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BIOREMEDIAL IMPACT OF *WITHANIA SOMNIFERA* ON ENDOSULFAN EXPOSED SPERMATOZOA OF MICE

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ABSTRACT

Background/Aim: Endosulfan is a pesticide of organochlorine group uses by 55 % of farmers in Bihar. Present study aims to illustrate effect of Endosulfan on biochemical, hormonal and sub cellular anomalies of spermatozoa of mice and their restoration through root extract of *Withania somnifera*.

Materials and Methods: Experimental mice were administered with endosulfan for eight weeks followed by eight weeks administration of *Withania somnifera*.

Results: Endosulfan administered group show degenerated mitochondria and plasmamembrane. Degenerated microtubule were also observed with rudimentary central hub and dynein arm. While eight weeks *Withania somnifera* 1000 mg/kg/b.w/day administered group show greater degree of sub cellular restoration on mitochondria and nuclear membrane. Microtubules were almost normal in structure. Lipid peroxidation level were also restored toward normal after ashwagandha administration.

Conclusion: These combined effect finally leading to restoration in structure of spermatozoa in mice. This is very effective in restoring male fertility by combating endosulfan toxicity.

INTRODUCTION: Chemical pesticides have become part of our agriculture since 1960s. Farmers have been exposed to toxic pesticides like DDT and endrin since then. Nineteen sixties officials from the government departments had to plead and cajole them to start using a pesticide. Since 1980, people living in the villages near and around the plantation found some abnormalities first in the environment and later in their own body and their new born babies for which they could not find any reason [1]. In the '90s it is realized that the spraying of pesticides in the cashew plantations near their homes could be the reason for the strange happenings [2]. Environmental contaminants due to the intensive use of pesticides as modern agrochemical have created a lot of problem to the animal [3]. In terms of biochemical and physiological alteration in their metabolism. It exerts their impact at cellular and sub-cellular level prior to any gross sign of damage, being apparent in the morphology of the animal. Endosulfan is a group of organochlorine which has been used primarily to kill mosquitoes or different insects. Endosulfan enters into mammalian body through food chain or direct contact through inhalation leading to various physiological or metabolic dysfunctions including infertility.

India has a rich history of using plants for medicinal purposes. The Indian System of Medicine, viz Ayurveda, Siddha, Unani and Homeopathic system predominantly use plant-based raw materials for most of their preparations and formulations. Ashwagandha (*Withania somnifera*) is a medicinal plant extensively used in Ayurveda as home remedy for various diseases [4].

Present study illustrates structural alteration of spermatozoa due to endosulfan toxicity. Testosterone, calcium ion and lipid per oxidation also show abnormal trend. Therapeutic action of Withania somnifera were also observed against pesticidal toxicity on sub cellular, biochemical and hormonal parameters. Structural restoration of spermatozoa through Withania treatment provide a ray of hope against pesticide induced infertility.

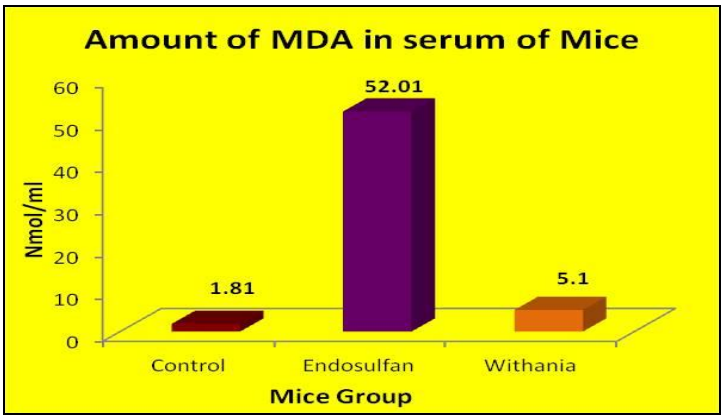
MATERIALS AND METHODS:

Animals: The mice were reared in our laboratory. The age group of mice selected for the study was 12 weeks old with 30±2 gm. b.w.

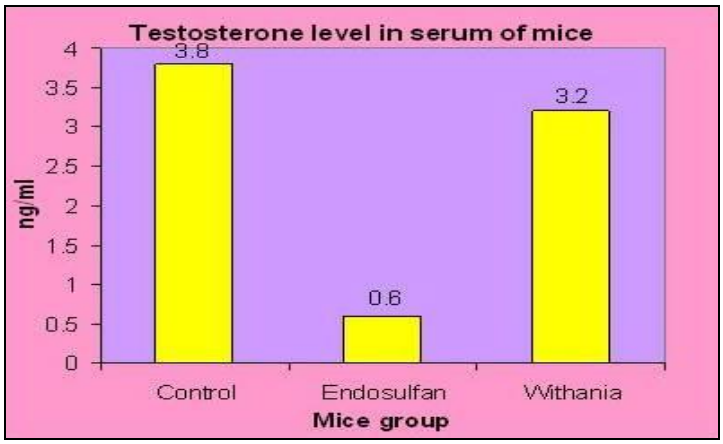
Chemicals: Pesticide Endosulfan, manufactured by Excel India Pvt. Ltd., Mumbai with EC 35% was utilized for the experiment. Aquous root extract of *Withania somnifera* is administered after pesticidal exposure.

Study groups & sampling: The control group of 10 mice received distilled water as drinking water. The 'treatment' groups (n=10) received Endosulfan 3 mg/kg b.w daily by gavage method for eight weeks followed by eight weeks administration of aqueous extract of roots of *Withania somnifera* (1000 mg/kg/b.w/day). Animals were sacrificed after the scheduled treatment. Serum was collected for testosterone, Calcium ion and Lipid peroxidation (MDA) assay. The testis from all the animals were removed and washed three times in isotonic saline (0.85 v/w%) and fixed in 2.5% gluteraldehyde for Transmission Electron Microscope (TEM) study.

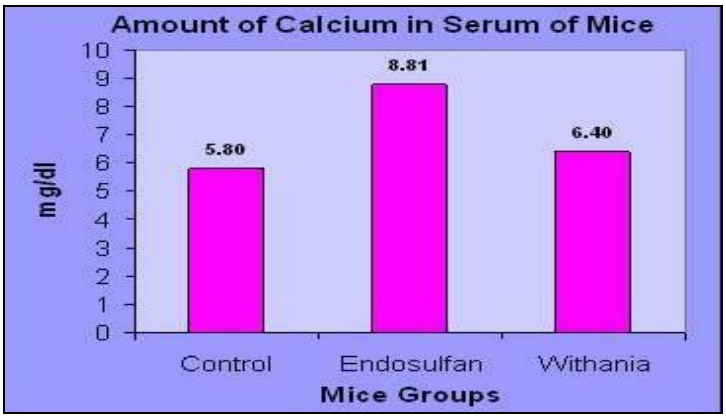
RESULTS: Lipid peroxidation (MDA) was 1.8 nmol/ml in control group, it was 52.0 nmol/ml after eight weeks of endosulfan and it was 5.1 nmol/ml after consecutive eight weeks of *Withania somnifera* administration (**Graph 1**). Testosterone level was 3.8 ng/ml in control group, while 0.6 ng/ml after eight weeks of endosulfan, 3.2 ng/ml after consecutive eight weeks of *Withania somnifera* administration (**Graph 2**). Calcium ions was 5.8 mg/dl in control group, it were 8.8 mg/dl after eight weeks of endosulfan and it were 6.4 mg/dl after consecutive eight weeks of *Withania somnifera* administration (**Graph 3**).



GRAPH 1: SHOWING LIPID PEROXIDATION (MDA) LEVEL IN DIFFERENT GROUP OF MICE



GRAPH 2: SHOWING TESTOSTERONE LEVEL IN DIFFERENT GROUP OF MICE



GRAPH 3: SHOWING CALCIUM ION LEVEL IN DIFFERENT GROUP OF MICE

Control group shows normal plasma membrane, Golgi complex, Mitochondria, Nuclear membrane and chromatin (**Figure 1**). Tail of spermatozoa with 9+2 organization of microtubules with distinct dynein arm were observed (**Figure 2**). While in Endosulfan eight weeks administered group degenerated plasma membrane were observed. Dissolved mitochondrial cristae were seen.

Deformed Golgi complex was also seen. Degenerated nuclear membrane were observed (**Figure 3**). Number of dilated nucleopore complex were observed with heterochromatinised nuclear material (**Figure 4**). Dispersed axonema, dynein arm, central hub and protofilament were observed (**Figure 5 & 6**).

While the group administered with eight weeks endosulfan followed by eight weeks ashwagandha administration shows restoration of these ultra structural deformities such as normal plasma membrane, almost normal Golgi complex with distinct secretary vesicles. Mitochondrial cristae were observed with well organized double membrane. Nucleopore complex were distinct with normal chromatin material (**Figure 7**). 9+2 organization of microtubules were restored with distinct dynein arm and protofilament, central hub were clearly seen (**Figure 8**).

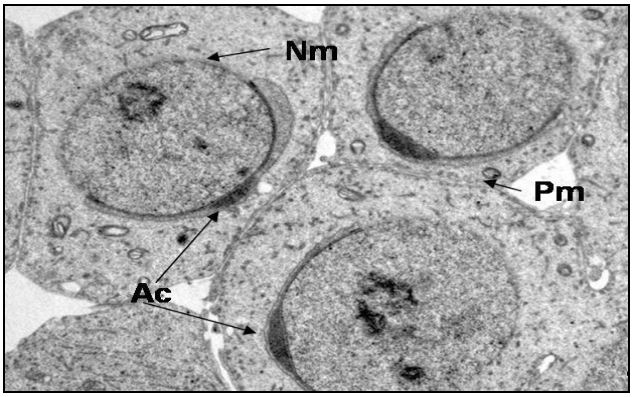


FIGURE 1: SHOWING NORMAL SPERMATOGONIA IN CONTROL GROUP

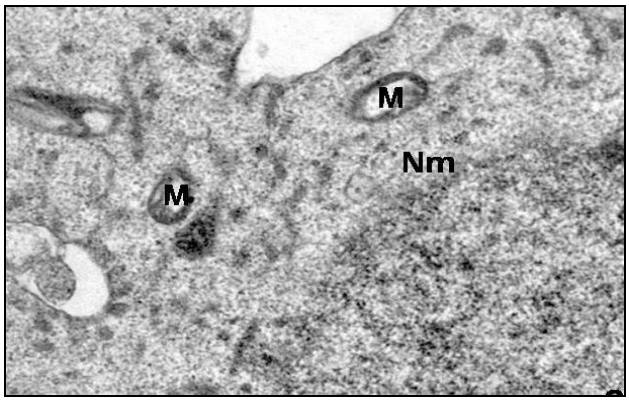


FIGURE- 2: SHOWING DEGENERATION OF SPERMATOGONIA AFTER ENDOSULFAN TREATMENT. DEFORMED MITOCHONDRIA ARE ALSO OBSERVED

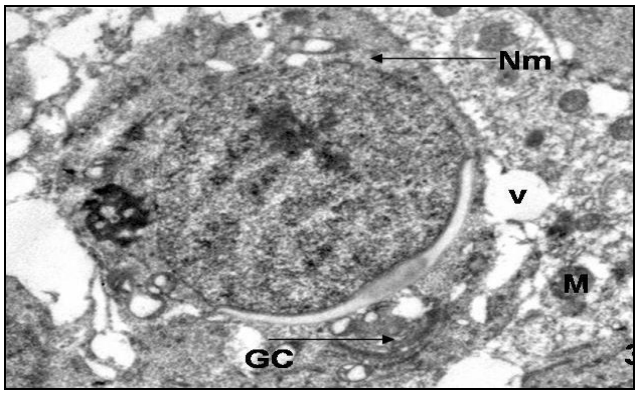


FIGURE 3: SHOWING DEGENERATION OF MITOCHONDRIA AS WELL AS GOLGI COMPLEX AFTER ENDOSULFAN TREATMENT

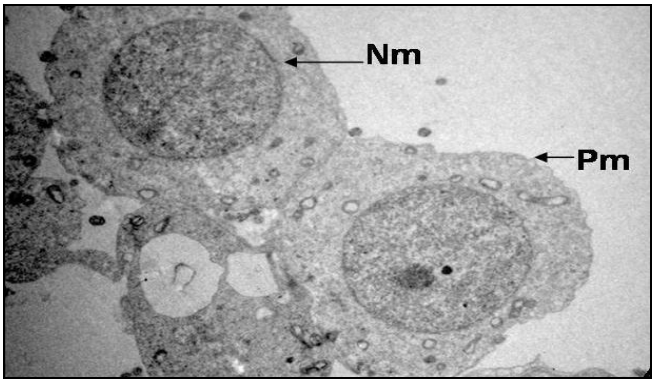


FIGURE 4: SHOWING RESTORATION OF ENDOSULFAN TOXICITY AFTER *WITHANIA SOMNIFERA* TREATMENT

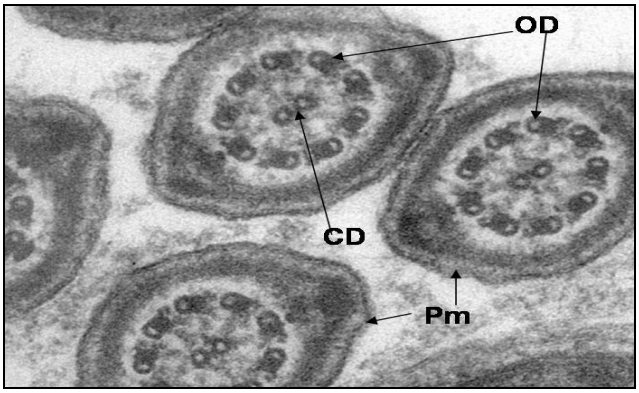


FIGURE 5: SHOWING NORMAL 9+2 ARRANGEMENT OF MICROTUBULE IN CONTROL GROUP

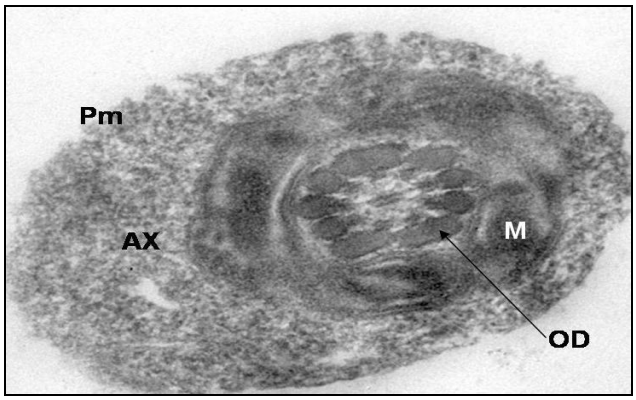


FIGURE 6: SHOWING DEGENERATION OF MICROTUBULE AFTER ENDOSULFAN TREATMENT

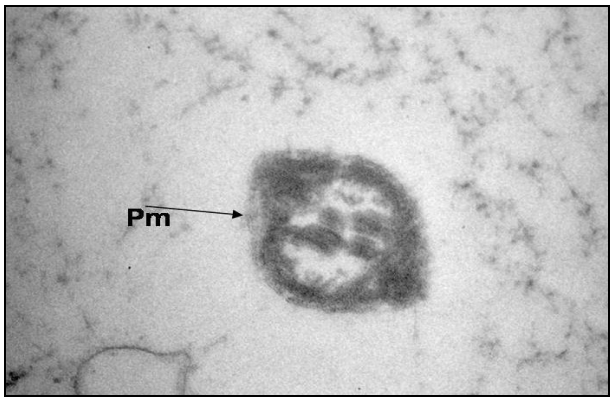


FIGURE 7: SHOWING COMPLETE DEGENERATION OF MICROTUBULE AFTER ENDOSULFAN TREATMENT

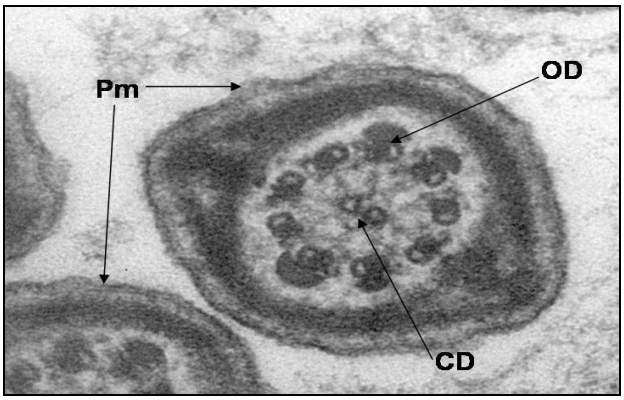


FIGURE 8: SHOWING RESTORATION OF ENDOSULFAN TOXICITY AFTER WITHANIA TREATMENT.

DISCUSSIONS: Endosulfan has also been shown to disrupt the reproductive endocrine system and affect reproduction [5]. Several research groups have demonstrated the harmful effects of this pesticide on various organs such as liver and gonads [6].

The biochemical and ultra structural observation on spermatozoa of mice treated with endosulfan clearly demonstrate that endosulfan causes 29 fold inclinations in MDA level as well as increased calcium Ion will be observed, while testosterone level declines up to 18 fold. These combined biochemical alteration were leading to malformed spermatozoa. Ultra structural degeneration of spermatozoa was also prominent after endosulfan exposure show correlation between calcium ion concentrations, testosterone and lipid per oxidation alteration with ultra structural degeneration.

Testosterone is made in the testes from cholesterol. The first step is to move cholesterol into the cell's mitochondria, the energy generating organelles located in a cell's liquid cytoplasm. A protein called steroidogenic acute regulatory protein (StAR) escorts cholesterol across the mitochondria's membrane. An enzyme called cytochrome p 450 side-chain cleavage (p 450 sec) converts cholesterol into pregnenolone. A series of steps controlled by other enzymes change pregnenolone into testosterone [7]. Testosterone level is found to be very low due to high amount of calcium released into serum. Testosterone is required for spermatogenesis while least testosterone level causes altered formation of spermatozoa.

It was found that Ca^{2+} stimulates respiration of mitochondria in a stoicheiometric and cyclic fashion, in such a manner that 2 Ca^{2+} ions yield the same amount of extra oxygen uptake as 1 molecule of ADP [8, 9]. Accumulation of Ca^{2+} during electron transport is accompanied by H^+ ejection [10, 11]. Due to increase in calcium ion concentration in serum accumulation of calcium does not occurs inside mitochondria, it decreases electromotive force generated in mitochondria causing least formation of ATP. More ATP is needed for detoxification by SER (Smooth Endoplasmic Reticulum) but very least were formed causing degeneration of mitochondria due to toxicity.

In the etiology of male infertility, there is growing evidence that damage to spermatozoa by reactive oxygen species (ROS) play a key role [12]. Spermatozoa contain large quantities of polyunsaturated fatty acids (PUFA). Therefore, they are susceptible to ROS-induced damage. It has been suggested that ROS induce membrane lipid per oxidation in sperm [13, 14, 15]. MDA level was found increased many times indicates that lipids were oozes out from membranous system of cell causing degeneration of spermatozoa.

Depletion of lipid was observed from microtubule causing non motility in spermatozoa. It has been observed through electron microscopic photographs that mitochondrial membrane is degenerated of mid piece of spermatozoa which is mentioned earlier. This may be the reason that after administration of endosulfan the level of testosterone declined drastically compared with control causing infertility in male mice. Jung *et al*., [16] have studied the effect of medicinal plant extract on swimming capacity of spermatozoa of mice. Mathur *et al*., [4] have observed the effect of *Withania somnifera* root extracts on cell cycle and angiogenesis.

CONCLUSIONS: Thus, it is evident from study that *Withania somnifera* restores cell organelles damage caused by endosulfan toxicity as well as maintains almost normal testosterone level, lipid preoxidation and calcium ions in serum like control group. These combined effect finally leading to restoration of normal structure of spermatozoa in Swiss albino mice. This is very effective in restoring male fertility by combating endosulfan toxicity.

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