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IN VIVO TOXICOLOGICAL EVALUATION OF ORGANOPHOSPHATE PESTICIDE ON FEMALE ALBINO MICE: THERAPEUTIC EFFECTS OF CURCUMIN

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ABSTRACT

Chlorpyrifos (CPF) an Organophosphate insecticide was evaluated for its potential to produce reproductive toxicity in rats following oral exposure. In the present study mice were given CPF at the dose level of 10 mg/kg body weight by Gavage method for 14 and 21 days. Morphological alterations of ovary were examined under LM. Its oxidative stress levels were also evaluated by Lipid peroxidation levels and hormonal changes (FSH) were also measured to evaluate the fertility rate in case of CPF induced toxicity mice. Bioremediation was done to the toxicity induced mice using extracted curcumin at 100 mg/kg body weight for 7 days. In control group, the germinal epithelium was continuous with prominent and well defined different stages of graffian follicles. While in chlorpyrifos treated group ruptured germinal epithelium with multiple nuclei were observed. Degenerated ovum was observed in matured graffian follicles. Rudimentary cells were observed in corpus leutium. Many vacuolated spaces were observed in ovarian cortex which is unusual. Significant increase in serum LPO levels and decrease in FSH levels were also observed. After treating the toxic mice with the curcumin restoration of all the parameters to the normal status was observed.

INTRODUCTION: Invention of biocides viz.; pesticides, fungicides and herbicides are considered as boom to the agricultural sector. They play a vital role and have wide applications in both agricultural and commercial pest control. They exhibit a broad spectrum of activity against different arthropod pests of plants and animals. As a result they improve the crop productivity and yield to meet the demands. They fight against disease causing vectors like malaria and prevent sickness that caused by mould food. On the other side pesticides are reported to show deleterious effects by contaminating every layer of ecosystem due to their high potency on non-targeted organism especially human beings either directly or indirectly.

The primary impact of pesticides is on hormonal imbalance and brain development and thereby consequently affects ability to learn. They owe their toxicity to their ability to attack Nervous system by oxidative desulfuration of cholinesterase enzyme [1] as a result neuronal dysfunction takes place and also effects other organs causing eye irritation, skin sensitization, carcinogenicity, Mutagenicity [2], teratogenicity, asthma, infertility [3] and immunosuppressant [4].

Among the organophosphates, chlorpyrifos chemically called as o, o-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate, is one which causes many hepatic and renal dysfunctions. As they are lipophilic in nature their main target is bilayer lipoidal membrane of the cell. CPF cause synoptosomal AChE activity in different parts of brain [5]. Organophosphate pesticides are known to alter the activity of Na^+/K^+-ATPase [6-8], Mg^{+2} ATPase, Ca^{+2}-ATPase, besides being potent anti-choline esterase compound [9]. Morphological changes produced by chlorpyrifos in brain and optic nerve tissues were also reported as well as a neurotoxicant [10].

As they are highly lipophilic in nature their main target is to attack the lipoidal membranes of the biological membranes and produces the reactive oxygen species (ROS) as a result of which oxidative stress is induced which leads to oxidation of lipid membrane and results in its degradation [11]. Curcumin analogues are known to be having tumerones modulate anti-inflammatory signaling and cell proliferation signaling [12]. Present study aims to unfold the histopathological and biochemical changes of ovary which take place while chlorpyrifos was administered at 10mg/Kg b. wt/day for 14 and 21 days to the Swiss albino mice, as well as to reveal the therapeutic effects of extracted curcumin for 7 days at 100 mg/kg b. wt.

MATERIALS AND METHOD:

Pesticide: Chlorpyrifos (CPF) (T_N –Dursban) were used as an E.C = 20% (w/v).

1) **Plant material:** Rhizomes powder of *Curcuma longa* was selected as a plant material for the extraction of the active ingredient called curcumin and extracted as per the protocol. Extracted curcumin was characterized by IR Spectroscopy and M.P was noted as 180-183˚C and close to the literature value.

2) **Experimental model:** Reared Sexually matured 6 week old age group female Swiss albino mice (*Mus musculus)* weighing 25-35g b. wt in the laboratory animal resource section of Mahavir Cancer Sansthan and Research center, Patna, were selected as an experimental model in the present study. The animals were housed at controlled environmental conditions 22±2˚C, relative humidity 50±10%, and 12h dark-light cycle. Animals were housed and allowed to free access to food and water. All experimental

procedures were conducted as per the guide lines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

3) Methodology:

a) Chronic Toxicity Studies: Selected pathogen free mice were sorted and CPF was administered at 10mg/kg b.wt dose level for 21 days. Sacrifice was done on 14th day and 21st day of treatment of CPF in each group.

TABLE 1: CHLORPYRIFOS TREATMENT SCHEDULE

N	CPF dose level (mg/kg b. wt)	Total no. of days administered	1st sacrifice	2nd sacrifice
10	12	21	After 14 days	After 21 days

(N= no. of animals in each group); CPF was administered to the mice (Gavage method) successively for 21 days

b) Bioremediation: After administering the mice with CPF at 10mg/kg b. wt for 21 days followed by the administering the extracted curcumin for 7 days at 100mg/kg b.wt, mice were sacrificed from each group for histological and biochemical analysis.

d) Histopathological Studies: Blood was collected by orbital puncture and centrifuged within 1hr for 15 min at 3500 rpm to separate the serum to carry out further biochemical analysis. The selected organ is dissected out and fixed it in 10% neutral formalin solution and tissue was processed. For each organ serial sections (4-6 µm) were made .These prepared slides were stained with Hematoxylin-Eosin (H & E) and examined morphometrical under LM.

e) Biochemical Assessment: With the separated serum following Biochemical analysis was performed to establish the effects of CPF induced toxicity and the remedial effect of the extracted curcumin.

i) Estimation of Lipid Peroxidation levels (LPO)

ii) Estimation of FSH levels

i) **Lipid Peroxidation Levels:** Lipid peroxidase test gives the amount of lipid oozes out from lipid biomembrane. It measures the levels of Melanaldehyde in blood sample. The LPO test was performed with separated serum of different groups of control, and treated mice according to Okhwa *et al* 1979 [13].

ii) **FSH Levels**: It influences the growth of follicle, stimulates the granulose and thecal cells which begin to secrete steroidal hormone principally estrogen into follicular lumen also responsible for the maturation of G.F as well as ovulation. It is done by Direct ELISA method.

Observation:

Histological observations of CPF treated Ovary: Control group of mice show normal ovary with continues germinal epithelium with well defined different stages of In Control ovary with continues Germinal Epithelium with well defined different stages of Graffian follicle, Mature Graffian follicles (**Plate I, Fig. A**) and Well defined ovarian cortex and medulla region (**Plate I, Fig. B**). While 14 days CPF administered mice show vacuolated spaces in Matured graffian follicle with clustered nuclei of granulosa cells (**Plate II, Fig. A**) and 21 days administered mice showing degeneration in ova were prominent with clustered nuclei of granulosa cells (**Plate II, Fig.**

B). The remedial effect of curcumin at 100mg/kg b. wt for 7 days after administering CPF showed restoration of Ova of Matured Graffian Follicles with prominent granulosa cells (**Plate III, Fig. A**). Showing restoration in cortex region of ovary with germinal epithelium. Little vacuolated spaces were observed. Matured graffian follicle show restoration but ova is not completely restored (**Plate III, Fig. B**).

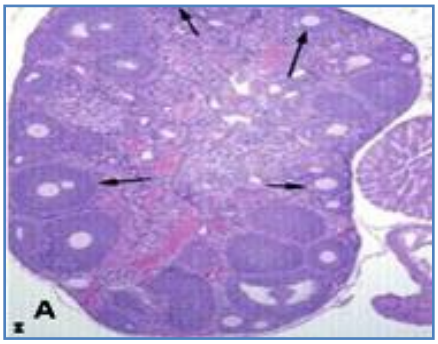

PLATE I FIG. A: IN CONTROL OVARY WITH CONTINUES GERMINAL EPITHELIUM WITH WELL DEFINED DIFFERENT STAGES OF GRAFFIAN FOLLICLE, MATURE GRAFFIAN FOLLICLE

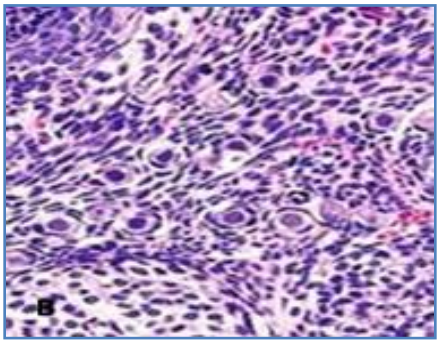

FIG B: WELL DEFINED OVARIAN CORTEX AND MEDULLA REGION

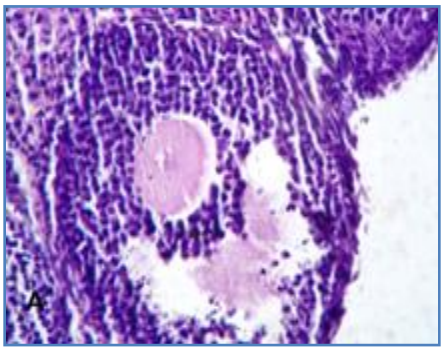

PLATE II FIG A: CPF TREATED @ 10MA/KG B. WT FOR 14 DAYS SHOWING VACUOLATED SPACES IN MATURED GRAFFIAN FOLLICLE WITH CLUSTERED NUCLEI OF GRANULOSA CELLS

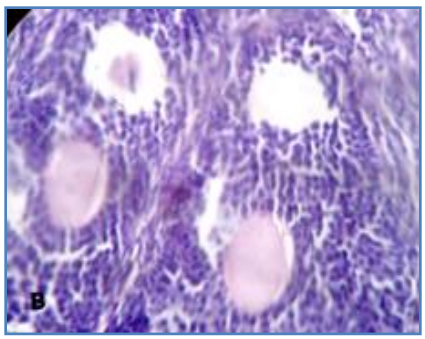

PLATE II FIG B: CPF TREATED @ 10MG/KG B.WT FOR 21 DAYS SHOWING DEGENERATION IN OVA WERE PROMINENT WITH CLUSTERED NUCLEI OF GRANULOSA CELLS

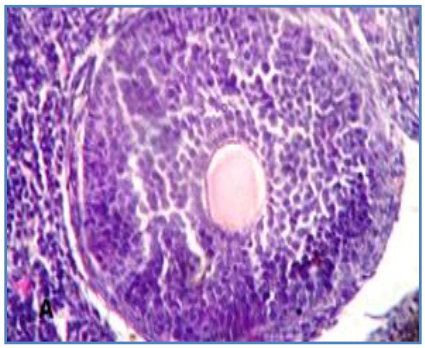

PLATE III FIG A: OVARY OF CPF AT 10MG/KG B.WT FOR 21 DAYS FOLLOWED BY CURCUMIN TREATED FOR 7 DAYS MICE SHOWING RESTORATION OF OVA OF MATURED GRAFFIAN FOLLICLES WITH PROMINENT GRANULOSA CELLS

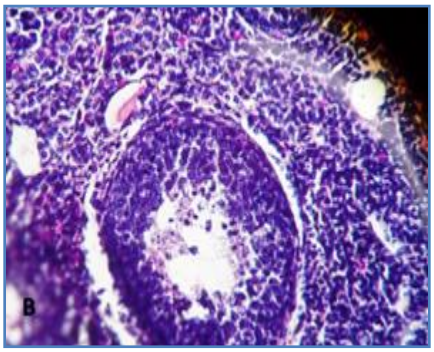

PLATE III FIG B: SHOWING RESTORATION IN CORTEX REGION OF OVARY WITH GERMINAL EPITHELIUM. LITTLE VACUOLATED SPACES WERE OBSERVED. MATURED GRAFFIAN FOLLICLE SHOW RESTORATION BUT OVA IS NOT COMPLETELY RESTORED

Determination of LPO Levels: When compared to control (1.6 nmol/ml), CPF treated mice at 10 mg/kg b. wt has 35 nmol/ml (14 days) which was a drastic increase from control and 37.5 nmol/ml (21 days) as serum lipid levels. After bioremediation with Curcumin, the lipid levels

were found to be as 35.26 nmol/ml 100 mg/kg b. wt for 7 days (**fig. 1**).

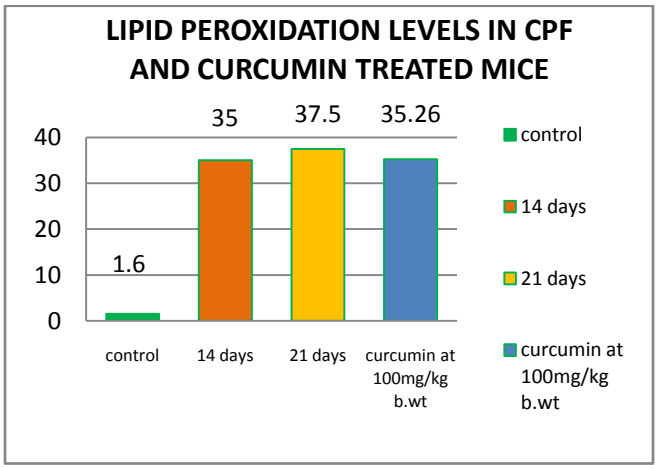

FIG. 1: GRAPHICAL REPRESENTATION OF LPO LEVELS IN CPF TREATED AND FOLLOWED BY EXTRACTED CURCUMIN

Determination of FSH Levels: Comparing with the control (0.05 ml U/ml), CPF administered mice at 10mg/kg b.wt for 14 and 21 days showed 0.029 ml U/ml which was an sudden decrease and 0.02 ml U/ml respectively. While treated with curcumin show marked 0.03 ml U/ml FSH levels (**fig. 2**).

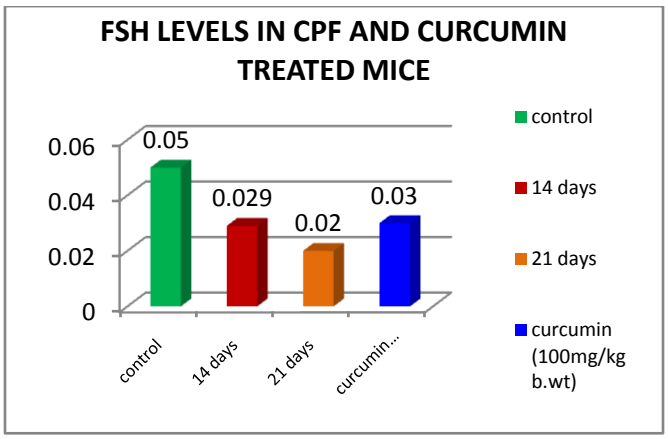

FIG. 2: SERUM FSH LEVELS IN MICE TREATED WITH 10MG/KG B. WT CPF

DISCUSSION: The study elucidated by the Sang-Hee Jeong et al., proved the Chlorpyrifos-methyl (CPM) exhibiting weak reproductive toxicity in F0 rats exposed at adulthood and negligible effects in F1 offspring exposed in uterus and via lactation at weanling also relative and absolute ovaries weights were decreased at 100 mg/kg CPM [14]. Suleiman F. Ambali *et al*., worked on CPF and reported that by the administering the CPF in a dose dependent manner and they observed the significant loss in pre-implantation potency which was correlated to the number of Corpus luteum [15], whereas, as per our results the CPF administered mice showed the degeneration in matured graffian follicle with disrupted germinal epithelium, clustered nuclei of granulosa cells.

Reproductive toxicity caused by organophosphates at cellular and molecular level in the ovaries of rats showed significant decrease in the concentration of cytoplasm as well as membrane bound proteins, total lipids, phospholipids and cholesterol [16]. Our study revealed there is a degeneration of cytoplasm in graffian follicle. In case of Organochlorine, organophosphate and Carbamates poisoning increased lipid peroxidation, coupled with altered levels of GSH and OFR scavenging enzymes in the blood were observed [17]. Some also reported the effect on both CM and CPF on different free radical mediated parameters [18].

Present study revealed that CPF increases the Lipid peroxidation levels in the serum from 1.6 nmol/ml(control) to greater extent to 35 nmol/ml (14 days) and 37.5 nmol/ml (21 days). The highest reduction testosterone of was observed in case of Diazinon-treated rats at 50 ppm (48.3%) followed by chlorpyrifos methyl (55%) and Profenofos (61.6 %). In addition, serum LH and FSH levels in all pesticides-treated rats were significantly decreased with the high concentration only [19]. The results in our study quite indicate that even by the administration of CPF at lower concentrations the serum FSH levels were decreased significantly. When the

ameliorative effect of curcumin for 7 days was studied, based on the histopathology results of ovary showed the restoration of ova of graffian follicle with continues germinal epithelium which inturn maintains the normal fertility functions confirming that curcumin play significant role against CPF toxicity on Graffian follicle. Curcumin bound iron, but did not block iron uptake or bioavailability in T51B cells given FAC.

However, it reduced cytotoxicity, blocked generation of ROS, and eliminated signaling to cellular stress pathways caused by iron. When they compared these with those of tocopherol, the effects of curcumin also differed from those of α-tocopherol, which did not bind iron and was less effective at blocking iron-stimulated ROS generation [20]. During the remedial effect of curcumin to the toxic mice in the present study, the sudden decrease in lipid levels is quite interesting to note (35.26 nmol/ml) which ensures that the curcumin acts as well as retains its antioxidant property effectively even after CPF induced toxic conditions by decreasing the oxidative stress. As per our observations the FSH level was retrieved to some extent by treating the toxic induced mice with curcumin.

CONCLUSION: This is concluded that Chlorpyrifos pesticide causes increased levels of lipid peroxidation and decreased levels of FSH in mice which causes improper ovulation leading to infertility of mice, while curcumin restores FSH levels and LPO levels to greater extent which causing restoration in fertility in mice. Pesticide also causes histopathological alteration through degeneration of germinal epithelium and graffian follicle while curcumin reports normal architecture of graffian follicle and germinal epithelium in mice, which strongly suggests that curcumin play very effective role against pesticidal toxicity and maintains normal histological, hormonal and biochemical parameters in ovary of mice.

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