BIO-REMEDIAL EFFECT OF CURCUMA LONGA ON ESTROGEN AND SUB-CELLULAR STRUCTURES OF OVARIES OF PESTICIDE EXPOSED MICE

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ABSTRACT

Pesticides are some of the most frequently released toxic chemicals into the environment. Although the use of pesticides enhances crop productivity, human beings also pay a price for the benefits. Chlorpyrifos (an organophosphate) is a broad-spectrum insecticide against a variety of insects and mites in agriculture and allied sectors. After absorption through the gut and lungs, it rapidly distributes to all organs exerting damaging effects at cellular and sub-cellular levels.

The present work is designed to study the bio-remedial effect of Curcuma longa (Turmeric) on estrogen and sub-cellular structure of ovary of Chlorpyrifos exposed Swiss albino mice. Chlorpyrifos administration at 6 mg/kg b.w./day for 8 weeks was followed by 8 weeks administration of alcoholic rhizome extract (5% Ethanol) of Curcuma longa at 50 mg/kg b.w./day. Estrogen level was increased in Chlorpyrifos administered group while it gradually decreased in Curcuma longa administered group. Degeneration of nucleus, nuclear membrane, mitochondria, endoplasmic reticulum and Golgi complex was observed in Chlorpyrifos administered group, while Curcuma longa restored mitochondria, nuclear membrane, nucleus, endoplasmic reticulum and Golgi complex effectively. Thus it is evident from the study that Curcuma longa plays an effective role against Chlorpyrifos induced toxicity on estrogen. It also maintains histology of ovary effectively. It restores cellular and sub-cellular toxicity of Chlorpyrifos exposed ovary effectively and restores normal function of ovary in female.

KEYWORDS: Chlorpyrifos, Curcuma longa, Nucleus, Mitochondria, Estrogen.
1. INTRODUCTION

Pesticides play a vital role in agricultural, industrial and residential pest control. They have offered the protection of crops in the field. Moreover, they have decreased public health concerns by reducing the level of vector-borne diseases. Environmental contaminants, due to intensive use of pesticides as modern agrochemicals, have created a lot of problem to the animals in terms of biochemical and physiological alterations in their metabolism. They exert their impact at cellular and sub-cellular levels prior to any gross sign of damage being apparent in the morphology of the animal. Exposure to pesticides can range from mild skin irritation to birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption, and even coma or death.[1]

Chlorpyrifos is one of the most widely used organophosphorus insecticides in the world. It has been used increasingly by farmers of Bihar, India for agricultural purposes. Chlorpyrifos is moderately toxic to humans and chronic exposure has been linked to neurological effects, developmental disorders, and autoimmune disorders.[2] Presence of organophosphorus pesticides in blood and breast milk of mothers has negative effects on newborns including mutagenic, carcinogenic and neurotoxic disorders.[3]

Curcuma longa is widely consumed as a dietary spice, a dietary pigment and a herbal medicine for the treatment of various illnesses. Several studies found turmeric to have anticarcinogenic, antioxidant, anti-inflammatory, anti-allergic, anti-mutagenic,[4] immunomodulatory properties,[5,6] with antiviral and antibacterial activities.[7] The active component in turmeric is curcumin, which target cells expressing phosphorylated Cdc27 prominent in proliferating cells to cause apoptosis.[8] Curcumin has also emerged as one of the promising targets of gastrointestinal tract, gastrointestinal diseases including inflammatory bowel diseases, hepatic fibrosis and gastrointestinal cancers.[9] Thus the present work is designed to study the bio-remedial effect of Curcuma longa on estrogen and subcellular structure of ovary of Chlorpyrifos exposed Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Pesticide

Chlorpyrifos (T_N–Dursban) was used at an effective concentration, EC = 20% (w/v).
2.2 Herbal Plant
Rhizome of *Curcuma longa* was selected as a plant material for bio-remedial study against pesticide induced toxicity.

2.3 Experimental Model
Female Swiss albino mice (*Mus musculus*) weighing 30±2gm were selected as an experimental model in the present study. The animals were housed at controlled environmental conditions – temperature 22±2°C, relative humidity 50±10% and 12h dark-light cycle. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). **Ethical clearance number: IAEC/2011/1d (dated: 26-12-2011).**

2.4 METHODOLOGY
2.4.1 Chronic Toxicity Studies
Selected pathogen-free mice were sorted and Chlorpyrifos was administered at 6 mg/kg b.w./day for 8 weeks by Gavage method. Sacrifice was done after 2nd week, 4th week and 8th week of Chlorpyrifos administration in each group.

2.4.2 Herbal Administration
Chlorpyrifos administration at 6 mg/kg b.w./day for 8 weeks was followed by 8 weeks administration of alcoholic rhizome extract (5% Ethanol) of *Curcuma longa* at 50 mg/kg b.w./day. Animals were sacrificed on 4th week and 8th week after herbal treatment in each group.

2.4.3 Sub-cellular Studies
Mice were sacrificed from each group for histological analysis. The ovaries were dissected out and washed three times in isotonic saline (0.85 w/v %). Tissues of ovary were fixed in 2.5% Glutaraldehyde for Transmission Electron Microscope study.

2.4.4 Hormonal Assessment
Blood was collected by orbital puncture and centrifuged to separate the serum for estrogen assay through ELISA techniques.

3. RESULTS AND DISCUSSION
Estrogen level in control group was 224.8±3.25 pg/ml, while it was 340.0±5.79 pg/ml, 494.0±2.58 pg/ml and 551.7±4.70 pg/ml in groups of mice after Chlorpyrifos administration.
for 2 weeks, 4 weeks and 8 weeks respectively. Estrogen level was 227.1±1.27 pg/ml and 285.4±1.83 pg/ml in groups of mice after Curcuma longa administration for 4 weeks and 8 weeks respectively (Graph).

Ovary of control mice showed well organized defined double membrane of nucleus. Chromatin material was normal. Endoplasmic reticulum and mitochondria were also normal in structure (Figure 1).

Ovary of two weeks Chlorpyrifos administered mice showed bulging of nuclear membrane. Many degenerated vacuoles were observed in cytoplasm. Dilated nucleopore complexes were also observed (Figure 2). Ovary of four weeks Chlorpyrifos administered mice showed degeneration in nucleus and nuclear membrane. Heterochromatinized chromatin materials were clearly observed. Nucleolus was observed in degenerated condition (Figure 3). Ovary of eight weeks Chlorpyrifos administered mice showed bulging of double membrane of nucleus. Nuclear membrane became wavy. Degenerated mitochondrial cristae were observed. Many vacuolated spaces were observed in cytoplasm. Rough endoplasmic reticulum was observed in fragmented condition (Figure 4).

Ovary of eight weeks Chlorpyrifos administered mice followed by eight weeks Curcuma longa administration showed well defined nuclear double membrane with restored chromatin material. Nucleopore complexes were also restored effectively. Ribosomes were also observed in normal condition (Figure 5).

![Graph: Estrogen level in serum of mice](image-url)
Photoplates

Figure 1: Shows Transmission Electron Microphotograph of ovary of control mice with double membrane of nucleus. Chromatin material was normal. Endoplasmic reticulum and mitochondria were also normal in structure.

Figure 2: Shows Transmission Electron Microphotograph of ovary of two weeks Chlorpyrifos administered mice with bulging of nuclear membrane. Many degenerated vacuoles were observed in cytoplasm. Dilated nucleopore complexes were also observed.
Figure 3: Shows Transmission Electron Microphotograph of ovary of four weeks Chlorpyrifos administered mice with degeneration in nucleus and nuclear membrane. Heterochromatinized chromatin materials were clearly observed. Nucleolus was observed in degenerated condition.

Figure 4: Shows Transmission Electron Microphotograph of ovary of eight weeks Chlorpyrifos administered mice with bulging of double membrane of nucleus. Nuclear membrane became wavy. Degenerated mitochondrial cristae were observed. Many vaculated spaces were observed in cytoplasm. Rough endoplasmic reticulum was observed in fragmented condition.

Figure 5: Shows Transmission Electron Microphotograph of ovary of eight weeks Chlorpyrifos administered mice followed by eight weeks Carvacrol long administration with well defined nuclear double membrane with restored chromatin material. Nucleopore complexes were also restored effectively. Ribosomes were also observed in normal condition.
Methoxychlor was shown to cause follicular atresia in the ovary of mice indicated by low FSH levels.\textsuperscript{[10]} Ohi \textit{et al.}\textsuperscript{[11]} showed that administration of an insecticide fipronil causes a significant reduction in estradiol level in female Wistar rats. Similarly, Gojmerac \textit{et al.}\textsuperscript{[12]} showed that lindane, atrazine and simazine cause a decrease in circulating estradiol levels in rats. Whereas, El-Kashoury \textit{et al.}\textsuperscript{[13]} reported that dicofol, an organochlorine acaricide, causes elevation in estradiol level. In our study also, we observed elevated level of estrogen in Chlorpyrifos administered mice, which finally hampers normal estrous cycle due to imbalance in estrogen and progesterone balance. Ovulation is also impaired due to increased estrogen in female.

Das \textit{et al.}\textsuperscript{[14]} observed significant decrease in matured ovarian follicle with a concomitant increase in the number of atretic follicles in Chlorpyrifos treated female rats. Moreover, the most of the matured follicles showed depletion of thecal and granulosa cell layers; and acoecytic. Sahay \textit{et al.}\textsuperscript{[15]} reported that endosulfan exposure leads to ovarian nuclear degeneration in mice. In our study also, degeneration in mitochondria, endoplasmic reticulum and Golgi complex was observed after exposure to Chlorpyrifos. Plasma membrane and nuclear membrane were also observed in degenerated condition, which finally causes degeneration in ova and ovulation leading to infertility in female mice.

Thakur \textit{et al.}\textsuperscript{[16]} reported that both aqueous and ethanolic extracts of rhizome of \textit{Curcuma longa} showed a significant antifertility activity in female albino rats. Also the amount of estrogen in ethanolic extract treated animals was found to have increased significantly. But our study shows reduced level of estrogen in \textit{C. longa} treated group. The present finding is supported by Kumar R \textit{et al.}\textsuperscript{[17]} who reported restoration of increased estrogen level toward normalcy in endosulfan exposed mice after \textit{C. longa} administration. Ghosh \textit{et al.}\textsuperscript{[18]} showed that extract of \textit{Curcuma longa} causes the suppression of ovulation by the inhibition of estrous phase. It was presumed that anti-ovulatory action was due to the anti-estrogenic property of phytochemicals, which either block the estrogen receptors or diminish estrogen synthesis due to decrease in cholesterol metabolism or both.

Al-Taee \textit{et al.}\textsuperscript{[19]} reported that aqueous extract of \textit{Curcuma longa} (5, 10 mg/kg b.wt. for two weeks) causes increase in the numbers of primary and secondary follicles and in the numbers of corpus luteum in ovary of female mice. Similarly, Kumar and Madhavi\textsuperscript{[20]} showed that curcumin maintains normal architecture of Graafian follicle and germinal epithelium in ovary of mice after Chlorpyrifos intoxication. Our study also shows restoration in mitochondria
with almost normal mitochondrial cristae after C. longa administration. Nucleus was well organized with double membrane of nucleus. Chromatin material was well organized. Endoplasmic reticulum and ribosomes were also observed in restored condition.

4. CONCLUSION
This study shows that Curcuma longa plays an effective role against Chlorpyrifos toxicity on estrogen and ovary. It restores cellular and sub-cellular toxicity of Chlorpyrifos exposed ovary effectively. This causes normal estrous cycle and proper ovulation leading to normal fertility in female.

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6. REFERENCES