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COMPARATIVE BIOREMEDIAL EFFECT OF *WITHANIA SOMNIFERA* AND *CURCUMA LONGA* ON OVARIES OF PESTICIDE INDUCED MICE

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ABSTRACT

Since last two decades, the phyto-remediation for pesticide borne diseases has remained a matter of great challenge. Pesticides are commonly used for protection of crops from different pests. Now a day's organophosphates are commonly used pesticide. Some of the pesticides persist in the environment and also bio-accumulate into different organisms. The present work is designed to study the bioremedial effect of *Withania somnifera* (Ashwagandha) and *Curcuma longa* (Turmeric) on ovaries of Chlorpyrifos exposed Swiss albino mice. The end point of bioremedial effect is measured by restoration of estrogen and cholesterol levels. The treatment groups received Chlorpyrifos at 6mg/kg b.w./day by Gavage method for eight weeks followed by eight weeks administration of alcoholic root extract of *Withania somnifera* and rhizome extract of *Curcuma longa* separately at the dose of 50mg/kg b.w./day. Chlorpyrifos administered groups showed increased level of Estrogen and Cholesterol. *Withania* and *Curcuma* treated groups showed restoration in Estrogen and Cholesterol levels. Restoration of Cholesterol level is more in *Withania* treated group while *Curcuma* treated group restores Estrogen level significantly. Ovaries of Chlorpyrifos administered groups showed degenerated germinal epithelium, Graafian follicles and corpus luteum. *Withania* treated group showed more restoration in germinal epithelium, Graafian follicles and corpus luteum than *Curcuma* treated group comparatively. Thus it is evident from the study that *Withania somnifera* plays effective role against Chlorpyrifos induced toxicity on biochemical, hormonal and histology of ovary in comparison to *Curcuma longa*.

KEYWORDS: Chlorpyrifos, Graafian follicle, Estrogen, Cholesterol.

INTRODUCTION

Due to rising human population, increase in the agricultural productivity is needed. Lots of crops were destroyed by rodents and insects in field. Pesticides are widely applied on these crops for their protection from rodents and insects. It is also used for preservation of different agricultural products. These pesticides were accumulated into living organisms. This bioaccumulation is one of the serious causes of mutation in living organisms.[1] Pesticides like DDT, BPA, endosulfan and other organochlorine compounds are persistent organic pollutants (POPs) which are being implicated for precocious puberty[2], higher incidence of breast cancer[3], lowered libido, impotency, sterility, fibroids, early menopause, endometriosis and osteoporosis in females[4] and oligospermia[5], testicular cancer, cryptorchidism, gynecomastia, sterility and prostatic problems in males.[6]

Chlorpyrifos is a broad spectrum organophosphate insecticide used for wide range of crops. Chlorpyrifos is widely used for mosquito control and other public health applications. Chlorpyrifos is moderately toxic to humans and chronic exposure has been linked to neurological effects, developmental disorders, and autoimmune disorders.[7] Presence of organophosphorus pesticides in blood and breast milk of mothers has negative effects on newborns including mutagenic, carcinogenic and neurotoxic disorders.[8]

Withania somnifera has been used as an antioxidant, adaptive, liver tonic, anti-inflammatory agent and more recently to treat ulcers, bacterial infection and senile dementia. Treatment with *Withania somnifera* has been found to partially reverse the Lead-induced suppressed reproduction in female rats.[9] *Withania somnifera* administration has been reported to cause restoration in glomerulus, bowmen's capsule, PCT and DCT as well as biochemical parameters of kidney of stress induced mice.[10]

Curcuma longa is widely consumed in India and Pakistan for a variety of uses, including as a dietary spice, a dietary pigment and an Indian folk medicine for the treatment of various illnesses. It is reported to have antioxidant, hepato-protective, anti-inflammatory, antimicrobial, cardiovascular and gastrointestinal properties. Curcumin is active ingredient of *Curcuma longa*, it has been reported to exert significant preventative effects against arsenic-induced

carcinogenesis in humans.[11] Curcumin treatment has been found to reduce the induction of diabetic retinopathy in rats significantly.[12]

Thus the present study is designed to evaluate the comparative effect of *Withania somnifera* and *Curcuma longa* on biochemical, hormonal and histology of ovaries of Chlorpyrifos induced mice.

MATERIALS AND METHODS

Pesticide

Chlorpyrifos (T_N –Dursban) was used at an effective concentration, EC = 20% (w/v).

Herbal Plant

Roots of *Withania somnifera* and rhizome of *Curcuma longa* were selected as the plant material for study against pesticide induced toxicity.

Experimental model

Female Swiss albino mice (*Mus musculus*) weighing 30±2gm were selected as an experimental model in the study. The animals were housed at controlled environmental conditions 22±2ºC, 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). Permission for conducting this study was obtained from Institutional Animal Ethical Committee (I.A.E.C.) of Mahavir Cancer Sansthan.

METHODOLOGY

Chronic Toxicity Studies

Selected pathogen-free mice were sorted and chlorpyrifos was administered at 6 mg/kg b.wt/day for 8 weeks by Gavage method. Sacrifice was done on 2nd week, 4th week and 8th week of chlorpyrifos administered group.

Herbal Administration

Chlorpyrifos administration at 6 mg/kg b.wt for 8 weeks was followed by the administration of alcoholic extract (5% Ethanol) of roots of *Withania somnifera* and rhizome of *Curcuma longa* separately for 8 weeks at 50 mg/kg b.wt. Animals were sacrificed on 4th week and 8th week of herbal treatment in each group.

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 %) and then fixed in neutral formalin solution and the tissue was processed for Light Microscope study.

Biochemical and Hormonal Assessment

Blood was collected by orbital puncture and centrifuged to separate the serum to carry out further biochemical analysis through ELISA and spectrophotometer.

RESULTS

Estrogen level in control group was 241.3 ± 6.88 pg/ml while it was 525.0 ± 14.00 pg/ml, 484.7 ± 4.97 pg/ml and 557.7 ± 7.53 pg/ml in chlorpyrifos 2 weeks, 4 weeks and 8 weeks administered group of mice. Estrogen level was 453.3 ± 2.67 pg/ml and 511.7 ± 4.18 pg/ml in *Withania* 4 weeks and 8 weeks administered group of mice while it was 259.0 ± 10.39 pg/ml and 287.0 ± 13.65 pg/ml in *Curcuma* 4 weeks and 8 weeks administered group of mice (Graph: I).

Cholesterol level in control group was 165 ± 3.49 mg/dl while it was 204.8 ± 3.53 mg/dl, 212.2 ± 3.71 mg/dl and 205.8 ± 7.19 mg/dl in chlorpyrifos 2 weeks, 4 weeks and 8 weeks administered group of mice. Cholesterol level was 202.8 ± 4.02 mg/dl and 189.3 ± 2.94 mg/dl in *Withania* 4 weeks and 8 weeks administered group of mice while it was 207 ± 2.32 mg/dl and 193.2 ± 3.65 mg/dl in *Curcuma* 4 weeks and 8 weeks administered group of mice (Graph: II).

Ovaries of control mice showed normal corpus luteum. Mature Graafian follicle was also observed with distinct ova. Different stages of follicles were observed (Fig-1). Ovaries of four weeks chlorpyrifos administered mice showed degenerated germinal epithelium. Many vacuolated spaces were observed in matured Graafian follicles with clustered nuclei of granulosa cells. Degeneration in ova was also prominent (Fig-2). Ovaries of eight weeks chlorpyrifos administered mice showed degeneration of germinal epithelium to the greater extent. Large vacuolated spaces were also observed in mature Graafian follicle (Fig-3). Ovaries of eight weeks chlorpyrifos administered mice followed by eight weeks administration of *Withania somnifera* showed restoration in ova and granulosa cells. Restoration in germinal epithelium was also observed. Mature Graafian follicle was restored effectively (Fig-4). Ovaries of eight weeks chlorpyrifos administered mice followed by eight weeks administration of *Curcuma longa* showed restorations in ova and mature Graafian follicle. Germinal epithelium was continuous. Corpus luteum was homogeneous with well differentiated nuclear and cytoplasmic material (Fig-5).

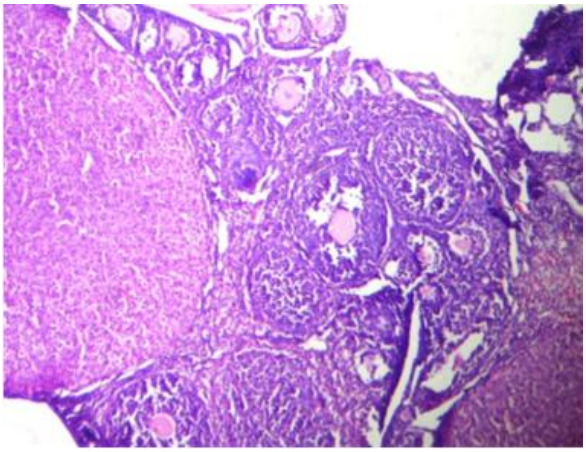


Fig 1: Ovary of control mice shows normal corpus luteum. Mature Graafian follicle was also observed with distinct ova. Different stages of follicles were visible. X200.

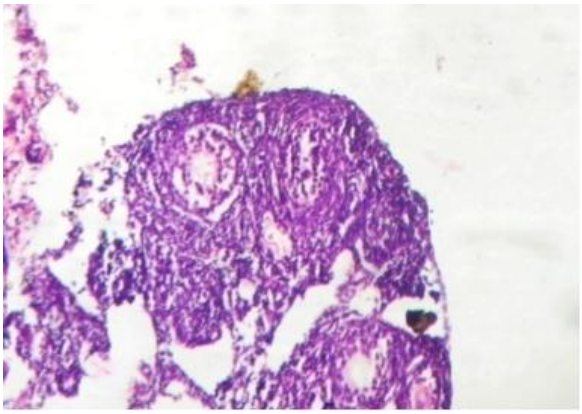


Fig 2: Ovary of four weeks chlorpyrifos administered mice shows degenerated germinal epithelium. Many vacuolated spaces were observed in matured Graafian follicles with clustered nuclei of granulosa cells. Degeneration in ova was also prominent. X150.

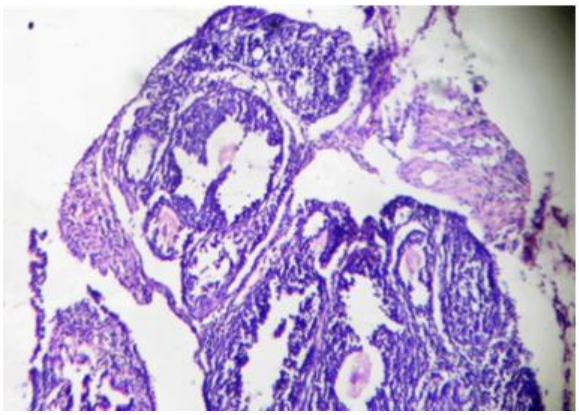


Fig 3: Ovary of eight weeks chlorpyrifos administered mice shows degeneration of germinal epithelium to the greater extent. Large vacuolated spaces were also observed. Greater degrees of degeneration were observed in granulosa cells. X200.

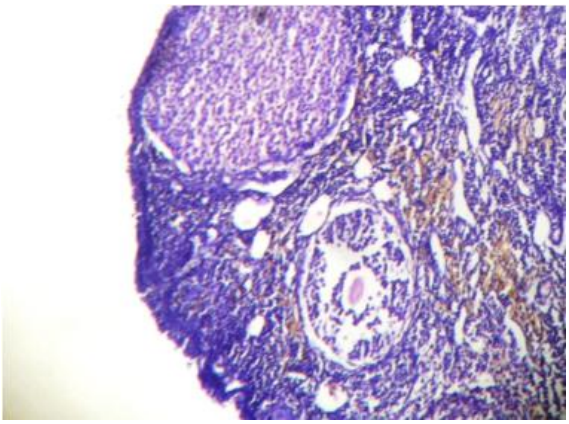


Fig 4: Ovary of eight weeks chlorpyrifos administered mice followed by eight weeks administration of *Withania somnifera* shows restoration of ova and granulosa cells. Restoration in germinal epithelium was also observed. Mature Graafian follicle was also prominent. X400.

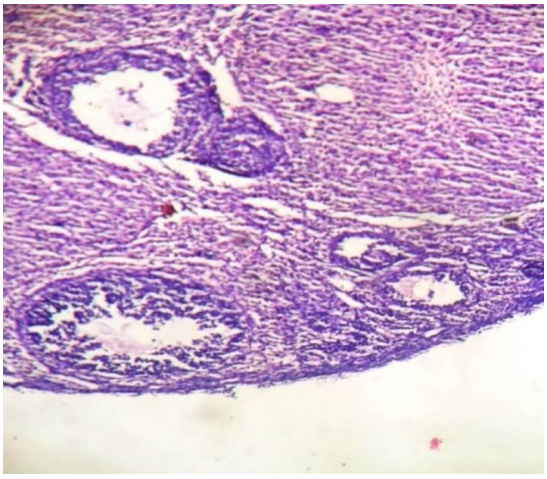
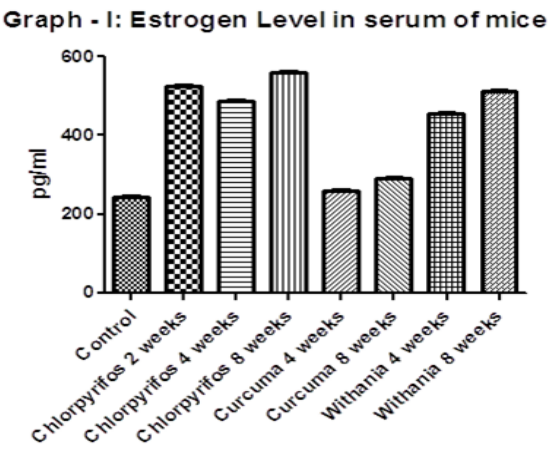
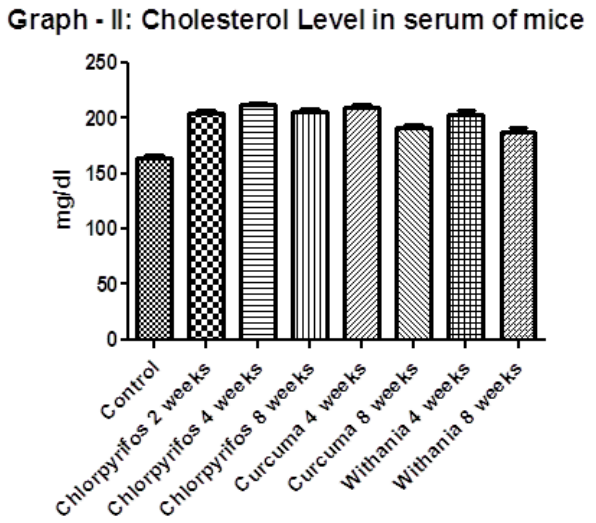


Fig 5: Ovary of eight weeks chlorpyrifos administered mice followed by eight weeks administration of *Curcuma longa* shows restorations in ova and mature Graafian follicle. Germinal epithelium was continuous. Corpus luteum was homogeneous with well differentiated nuclear and cytoplasmic material. X200.





DISCUSSION

Fipronil administration causes a significant reduction in estradiol level in female Wistar rats.[13] Similarly Oduma[14] reported that treatment of rats with the insecticide heptachlor also suppresses estradiol concentrations in blood and reduces the production of estradiol by ovarian cells of treated rats. In our study, 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.

Kammon[15] reported that oral administration of chlorpyrifos significantly reduces plasma cholesterol level in layer chickens. The present study showed elevated level of cholesterol in chlorpyrifos administered group of mice. The present finding is consistent with the findings of Newairy[16], Nashwah[17] and Ogutcu[18] who reported an elevation in serum total cholesterol level of rats treated with Chlorpyrifos, Profenofos and Dichlorvos, respectively. Estrogens readily diffuse across the cell membrane. Once inside the cell, they bind to and activate estrogen receptors which in turn modulate the expression of many genes.[19] Additionally, estrogens have been shown to activate a G protein-coupled receptor, GPR30.[20] In the present study, increase in cholesterol level facilitates estrogen synthesis in mice because cholesterol acts as a precursor for estrogen biosynthesis. This indicates why estrogen level is elevated in chloropyrifos administered group. The present study also showed that Chlorpyrifos administered mice showed degeneration of germinal epithelium to a greater extent. Large vacuolated spaces were also observed in mature Graafian follicle. Degeneration in ova was observed with clustered nuclei of granulosa cells.

Zade[21] reported that alcoholic extract of *Cannabis sativa* leaves causes slight increment in serum progesterone level and decrement in serum estrogen level in rats. Ghosh[22] showed that extract of *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 diminished estrogen synthesis due to decrease in cholesterol metabolism or both. In the present study, alcoholic extracts of both *Withania somnifera* and *Curcuma longa* also reduced estrogen level in Chlorpyrifos administered group of mice, restoration was more in *Curcuma* treated group.

In a study on 18 healthy volunteers for safety, tolerability and activity of *W. somnifera*, they observed a significant decrease in total cholesterol and decreasing trend was observed in triglycerides.[23] Our study also shows reduced cholesterol level in *W. somnifera* treated group. Turmeric increases cholesterol, LDL-C and HDL-C in rats.[24] In our study, we observed reduced level of cholesterol after *C. longa* administration. This result is supported by Varalakshmi[25], who showed that aqueous extract of *Curcuma longa* significantly reduced serum levels of total cholesterol and triglycerides in ethanol-induced rats. In our study more restoration of cholesterol level was observed in *Withania* treated group. *Withania somnifera* and *Curcuma longa* administration showed restoration in ova and granulosa cells. Restoration in germinal epithelium was also observed. Mature Graafian follicle was restored effectively. Corpus luteum was homogeneous with well differentiated nuclear and cytoplasmic material. *Withania* treated group showed more restoration in histology of ovary than *Curcuma* treated group in the present study.

CONCLUSION

This study concludes that *Withania somnifera* and *Curcuma longa* both play effective role against Chlorpyrifos induced toxicity on biochemical, hormonal and histology of ovary, however *Withania somnifera* has great restorative effect against Chlorpyrifos induced toxicity than *Curcuma longa* comparatively. It restores cellular and sub-cellular toxicity of Chlorpyrifos exposed ovary effectively.

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