Effect of Curcuma longa on Ovary of Endosulfan Exposed mice

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ABSTRACT

India being a developing country, nearly 67% of the population strives on livelihood through agriculture. Endosulfan is a pesticide of organochlorine group commonly used in this region. Now a day’s herbal medicine were used extensively for treatment of different disease. Curcuma longa is very common herbal medicine used as spice commonly. Thus the present study is designed to evaluate bioremedial effect of Curcuma longa on estrogen and ovary of endosulfan exposed mice. The control group of mice received distilled water as drinking water. The ‘treatment’ groups received Endosulfan 3 mg/kg body weight daily by Gavage method for eight weeks followed by eight weeks administration of aqueous extract of rhizome of Curcuma longa (200 mg/kg/b.w/day). Animals were sacrificed after the scheduled treatment. Estrogen level was increased in endosulfan administered group of mice. Graffian follicle and granulosa cells were degenerated. Germinal epithelium and ova was also degenerated. While Curcuma longa administered group show restoration in estrogen level. Graffian follicle and germinal epithelium were also restored to greater extent. Ova were observed in normal shape. Thus it is concluded from entire study that endosulfan causes degeneration in graffian follicle, granulose cells and germinal epithelium layer. Ova were also degenerated with frequent vacuolization. Estrogen levels were also increased to greater extent. While Curcuma longa administration causes restoration in ova and graffian follicule. Germinal epithelium was also restored in structure to greater extent which indicates that Curcuma longa plays vital role against endosulfan toxicity on ovary and estrogen level to greater extent and restores normal fertility.

Key word: Organochlorine, graffian follicle, germinal epithelium, Gavage, ova.

INTRODUCTION

Agricultural industry being the prime source of national revenue, consumption of pesticides for high crop production has also increased. India being a developing country, nearly 67% of the population strives on livelihood through agriculture. Diverse environmental pollutants which mainly include a wide range of pesticides cause severe neuroendocrine disruption in both vertebrates and invertebrates [1]. Pesticides include various chemicals that protect the crops from pests such as weeds, fungus, insects and bacteria. A pesticide when applied in the field gets distributed majorly in air, water, soil and living organisms. Neurobehavioral deficits were also observed in rats administered with the same insecticide [2]. Diuron induced cytotoxicity has also been observed in male wistar rats causing urinary bladder carcinogenesis [3]. Shin et al. [4] conducted studies on uterus on different pesticides which revealed increase in the weight of the uterus of the rats on administration of Ethinyl estradiol and administration of Methoxychlor induces changes in the uterotrophic responses. Methoxychlor treated mice have also been studied by Tomic et al.[5] and this pesticide was shown to cause follicular atresia in the ovary indicated by low FSH levels.

Endosulfan has been a routinely used pesticide since 1960. Clinical examination has revealed hazardous consequences of endosulfan exposure which can lead to severe neurotoxicity, immunotoxicity, even causing chromosomal aberrations in humans [6]. Endosulfan-induced toxicity on the reproductive system of male rats illustrated disturbances in the serum hormonal levels suggesting deteriorating effect of endosulfan on the male reproductive system [7]. Studies conducted by Sarma et al.[8] explain the
effect of endosulfan on Testes and uterus of the swiss albino mice. High doses of endosulfan reduced the weight of the testes and lead to a decline in the sperm-count. The uterine weight and the uterine protein concentration of the treated mice also show a declining trend.

Now a day’s herbal medicine were used extensively for treatment of different disease. *Curcuma longa* is very common herbal medicine used as spice commonly. Curcumin is active ingredient of *Curcuma longa* it also target cells expressing phosphorylated Cdc27 prominent in proliferating cells to cause apoptosis [9]. Curcumin has also emerged as one of the promising targets of gastrointestinal tract, gastrointestinal diseases including inflammatory bowel diseases, hepatic fibrosis and gastrointestinal cancers [10]. Thus the present study is designed to evaluate effect of *Curcuma longa* on estrogen and ovary of endosulfan exposed swiss albino mice.

**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 rhizome extract of *Curcuma longa* is administered after pesticide exposure. Fresh rhizome of *Curcuma longa* was purchased from local herbal store in Patna, India. The identity of the rhizome of *Curcuma longa* was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India.

**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 rhizome of *Curcuma longa* (200 mg/kg/b.w/day). Animals were sacrificed after the scheduled treatment. Serum was collected for estrogen assay through ELISA techniques. The ovary from all the animals were removed and washed three times in isotonic saline (0.85 v/w %) and fixed in neutral formalin for Light Microscope (LM) study.

**RESULTS**

Estrogen level in control mice was 32.4 pg/ml. In endosulfan 4 weeks administered mice it was 42.4 pg/ml while after 8 weeks endosulfan administration it was 89.3 pg/ml. Endosulfan 8 weeks administered group followed by 8 weeks *Curcuma longa* administration it was 40.1 pg/ml (P Value: 0.0001) (Graph 1).

Ovary of control mice shows different stages of graffian follicle. Corpus luteum and germinal epithelium was also continuous (Fig 1). Normal structures of Corpus luteum were observed (Fig 2). Ovary of 8 weeks endosulfan administered mice show clustered nuclei in germinal epithelium. Ova become crescent shaped. Degenerated mature graffian follicle was also observed. Vacuolated nuclei were observed in corpus luteum (Fig 3). Ovary of 8 weeks endosulfan administered mice with degenerated mature graffian follicle, ova were degenerated. Degenerated cytoplasm was observed in germinal epithelium (Fig 4). Ovary of 8 weeks endosulfan administered mice followed by 8 weeks administration of *Curcuma longa* show restoration in corpus luteum. Germinal epithelium was also continuous. Ovum was also observed in restorative condition (Fig 5). Ovary of 8 weeks endosulfan administered mice followed by 8 weeks administration of *Curcuma longa* show restoration in corpus luteum. Germinal epithelium was also normal in structure (Fig 6).

**DISCUSSION**

Endosulfan is also known to influence the human estrogen-sensitive cells causing degenerative effects on the female reproductive system [11]. Work on Eker rats cell lines exposed with several organochlorides including endosulfan stimulated uterine leiomyoma proliferation. These organochlorides were seen to influence transcription of the estrogen-responsive genes and the progesterone receptor [12]. The alterations in liver tissue such as the increase in vacuolation, sinusoidal dialation and formation of bile plugs, has been also reported by Sakr et al. [13] in fish. Sinusoidal dilation in the liver is attributed to the impairment of outflow of the hepatic veins [14], while the formation of vacuoles in hepatocytes is due to the degeneration of cell membranes [15] and an imbalance between the rate of synthesis and utilization of substances in cells [16]. Marked hyperplasia and hypertrophy of tubuler cells in kidney are seen as a result of the effect of chronic exposure some pesticides such as malathion. Besides, an increase in excretion of potassium was significant [17]. Endosulfan causes spermatozoa degeneration [18] as well as declined testosterone level. Endosulfan exposure leads to ovarian nuclear degeneration [19]. In present study degeneration in graffian follicle and germinal epithelium were observed. Ova and granulose...
cells were also degenerated. Estrogen levels were also increased to greater extent after endosulfan exposure.

Evidence suggests that cell death induced by curcumin involves the activation of cell death pathways and inhibition of growth/proliferation pathways [20]. It down-regulates transcription factors NF-kappa B, AP-1 and Egr-1, and the expression of COX2, LOX, iNOS, MMP-9, TNF, chemokines, cell surface adhesion molecules and cyclin D1 factors and prevents nuclear translocation of NF-κB, thereby causing cancer cells to commit suicide [21]. In present study Curcuma longa administration causes increase in body weight of mice. It causes greater degree of restoration in both graffian follicle and ova. Germinal epithelium was also restored to greater extent. Granulose cells were also restored. Estrogen levels tend toward normalcy in Curcuma longa treated group of mice. Studies also suggest that curcumin is effective against bladder cancer caused by cigarette smoking [22].

Thus it is concluded from entire study that endosulfan causes degeneration in graffian follicle and ova. Germinal epithelium was also degenerated with frequent vacuolisartion. Clustered nuclei were observed in germinal epithelium. Estrogen level was increased many times. While Curcuma longa administration causes restoration in graffian follicle and germinal epithelium layer. Ova were also restored in structure to greater extent. Estrogen level also tends toward normalcy, which indicates that Curcuma longa plays vital role against endosulfan toxicity on estrogen and ovary and restores normal fertility in female.

Graph 1: Estrogen level in serum of mice

![Graph 1: Estrogen level in serum of mice](image)

Figure 1: show ovary of control mice with different stages of graffian follicle. Corpus luteum and germinal epithelium were also continuous.

Figure 2: show ovary of control mice with normal corpus luteum.

Figure 3: show ovary of 8 weeks endosulfan administered mice with degenerated mature graffian follicle. Ova become crescent shaped.

Figure 4: show ovary of 8 weeks endosulfan administered mice with degenerated mature graffian follicle, ova were also degenerated.
Figure 5: Ovary of 8 weeks *Curcuma longa* show restoration in corpus luteum. Germinal epithelium was also continuous. Ovum was also observed in restorative condition.

Figure 6: Ovary of 8 weeks administration of *Curcuma longa* show restoration in corpus luteum. Germinal epithelium was also normal in structure.

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REFERENCES


