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PHYTOREMEDIAL EFFECT OF *WITHANIA SOMNIFERA* AGAINST ENDOSULFAN INDUCED TOXICITY IN SERTOLI CELLS OF MICE

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ABSTRACT

The main objective of the study was to observe the ameliorative effect of *Withania somnifera* on Endosulfan induced toxicity in sertoli cells of mice. In the present study, Endosulfan at the dose of 3mg/Kg body weight was administered orally to male mice for 5 weeks. Thereafter, crude root extract of *Withania somnifera* at the dose of 800 mg/Kg body weight was administered for 5 weeks to observe the ameliorative effect of it on sertoli cells. The study reveals that after the administration of Endosulfan, there significant damage at the sub cellular level in sertoli cells of mice. There were significant changes in the testosterone level in endosulfan exposed group. Administration of *Withania somnifera*, caused significant reversal at the sub cellular levels denotes that it not only possesses antioxidant and rejuvenating property but also maintains the cellular integrity and ultrastructure of sertoli cells. Testosterone levels were also restored effectively in withania administered group. It is one of the best antidote against endosulfan induced sub cellular toxicity.

KEYWORDS: Endosulfan, *Withania somnifera*, mice, Testosterone, Sertoli cells.

INTRODUCTION

Agrochemicals pollute the environment primarily because of their wasteful application and due to the fact that crops use them inefficiently. Indiscriminate use of agrochemicals under conventional agriculture not only causes severe health hazards for human beings but also has numerous other side effects on the environment including destruction of the biodiversity.

Endocrine disrupting chemicals 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.[1] It has been suggested that endocrine disrupting chemicals pose a potential risk and can alter the hormone balance in humans and wildlife.[2] These environmental xenobiotics may impair the normal embryonic development and disrupt normal reproductive functions in adulthood.[3,4] Organochlorine pesticide, such as 2, 2-bis (4-Chlorophenyl)-1,1,1-trichloroethane (DDT), is a widespread environmental xenobiotics. DDT is a persistent organic pollutant with the features of wide pollution, huge harm and longtime persistence in environment, and entering the living system via bio-magnification of food chain. It may persist mainly by the forms of metabolite 1,1-dichloro- 2,2 bis(p-chlorophenyl) ethylene (p,p_-DDE) in the blood lipid and adipose tissue for several decades.[5,6,7]

Withania somnifera (WS) also called as Ashwagandha, winter cherry or Indian Ginseng is one of the important medicinal herb in Ayurveda and indigenous medical systems for over 3000 years. Studies indicate Ashwagandha possesses antioxidant.[8], anti-inflammatory[9], immunomodulatory[10], antitumor[11], antistress[12], adaptogenic[13], antiulcer[14] and rejuvenating properties.[15, 16] However, no studies have been reported effect of *W.somnifera* root extract as antidote against arsenic induced male reproductive toxicity in mice. Therefore, present study aims to observe the phytoremedial effect of *Withania somnifera* on Endosulfan induced toxicity in sertoli cells of mice.

MATERIALS AND METHODS

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

Animals

Swiss albino mice were bred at the mice room of Prof. A.Nath, Department of Zoology, Patna University, Patna, Bihar, India. The age of mice for the experiment was 12 weeks old. The average body weight of experimental mice was 30 ± 2 g. Food and water to mice were provided *adlibitum* (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups (6 each). The mice were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22±2$^{\theta}$C with 12 h light/dark cycle.

Preparation of crude extract: In the present study, dry root of *W.somnifera* were purchased from Haridwar Medicinal Store, Haridwar, Uttrakahand, India. The collected root of *W. somnifera* were dried at 37 ^{0}C and were grinded to fine powder. The crude extract dose was 800 mg kg^{-1} body weight.

Study groups & sampling*:* The control group of mice received distilled water as drinking water. The treatment groups received Endosulfan 3 mg/kg b.w daily by gavage method for five weeks followed by five weeks administration of aqueous extract of roots of *Withania somnifera* (800 mg/kg/b.w/day). Animals were sacrificed after the scheduled treatment and their blood serum were collected for testosterone assay. The testis from all the animals were dissected and washed three times in isotonic saline (0.85 v/w%) and fixed in 2.5% gluteraldehyde for Transmission Electron Microscope (TEM) study.

RESULTS

The serum testosterone levels in control group was 3.637 ± 0.1126 ng/ml, 0.9683 ± 0.1314 ng/ml in endosulfan 5 weeks administered mice while 3.412 ± 0.1304 ng/ml in *Withania somnifera* administered to Endosulfan 5 weeks pretreated mice (Graph: I, Table: I).

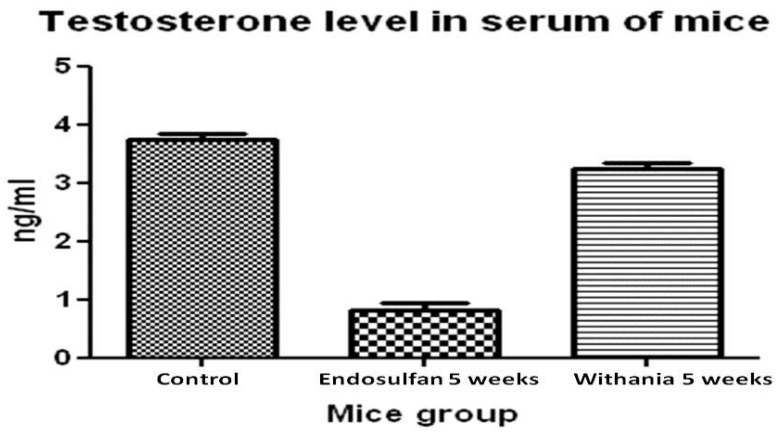
Sertoli cells of control mice show well organized nucleus with nuclear membrane (Figure -1). Sertoli cells of five weeks endosulfan administered mice show many osmiophilic granules condensed in cytoplasm. Degenerated plasma membrane of spermatozoa were also observed (Figure - 2). Deshaped nucleus and degenerated mitochondria were clearly observed with many vacuolated spaces. Degenerated plasma membrane of sertoli cells were observed. Nourishing spermatozoa were clearly observed with degenerated acrosome (Figure - 3). Sertoli cells of five weeks endosulfan administered mice followed by five weeks administration of *Withania somnifera* show almost normal plasma membrane. Restoration was prominently observed in mitochondria. Tail of spermatozoa were also restored inside sertoli cells (Figure - 4). Well nourished spermatozoa with normal chromatin structure were also observed in *Withania somnifera* administered group. Plasma membrane of sertoli cells was continuous and normal while acrosome were almost normal in structure (Figure - 5).

DISCUSSION

Abnormalities sexual development in mice and wildlife might be associated with exposure to organochlorine pesticide is antiandrogenic and can inhibit androgen binding to the androgen receptor.[17]

Sertoli cells are the first cell type to become recognizably differentiated in the fetal gonad, an event which enables seminiferous cord formation, prevents germ cell entry into meiosis differentiation, and enables the function of leydig cells.[18] At puberty, the sertoli cells responds to androgen and takes on roles supporting spermatogenesis, and likely affects the function of the steroid-producing leydig cells through complex cell–cell interactions. Spermatogenesis is a multistep process, with mature spermatozoa developing from spermatogonia, the stem cells of the germ cell lineage.[19] This process involves the complex interaction of germ cells and sertoli cells within the seminiferous tubules.[20,21] Co-culture of sertoli cells with spermatogenic cells results in stimulation of germ cell RNA and DNA synthesis[22], induction of germ cell surface antigen presentation.[23], and maintenance of glutathione synthesis in developing germ cells.[24] In addition to interacting with and providing essential cellular factors to germ cells, sertoli cells are able to functionally communicate with leydig cells. The location of leydig cells in the interstitium between seminiferous tubules, and the presence of peritubular myoid cells at the basement membrane of the seminiferous tubules prevent direct physical contact between sertoli and leydig cells. However, damage to seminiferous tubule function, with cytotoxic agents or fetal irradiation, causes abnormal cytological features and function in adjacent leydig cells[25], suggesting that a regulatory interaction exists between sertoli cells and leydig cells.

Withania somnifera exhibits both antioxidant and pro-oxidant activity. Tumor-bearing animals treated with both IP and PO doses of WS showed increased GSH, SOD, GPx, and catalase in the liver and skin.[26] These effects could clearly repair oxidative damage caused by tumor growth and inflammation, thus reducing the likelihood of disease progression. This antioxidant activity is enhanced by the potential of *Withania somnifera* to up-regulate phase II liver enzymes.[27] In present study bioremediation on plasma membrane and nuclear membrane of sertoli cell were distinct. Experiment on Swiss albino mice fed with 2.5- and 5.0-percent Withania root extract diet showed 1.67- and 1.26-fold up-regulation of DT-diaphorase (DTD) and GST, respectively. Both are phase II liver enzymes that conjugate metabolites of cytochrome p450, which aids in liver detoxification of toxic phase I byproducts. In present study mitochondria were also restored to greater extent. Cytochrome p450 directs testosterone formation due to which testosterone level in mice was almost normal after *Withania somnifera* treatment.[28]



Graph Figure –I: Testosterone levels in serum of mice

Figure legends.

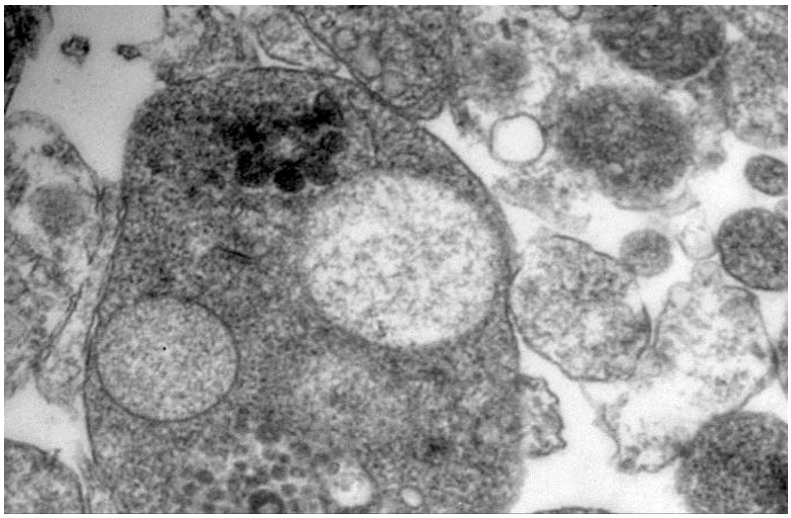


Figure 1: Showing sertoli cells of control mice with well organized nucleus.

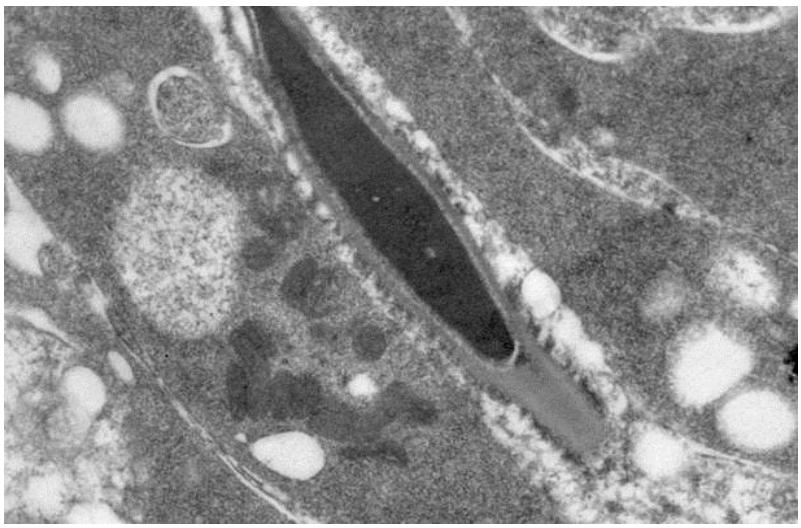


Figure-2: showing sertoli cells of five weeks endosulfan administered mice with many osmiophilic granules condensed in cytoplasm. Degenerated tail of spermatozoa is also observed. Degenerated mitochondria is clearly observed with many vacuolated spaces.

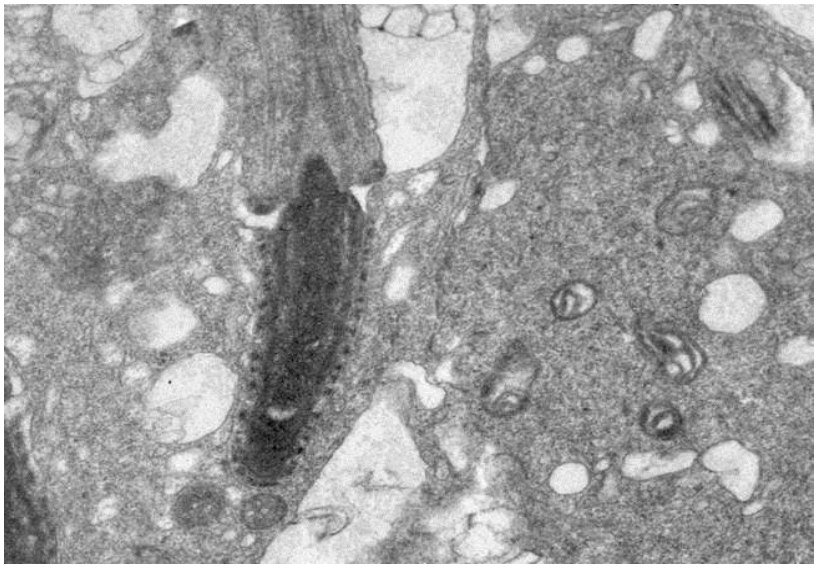


Figure - 3: showing sertoli cells of five weeks endosulfan administered mice with degenerated plasma membrane of sertoli cells. Nourishing spermatozoa is clearly observed with degenerated acrosome.

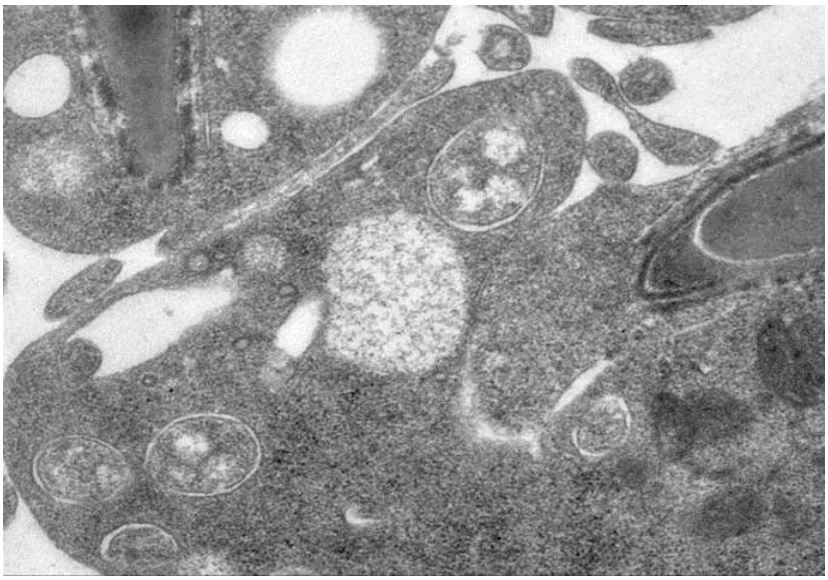


Figure - 4: showing sertoli cells of five weeks endosulfan administered mice followed by five weeks administration of *Withania somnifera*. Plasma membrane is almost normal. Restoration is prominently observed in mitochondria. Tail of spermatozoa is also restored.

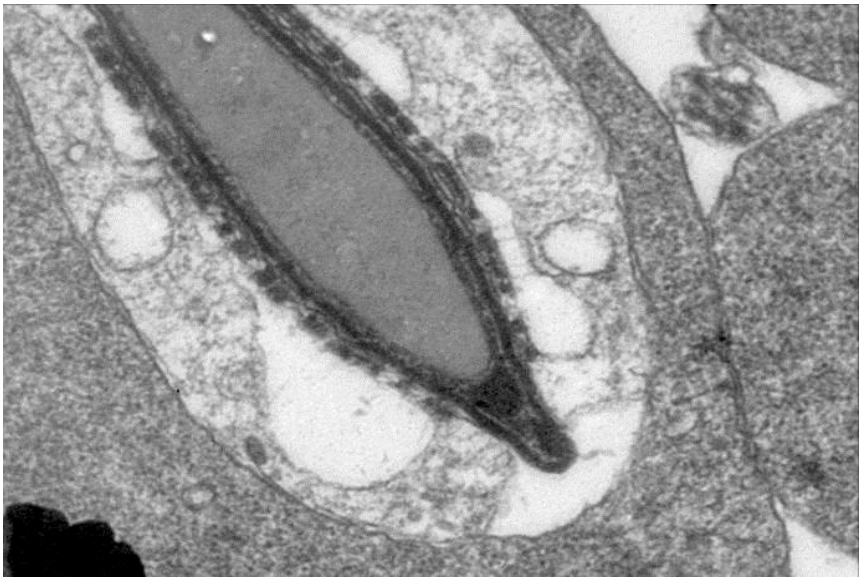


Figure - 5: showing sertoli cells of five weeks endosulfan administered mice followed by five weeks administration of *Withania somnifera*. Showing well nourished spermatozoa with normal chromatin structure. Plasma membranes of sertoli cells is almost normal. Plasma membrane of spermatozoa is fragmented, while acrosome is almost normal.

CONCLUSION

It is evident from study that endosulfan causes decrease in the levels of testosterone in mice. It also causes degeneration in nuclear material with many vacuolated spaces. Plasma membrane with mitochondrial membrane are highly degenerated while *Withania somnifera* show greater restoration in testosterone levels upto normal level. Plasma membrane, nuclear membrane and mitochondria were restored as good as normal. Thus, from the above study it can be concluded that *Withania somnifera* plays vital role in phytoremediation of endosulfan toxicity on ultra-structure of sertoli cells and testosterone of mice.

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