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Ameliorating Effect of Turmeric against Endosulfan Induced Toxicity in Ovarian Cells of Mice

Open Access **Research Article**

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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 utilising 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 therefore focused to know the ameliorating effect of turmeric on endosulfan induced reproductive toxicity in female mice. Endosulfan at the dose of 3mg/Kg body weight was administered by Gavage method to female mice for1 week, 2 weeks & 4 weeks. Thereafter, aqueous extract of turmeric at the dose of 200 mg/Kg body weight per day orally was administered for 4 weeks to observe the ameliorative effect of it on ovarian cells. The study reveals that after the exposure of endosulfan, there was significant damage at the sub cellular level in ovarian cells of mice along with hormonal imbalance. But, after the administration of turmeric, there was significant normalisation at the sub cellular levels denotes that it not only possesses ameliorating and rejuvenating property but also maintains the cellular integrity of the ovarian cells leading to normal functioning of it. Thus, it proves to be one of the best antidote against endosulfan induced toxicity.

Keywords: Endosulfan; Turmeric; Hormonal Imbalance; Mice; Electron Microscopy

Introduction

The wide use of agrochemicals under conventional agriculture has caused severe health hazards for human beings. It also has caused numerous other side effects on the environment including destruction of the biodiversity. Endosulfan is an organochlorine insecticide effective against a wide range of pests like cereals, vegetables, fruits, coffee, cotton, oilseeds etc. It is easily absorbed in the gastro intestine by the stomach, lungs and skin and exposure through any route can be hazardous. The commercially produced endosulfan is composed of two isomers α- endosulfan and β- endosulfan and both these forms are genotoxic to human gonads *[1,2]*. According to World Health Organisation (WHO), endosulfan is currently classified as Class II – moderately hazardous to human health while the United States' Environmental Protection Agency (EPA) rates endosulfan as Category Ib – highly hazardous *[3]*. The LD_{50} data for endosulfan are in conformity with some published results indicating that the chemical should be in the WHO's Class Ib, according to the organisation's own criteria. Evidence of the threats to human health posed by endosulfan are abundant, and the chemical has been banned outright or severely restricted in a number of countries as a result. Independent of LD_{50} results, these threats warrant the immediate upgrading of endosulfan to WHO Class Ib *[4]*. Endosulfan has also been associated with estrogenic activity both *in vivo* and *in vitro [5-7]*.

The active constituent of turmeric is known as curcumin. It has been shown to have a wide range of therapeutic actions. First, it protects against free radical damage because it is a strong antioxidant (Sreejayan& Rao, 1996). Second, it reduces inflammation by lowering histamine levels and possibly by increasing production of natural cortisone by the adrenal glands *[8]*. Curcumin is also reported to have antibacterial *[9]*, antiamoebic *[10]* and anti HIV *[11]* activities. Curcumin also shows antioxidant activity *[12-15]*. It also shows antitumour, *[16-18]* and anticarcinogenic *[19-21]* activities.

Therefore, in the present study turmeric has been used to observe its ameliorating efficacy against the pesticide endosulfan.

Materials and Methods

Animals: Swiss albino mice were bred at the Mice Room of Prof. A.Nath, Department of Zoology, Patna University, Patna, Bihar, India. The animals had free access to water and feed pellets (mixed formulated feed prepared by the laboratory itself). The ethical approval was obtained from the Post Graduate Research Council of Patna University.

The age of mice for the experiments was 12 weeks old. The average body weight of experimental mice was 30 + 2 g.

Test Chemical: Pesticide endosulfan, manufactured by Excel

India Pvt. Ltd., Mumbai with EC 35% was utilized for the experiment.

Preparation of Turmeric dose: In the present study, turmeric was purchased from the local market and aqueous dose of 200 mg/Kg body weight was prepared for oral administration after estimation of LD_{50} value which was 1600 mg/Kg 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 7days, 14 days & 4 weeks followed by 4 weeks administration of turmeric (200 mg/Kg body weight /day). Animals were sacrificed after the scheduled treatment. Serum were collected for estrogen & progesterone assay. The ovary 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.

Hormonal Assay: Using the ELISA method Estrogen & Progesterone kit of LILAC Medicare (P) Ltd., Mumbai was utilized for the experiment.

Statistical Analysis: Results are presented as mean ± SD and total variation present in a set of data was analysed through one way analysis of variance (ANOVA). Difference among mean values has been analysed by applying Dunnett's test. Calculations were performed with the Graph Pad Prism Program (Graph Pad software, Inc., San Diego, U.S.A.). The criterion for statistical significance was set at $P< 0.05$.

Results

Hormonal Assay : The estrogen levels in control mice was 32.1± 0.078pg/ml, while in the endosulfan treated group there was significant decrease in the hormone levels as in 7 days, 14 days and for 28 days were 26.3 ± 0.011 pg/ml, 18.2 ± 0.029 pg/ml and 13.5± 0.098 pg/ml respectively. But, after the 28 days of turmeric treatment there was significant normalisation in the estrogen level after Endosulfan toxicity as 26.3± 0.314 pg/ml. (Figure 1). Similarly in the progesterone levels in control mice was 4.0±0.352 pg/ml, while in the endosulfan treated group there was significant decrease in the hormone levels as in 7 days, 14 days and for 28 days were 3.1 ± 0.221 ng/ml, 2.5 ± 0.189 ng/ml, 1.9± 0.041 ng/ml respectively. But, after the 28 days of turmeric treatment there was significant normalisation in the progesterone level after endosulfan toxicity as3.3± 0.124 ng/ml (*Figure 2*).

Electron Microscopic Study: Transmission Electron Micrographs of control ovary of mice showed double membrane of nucleus with normal chromatin material. Mitochondria as well as the ribosome were distinct while mitochondrial cristae and lipid droplets were clearly visible with normal endoplasmic reticulum (*Figure 3*). Ovary of mice treated with Endosulfan for 1 week showed nucleus with intact nuclear membrane with dilated nuclear pore complex with increased heterochromatisation. Dissolved plasma membrane were clearly visible with vacuolated spaces and dilation in nuclear pore complex with degeneration in mitochondria (*Figure 4*). Ovary of mice treated with Endosulfan for 2 weeks showed degenerated and deshaped nucleus. Mitochondrial membrane was highly degenerated in mitochondria. Wavy nuclear membranes with heterochromatinised elongated nucleus were observed. The nuclear pore complexes were 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. 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*). Turmeric administration for 4 weeks has played a promising role in combating the toxic effect of endosulfan. Turmeric 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 were distinct. Restoration was also seen in plasma membrane. Lipid droplets were prominent in cytoplasm; mitochondria were distinct with its double membrane covering, cristae and matrix (*Figure 7*).

Hormonal Assay Study

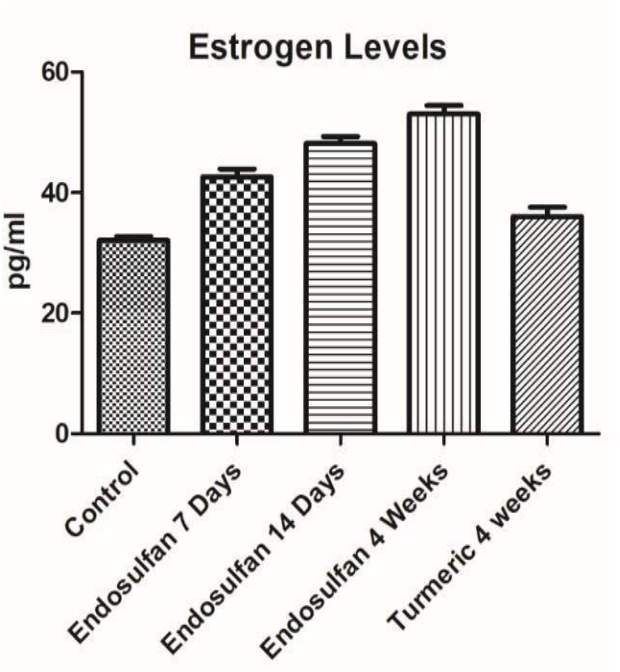

Figure 1: Showing Estrogen hormone levels in different groups in which seleniumshows significant amelioration. The data are presented as mean ± S.D, n = 6, significance at P< 0.001.

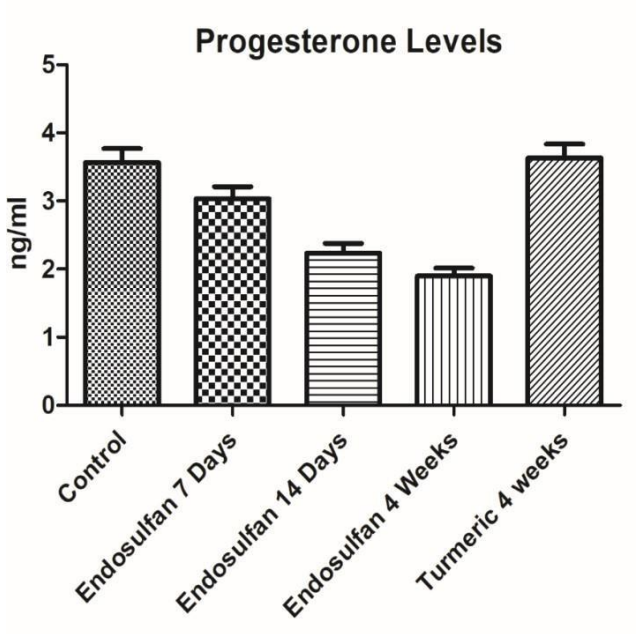

Figure 2: Showing Estrogen hormone levels in different groups in which selenium shows significant amelioration. The data are presented as mean ± S.D, n = 6, significance at P< 0.001.

Transmission Electron Microscopic Study

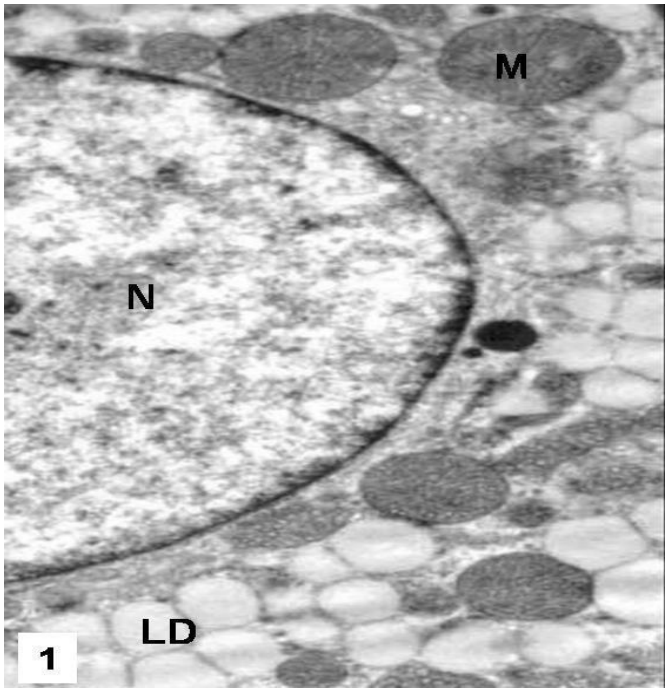

Figure 3: Transmission Electron Micrographs of Control ovary of mice showing normal architecture of double membrane of nucleus, chromatin material. Mitochondria as well as the ribosomes are very distinct.Mitochondrial cristae with lipid droplets are clearly visible.

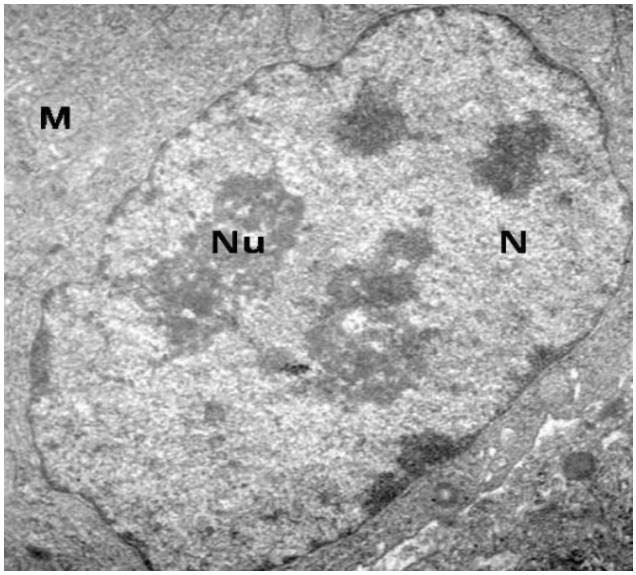

Figure 4: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 1 week showing nucleus with invagination. Dilated nuclear pore complex were observed with increased heterochromatisation. Dissolved plasma membrane are clearly visible with vacuolated spaces. Degeneration in mitochondria are clearly visible.

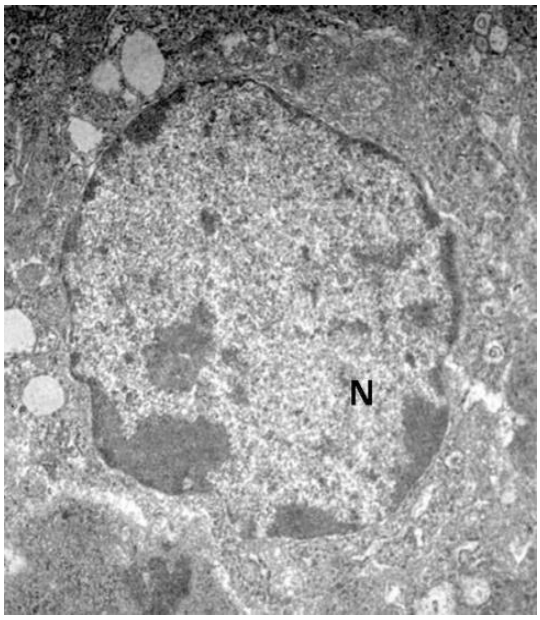

Figure 5: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for2 weeks showing degenerated and deshaped nucleus. Mitochondrial membranes were fragmented. Wavy nuclear membrane was clearly visible. Heterochromatinised elongated nucleus with patch like nucleolus and dilated nuclear pore complex are clearly observed. RER are in highly degenerated condition.

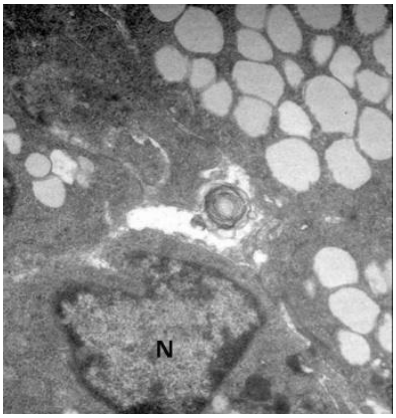

Figure 6: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 4 weeks showing nucleus in degenerated condition. Nuclear pore complex is highly dilated. Nucleolus is not prominent. Lipid droplets are increased in the cytoplasmic region.

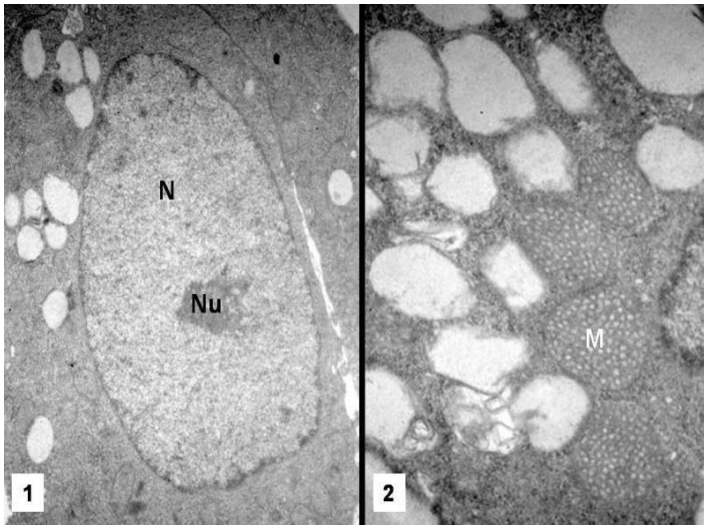

Figure 7: Transmission Electron Micrographs of ovary of mice treated with Endosulfan for 4 weeks followed by Turmeric administered for 4 weeks showing normal shape of nucleus with mitochondria and RER. Mitochondria with mitochondrial cristae are clearly visible. Double membrane of nucleus is clearly visible denotes recovery in cells.

Discussion

In the recent times, pesticides through food chain has caused serious health hazards in the human population. Endosulfan suppresses the estradiol concentration in female rats *[22]*. Estrogen plays an important role in increasing the activity of alkaline phosphatase activity in female genital organs (Filipe and Dowson, 1968) and also estrogen produced an increase in the endometrial mucopolysaccharide concentration (Huber, 1965). The functional relationship between DNA and RNA mediated protein synthesis was under the control of estrogen (Edwards 1967). Laguens (1964) suggested that estrogen acts upon the nuclear synthesis and secondary release of nuclear RNA into the cytoplasm. Progesterone is an important intermediate in the synthesis of androgens and oestrogens. It plays an important role in ovulation, atresia and luteinization *in vivo* (Telleria and Deis, 1994; Chaffin and Stouffer, 2000), and is essential for the continuation of early pregnancy. The functioning of vitellogenesis also depends upon the normal functioning of the liver. But the study also shows that endosulfan exposure causes deleterious effect on liver which hampers the normal functioning of liver (Kumar, 2008).In the present study, there was significant increase in the estrogen levels while significant decrease in the progesterone level denotes that these hormonal imbalances caused reproductive disorder in them. Similar study has been observed by *[23]*.

Endosulfan treatment inpubertal rate inhibits testicular functions *[24]*. 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.

Turmeric in the recent times has proven its efficacy as a good antioxidant in traditional medicine group. Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. Its anticancer effect is mainly mediated through induction of apoptosis (Ammon 1992). In the present study, turmeric maintains both estrogen and progesterone level in mice as it normalises the hormonal imbalance. Since its antioxidant activities are well established hence it plays the vital role to combat the toxicity *[25]*. However, the other studies have established the protective effect of turmeric on Endosulfan induced toxicity *[26]* (Farag 2014). In present study turmeric restores sub cellular structure of ovary in mice very effectively proves that it possesses antidote effect against endosulfan induced toxicity at hormonal level as well as at histopathological level *[27-33]*.

Conclusion

Therefore, from the present study it can be concluded that Turmeric plays the vital role to restore the Endosulfan induced toxicity athormone levels as estrogen, progesterone and at sub cellular level of ovary of mice

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