

RESEARCH ARTICLE

Effect of *Curcuma longa* on Ovary of Endosulfan Exposed mice

Ranjit Kumar ^{*1}, Divya Gupta², Somesh Mukul², Arun Kumar Singh², Anant Kumar³, Md Ali¹, A. Nath¹, Arun Kumar¹ and J.K. Singh¹

¹Mahavir Cancer Institute & Research Centre, Phulwarisharif, Patna (Bihar), India

²P.G. Dept. of Botany, T.M.B.U., Bhagalpur, Bihar

³Bihar Council on Science and Technology, Patna, Bihar

Received 14 Mar 2012; Revised 07 Jun 2012; Accepted 14 Jun 2012

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

*Corresponding Author: Dr. Ranjit Kumar, Email: ranjitool17@gmail.com , Phone No: +91-9304450602

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.

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 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 vacuolisation. 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

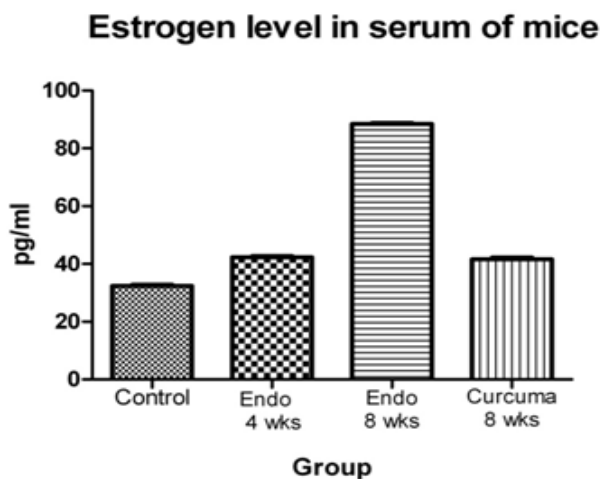


Figure 1: show ovary of control mice with different stages of graffian follicle. Corpus luteum and germinal epithelium were also continuous.

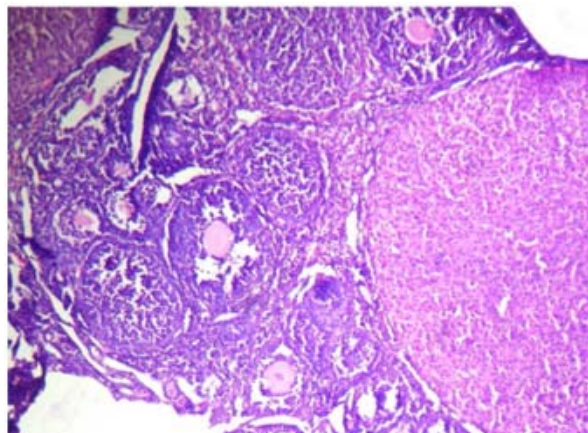


Figure 2: show ovary of control mice with normal corpus luteum.

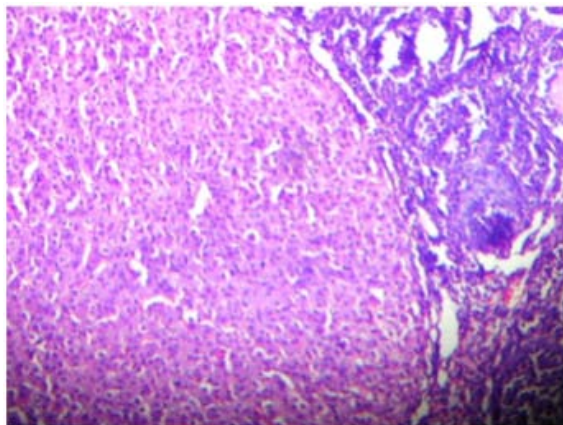


Figure 3: show ovary of 8 weeks endosulfan administered mice with degenerated mature graffian follicle. Ova become crescent shaped.

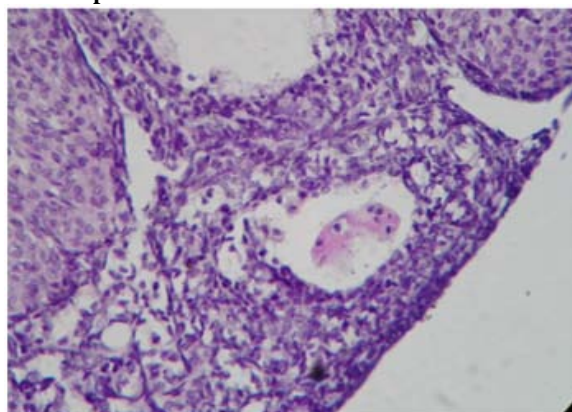


Figure 4: show ovary of 8 weeks endosulfan administered mice with degenerated mature graffian follicle, ova were also degenerated.

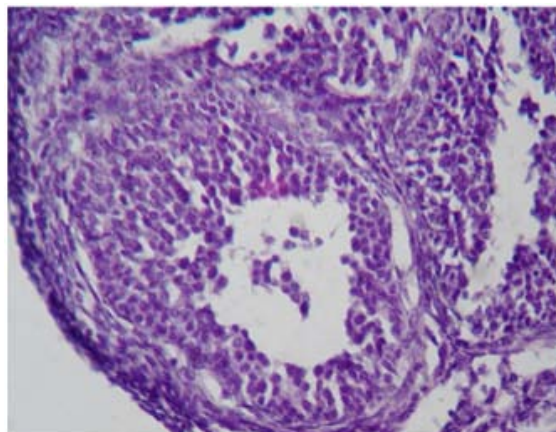


Figure 5: Ovary of 8 weeks *Curcuma longa* show restoration in corpus luteum. Germinal epithelium was also continuous. Ovum was also observed in restorative condition.

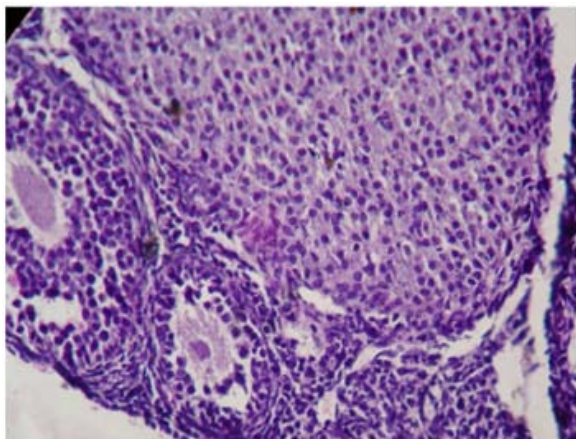
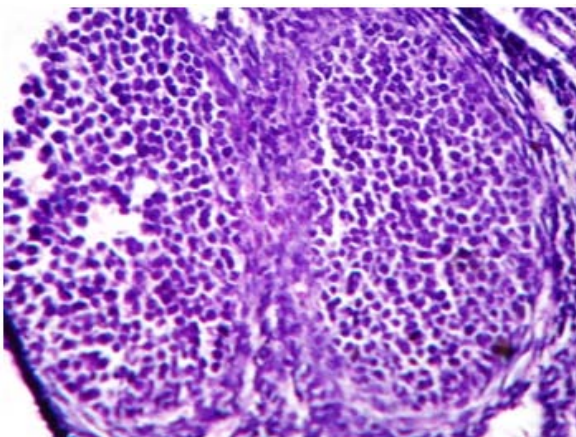


Figure 6: Ovary of 8 weeks administration of *Curcuma longa* show restoration in corpus luteum. Germinal epithelium was also normal in structure.



ACKNOWLEDGEMENT

The authors are grateful to Mahavir Cancer Institute and Research Centre, Patna for providing research facilities and we are also thankful to all research laboratory staff and animal house staff for their proper support during study.

REFERENCES

- Waye A., Vance L. Trudeau; NEUROENDOCRINE DISRUPTION: MORE THAN HORMONES ARE UPSET, *Journal of Toxicology and Environmental Health*, (2011) Part B, 14:270–291.
- Abou-Donia M.B., Goldstein L.B., Bullman S., Tu T., Khan W.A., Dechkovskaia A.M., Abdel-Rahman A.A., Imidacloprid Induces Neurobehavioral Deficits and Increases Expression of Glial Fibrillary Acidic Protein in the Motor Cortex and Hippocampus in Offspring Rats Following in Utero Exposure, *Journal of Toxicology*

- and Environmental Health, (2008) Part A, 71: 119–130.
- Rocha M. S., Merielen G. Nascimento, Ana Paula F. Cardoso, Patrícia L. A. de Lima, Edneia A. Zelandi, João Lauro V. de Camargo,1 and Maria Luiza C. S. de Oliveira; Cytotoxicity and Regenerative Proliferation as the Mode of Action for Diuron-Induced Urothelial Carcinogenesis in the Rat, *TOXICOLOGICAL SCIENCES*(2010) 113(1), 37–44.
- Shin Jae-Ho, Moon H. Ju, Kang H., Kim T. S., Lee S. J., Young J, Lee Y. J., Hong E.J., Jeung E. B., Han S.Y., Calbindin-D9k mRNA Expression in the Rat Uterus Following Exposure to Methoxychlor: A Comparison of Oral and Subcutaneous Exposure, *Journal of Reproduction and Development* (2007) Vol. 53, No. 2 April P 179-188.
- Tomic D.,* Frech M.S.,† Babus J.K.,* Gupta R.K.,* Furth P.A, Koos R.D.,‡ and Flaws J.A., Methoxychlor Induces Atresia of Antral Follicles in ERα-Overexpressing Mice, *TOXICOLOGICAL SCIENCES* (2006) 93(1), 196–204.
- Saraswathy R., Alex G., Basil B.M., Badarinath A.R.S., Girish R., Ribu G., Abilash V.G., Cruz G.D.J. and Marimuthu K.M., Clinical and Cytogenetic Effects in Habitants under Large Duration Exposure of Endosulfan, *Asian Journal of Medical Sciences* (2011) 3(1): 17-22.
- Saiyed H, Dewan A, Bhatnagar V., Shenoy U., Shenoy R., Rajmohan H., Patel K., Kashyap R., Kulkarni P., Rajan B. and Lakkad B., Effect of Endosulfan on Male Reproductive Development, *Environmental Health Perspectives VOLUME 111, NUMBER 16, December 2003, 1958-1962.*
- Sarma Q. and Kalita J., Estrogenic nature and Effects of Endosulfan in white Swiss albino mice, *International Journal of Scientific and Research Publications*, (2012) vol 2 issue 1, 78-82
- Lee S.J. and Langhans S.A., Anaphase-promoting complex/cyclosome protein Cdc27 is a target for curcumin-induced cell cycle arrest and apoptosis, *BMC Cancer*, (2012) 12:44.
- Rajasekaran S A, Therapeutic potential of curcumin in gastrointestinal diseases, *World J Gastrointest Pathophysiol*, February (2011) 15; 2(1): 1-14.

11. Soto A.M., Chung K.L., and Sonnenschein C., The Pesticides Endosulfan, Toxaphene, and Dieldrin Have Estrogenic Effects on Human Estrogen-Sensitive Cells, *Environment Health Perspectives*, (1994) Volume 102, Supplement 1, pp 148-153
12. Hodges L.C., Bergerson J.S., Hunter D.S., and Walker C. L., Estrogenic Effects of Organochlorine Pesticides on Uterine Leiomyoma Cells in Vitro, *TOXICOLOGICAL SCIENCES* (2000) 54, 355–364.
13. Sakr, S.A., Hanafy, S.M., ElDesouky, N.E., Histopathological, histochemical and physiological studies on the effect of the insecticide, Hostathion on the liver of the catfish *Clarias gariepinus*. Egypt. , *Journal of Aquatic Biology and Fishery*, (2001) 6, pp 103-124.
14. Tanaka, M., Wanless, I.R., Pathology of the liver in Budd Chiari syndrome: portal vein thrombosis and the histogenesis of venocentric cirrhosis, venoportal cirrhosis, and large regenerative nodules. *Herpetology* (1998) 27, pp 488–496.
15. Olurin, K.B., Olojo, E.A.A.1., Mbaka, G.O., Akindele, A.T.1., Histopathological responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate. *Afr. J. Biotech.* (2006) 5, pp 2480-2487.
16. Gingerich, W.H., Hepatic toxicology of fishes. In: Weber, L.J.(Ed.), *Aquatic Toxicology*. Raven Press, New York, (1982) pp 55-105.
17. Bosco C., Rodrigo R., Diaz S., Borax J. Renal effects of chronic exposure to malathion in octodon degus. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* (1997) 118, 247-253.
18. Nath, A. And R. Kumar, Interference of Endosulfan on Golgi complex action during acrosome formation in spermatozoa of mice. *Embryotalk*, (2007) 2: 72-76.
19. Sahay, R., R. Kumar and A. Nath,; Transmission electron microscopic study of ovarian follicular degeneration due to endosulfan treatment in Swiss albino mice, *Embryo talk*, (2007) (2): 67-71.
20. Ravindran J., Prasad S. and Aggarwal B.B., Curcumin and Cancer Cells: How Many Ways Can Curry Kill Tumor Cells Selectively? ; *The AAPS Journal*, (2009) Vol. 11, No. 3, September.78-86
21. Oyagbemi A.A., Saba A.B., Ibraheem A.O.; Curcumin: From Food Spice to Cancer Prevention, *Asian Pacific Journal of Cancer Prevention*, ,(2009) Vol 10, 253-256
22. Leite K.R. M., Chade D. C., Sanudo A., Sakiyama B.Y. P., Gustavo Batocchio, Miguel Srougi, Effects of Curcumin in an Orthotopic Murine Bladder Tumor Model ; *International Braz J Urol*, (2009) Vol. 35 (5): 599-607.