



## BIOREMEDIATIONAL IMPACT OF *CURCUMA LONGA* ON UTERUS OF PESTICIDE 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 uterus of endosulfan exposed mice. The control group of mice received distilled water. The 'treatment' groups received Endosulfan 2 mg/kg b.w daily by Gavage method for four weeks followed by four weeks administration of aqueous extract of rhizome of *Curcuma longa* (200 mg/kgb.w/day). Animals were sacrificed after the scheduled treatment. Endosulfan four weeks administered group show clustered nuclei with degenerated cytoplasm in muscular structure of uterus mainly in stratum basale. Circular muscles were observed in more degenerated condition. Degenerated cytoplasm was observed in glands of uterus. *Curcuma longa* show restoration in muscular layer of uterus with well-defined myometrium and endometrium. Glands were also observed in restored condition. Thus it is concluded from entire study that endosulfan causes degeneration in endometrium and myometrium layer with degenerated glands. Muscular layer were also degenerated with frequent vacuolization. While *Curcuma longa* administration causes restoration in endometrial layer and myometrium layer. Glands was also restored in structure to greater extent which indicate that *Curcuma longa* plays vital role against endosulfan toxicity on uterus and restores normal function of uterus including implantation.

**Key word:** Organochlorine, myometrium, endometrium, Gavage, implantation.

### INTRODUCTION

India being a developing country, nearly 67% of the population strives on livelihood through agriculture. Agricultural industry being the prime source of national revenue, consumption of pesticides for high crop production has also increased. 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. Diverse environmental pollutants which mainly include a wide range of pesticides cause severe neuroendocrine disruption in both vertebrates and invertebrates<sup>1</sup>. Neurobehavioral deficits were also observed in rats administered with the same insecticide<sup>2</sup>. Diuron induced cytotoxicity has also been observed in male wistar rats causing urinary bladder carcinogenesis<sup>3</sup>. Shin et al<sup>4</sup> 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.<sup>5</sup> 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<sup>6</sup>. 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<sup>7</sup>. Studies conducted by Sarma et al.<sup>8</sup> 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<sup>9</sup>. Curcumin has also emerged as one of the promising targets of gastrointestinal tract, gastrointestinal diseases including inflammatory bowel diseases, hepatic fibrosis and gastrointestinal cancers<sup>10</sup>.

Thus the present study is designed to evaluate bioremedial effect of *Curcuma longa* on uterus 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. \*\*Ethical clearance number: IAEC-2008-03-28

**Chemicals:** Pesticide Endosulfan, manufactured by Excel India Pvt. Ltd., Mumbai with EC 35% was utilized for the experiment.

Aqueous 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 2 mg/kg b.w daily by gavage method for four weeks followed by four 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 lipid per oxidation (MDA) assay. The uterus 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.

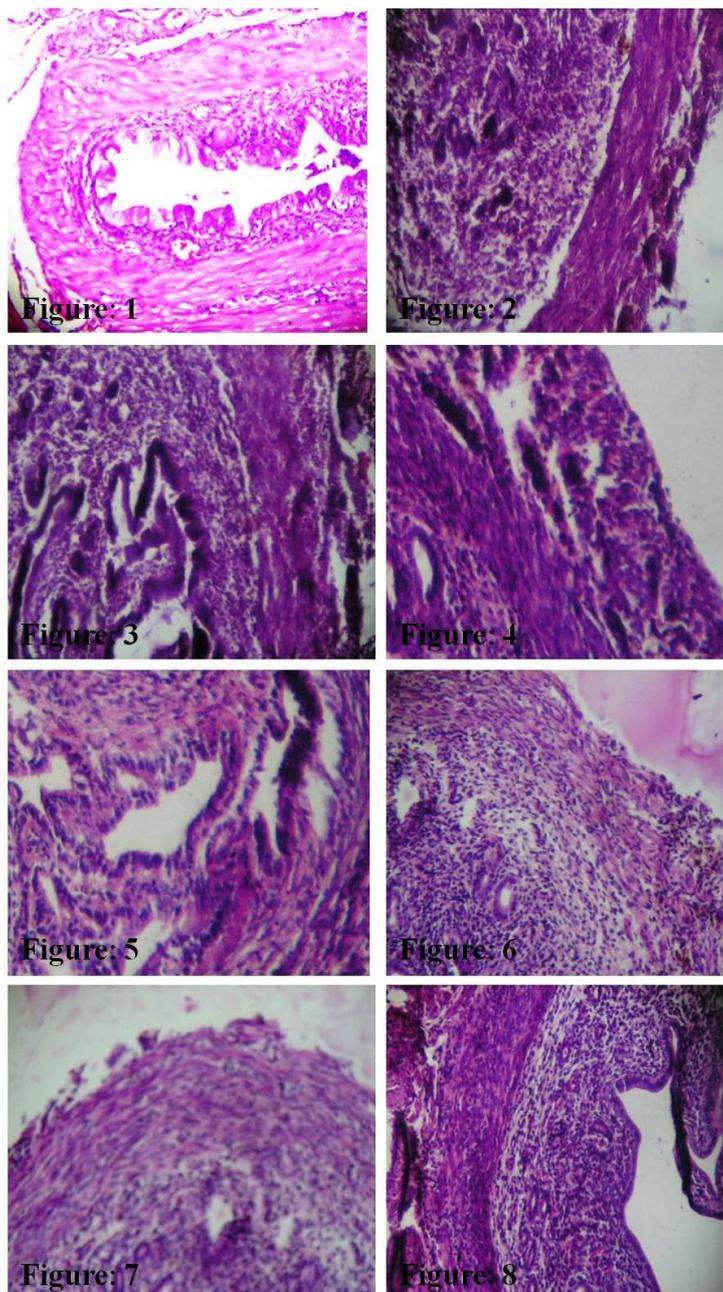


Figure 1: Shows uterus of control mice with well defined endometrium. Myometrium and glands are normal in structure. Muscle layers are also well defined. X 200

Figure 2: Shows uterus of endosulfan 2 weeks administered mice with degeneration in muscular layer of uterus. Degeneration in glands was also observed. X 300

Figure 3: Shows uterus of endosulfan 2 weeks administered mice with degenerated muscular layer with clustered nuclei. Degenerated endometrial layer was also observed in uterus. X 300

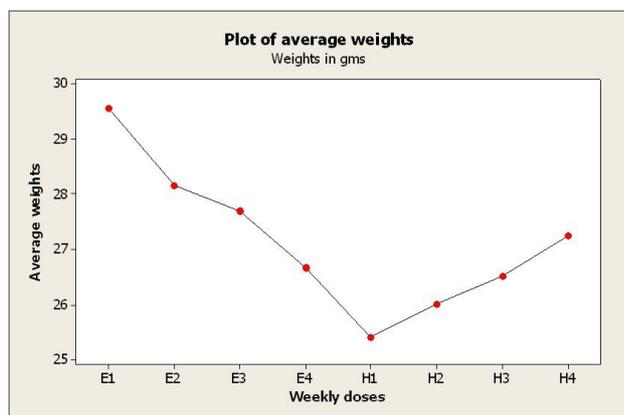
Figure 4: Shows uterus of endosulfan 4 weeks administered mice with circular muscles were observed in more degenerated condition. Degenerated cytoplasm was observed. X 400

Figure 5: Shows uterus of endosulfan 4 weeks administered mice with frequent vacuolization in endometrial layer. Cytoplasm was observed in degenerated condition. X 300

Figure 6: Shows uterus of 2 weeks *Curcuma* administered mice with restoration in both circular and longitudinal muscle to greater extent with well-defined glands of uterus. X 200

Figure 7: Shows uterus of 4 weeks *Curcuma longa* administered mice with well defined longitudinal muscle of uterus. Myometrium is also restored to greater extent. X 400

Figure 8: Shows uterus of 4 weeks *Curcuma longa* administered mice with restoration in muscular layer of uterus with well-defined myometrium and endometrium. X 400



**Graph 1: Decrease in body weight in endosulfan administered group (E1, E2, E3 and E4) while it increases in *Curcuma longa* treated group (H1, H2, H3 and H4).**

\*Note: E1 – E4 = Endosulfan 1 week to 4 weeks, H1 – H4 = *Curcuma longa* 1 week to 4 weeks

## RESULTS

In endosulfan administered group of mice body weight decreases, while after administration of *Curcuma longa* body weight of mice increases (Graph 1).

In control group of uterus of mice endometrium, myometrium, stratum functionale and stratum basale are normal. Glands were also observed in normal condition (Figure 1). Endosulfan two weeks administered group show degeneration in stratum basale of uterus with little vacuolization. Degeneration in glands was also observed (Figure 2). Degenerated muscular layer with clustered nuclei were observed in myometrium. Degenerated endometrial layer was also observed in uterus with clustered nuclei (Figure 3). Endosulfan four weeks administered group show clustered nuclei with degenerated cytoplasm in muscular structure of uterus mainly in stratum basale. Circular muscles were observed in more degenerated condition. Degenerated cytoplasm was observed in glands of uterus (Figure 4). Frequent vacuolisation in endometrial layer with clustered nuclei were observed. Cytoplasm was observed in degenerated condition (Figure 5). Endosulfan four weeks administered group followed by two weeks administration of *Curcuma longa* show restoration in both circular and longitudinal muscle of stratum basale to greater extent with well-defined glands of uterus (Figure 6). Well defined longitudinal muscle of uterus were observed in stratum basale. Myometrium is also restored to greater extent (Figure 7). Endosulfan four weeks administered group followed by two weeks administration of *Curcuma longa* show restoration in muscular layer of uterus with well-defined myometrium and endometrium. Glands were also observed in restored condition (Figure 8). Enlarged view of glands of uterus showing restoration in nuclear and cytoplasmic material of gland with restoration in myometrial layer (Figure 9).

## DISCUSSION

Endosulfan is also known to influence the human estrogen-sensitive cells causing degenerative effects on the female reproductive system<sup>11</sup>. 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<sup>12</sup>. The alterations in liver tissue such as the increase in vacuolation, sinusoidal dilation and formation of bile plugs, has been also reported by Sakr *et al.*<sup>13</sup> in fish. Sinusoidal dilation in the liver is attributed to the impairment of outflow of the hepatic veins<sup>14</sup>, while the formation of vacuoles in hepatocytes is due to the degeneration of cell membranes<sup>15</sup> and an imbalance between the rate of synthesis and utilization of substances in cells<sup>16</sup>. Marked hyperplasia and hypertrophy of tubular 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<sup>17</sup>. Endosulfan causes spermatozoa degeneration<sup>18</sup> as well as declined testosterone level. Endosulfan exposure leads to ovarian nuclear degeneration<sup>19</sup>. In present study degeneration in endometrium, myometrium and glands were observed. Stratum functionale and stratum basal were also degenerated to greater extent.

Evidence suggests that cell death induced by curcumin involves the activation of cell death pathways and inhibition of growth/proliferation pathways<sup>20</sup>. 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<sup>21</sup>. In present study *Curcuma longa* administration causes increase in body weight of mice. It causes greater degree of restoration in both endometrium and myometrium layer. Stratum functionale and stratum basale are normal in structure. Studies also suggest that curcumin is effective against bladder cancer caused by cigarette smoking<sup>22</sup>.

Thus it is concluded from entire study that endosulfan causes degeneration in endometrium and myometrium layer with degenerated glands. Muscular layer were also degenerated with frequent vacuolisation. Clustered nuclei were observed in muscular layer which finally causes improper movement of muscles in uterus and adversely affect implantation. While *Curcuma longa* administration causes restoration in endometrial layer and myometrium layer. Glands was also restored in structure to greater extent which indicate that *Curcuma longa* plays vital role against endosulfan toxicity on uterus and restores normal function of uterus including implantation.

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