology so a more precise and decisive conclusion can be made.

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