IJPSR (2011), Vol. 2, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 September, 2010; received in revised form 25 November, 2010; accepted 23 January, 2010

IN VIVO TOXICOLOGICAL EVALUATION OF CHLORPYRIFOS PESTICIDE ON FEMALE ALBINO MICE: THERAPEUTIC EFFECTS OF *CURCUMA LONGA*

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Keywords:

Chlorpyrifos,
Lipid peroxidation,
FSH,
Curcumin,
Bioremediation

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ABSTRACT

Chlorpyrifos (CPF) an Organophosphate insecticide was evaluated for its toxicity to produce reproductive disorders in rats following oral exposure. In the present study CPF was given to the mice at the dose levels of 10 and 20mg/kg body weight by Gavage method. **A** histological alteration of ovary and uterus was examined under **l**ight **m**icroscope. Its oxidative stress levels were studied by noting **l**ipid peroxidation levels and hormonal changes (FSH) were also checked to evaluate the fertility rate in CPF induces toxicity mice. LD_{50} CPF was established for **S**wiss **a**lbino **m**ice. Bioremediation was done to toxic induced mice using crude and extracted curcumin at 100 and 200 mg/kg body weight respectively. In control group, the germinal epithelium was continuous with prominent and well defined different stages of graffian follicles. On the other hand chlorpyrifos **(CPF)** treated group showed ruptured germinal epithelium with multiple nuclei and matured graffian follicles with degenerated ovum. Corpus leutium cells were observed to be **r**udimentary. Unusual number of vacuolated spaces was observed in ovarian cortex. Thus, the present study reveals that chlorpyrifos causes degeneration of graffian follicle and germinal epithelium of ovary leading infertility in female. Control **u**terus shows well defined longitudinal and circular muscles on periphery with well structured endometrial cells and glands. In case of drug treated, degeneration of circular and longitudinal muscles was observed on periphery with clustered endothelial cells at lower concentration. At higher concentrations, degeneration in circular muscles is prominent. Longitudinal muscles were also on periphery with many vacuolated spaces along with vacuolated endometrial cells and glands were also rudimentary in structure. The remediation effect with crude and curcumin it showed the better and satisfactory results in histopathology as well as biochemical aspects.

INTRODUCTION: Invention of biocides viz.; pesticides, fungicides and herbicides are considered as boom to the agricultural sector on one side as 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 plant and animal pests. Resulting in improving in productivity of crops and meet the demands.

On the other side pesticides are reported to show deleterious effects by contaminating each and 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 resulting in neuronal dysfunction 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 cellular bilayer lipoidal membrane. CPF shows different synoptosomal Acetylcholinesterase enzyme, AChE activity in different parts of brain[5]. Organophosphate pesticides are known to alter Na^+/K^+-ATPase[6-8] activity, Mg^{+2} ATPase activity, Ca^{+2}-ATPase activity, besides being a very potent anti-choline esterase compound[9]. 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 [10]. Curcumin analogues are known to be have tumerones modulate anti-inflammatory signaling and cell proliferation signaling [11]. In the present study chlorpyrifos was administered at 10 and 20mg/Kg body weight /day for 14 and 21 days to the Swiss albino mice. Successively biochemical and histopathological analysis was done.

MATERIALS AND METHODS:

Pesticide: Chlorpyrifos pesticide (T_N- Dursban) is used as an E. C. containing 20% (w/w).

Plant material: Commercially available rhizomes powder of *Curcuma longa* was selected as a plant material for the extraction of the active ingredient called curcumin.

Preparation of Extract: Crude turmeric powder contains maximum percentage of curcumin (approximately 80%) and the rest constitutes volatile oils and resinous substances. Curcumin was separated by continues hot extraction process using soxhlet apparatus. 30g of crude turmeric powder with 95% ethanol in a soxhlet extraction until all the coloring material is extracted. Distill off the alcoholic extract to a semi solid brown color mass. Distill with 50ml of hexane/pet. Ether and extract twice with equal volume of 0.1% NaOH solution. Oil extract was combined and acidified with dilute HCl until yellow color precipitate is formed. Allow it to settle for 15 minutes. After the settling of the precipitate for 15 minutes, concentrate the extract by boiling in boiling water bath. During the process of boiling resinous material would agglutinate and form lumpy mass. Filter the solution in hot conditions and concentrate the filtrate to very small volume and finally cool to get the crystals of curcumin. This extracted curcumin was characterized by Infrared (IR) Spectroscopy and melting point was noted as 180-183˚C and close to the literature value.

Experimental model: Reared sexually matured female Swiss albino mice (*Mus musculus)*, of about 6 week old age group weighing 20-35g body weight, 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).

Acute Toxicity Studies (LD_{50}): The acute oral toxicity study was carried out as per guidelines set by Organization for Economic Cooperation and Development (OECD).

Methodology: Selected pathogen free mice were sorted into two groups, group I and group II. CPF was administered according to 10 and 20mg/kg body weight dose level (**Table 1**).

Acute Toxicity Studies: The mice were administers in increasing dose levels of CPF at 35, 45, 55, 65, 75 mg/kg body weight respectively and LD_{50} was evaluated.

Bioremediation: After treating the mice with CPF of different concentrations for 21 days the crude and extracted curcumin was given as a dose (**Table 2**). After 7 days of remediation the mice were sacrificed from each group for histological and biochemical analysis.

TABLE 1: CPF ADMINISTRATION

Group	n*	CPF dose level (mg/kg body weight)	Total no. of days administered	Ist sacrifice	IIst sacrifice
I	12	I	12	After 14 days	After 21 days
II	12	20	21	After 14 days	After 21 days

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

TABLE 2: HERBAL TREATMENT SCHEDULE

Group	CPF Dose (mg/kg body weight)	Duration of CPF in days	Herbal Treatment	Herbal dose	Number of days
Group Ib	10	21	Curcumin	100	7
Group IIb	20	21	Crude Turmeric	200	7

Histopathological Studies: After every sacrifice the collected blood by ocular puncture is centrifuged within 1h for 15 min at 3500 rpm to separate the serum to carry out further biochemical analysis. The selected organ is dissected out axed 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 Hemat-oxylin- Eosin (H & E) and examined morphometrical under LM.

Biochemical Assessment: With the separated serum following biochemical analysis was performed to establish the effects of CPF induced toxicity and the remedial effects of the turmeric extracts.

- Estimation of Lipid Peroxidation levels (LPO)
- Estimation of FSH levels

Lipid Peroxidation Levels: LPO gives the amount of lipid oozes out from lipid biomembrane. It measures the melanaldehyde in blood sample. LPO test was performed with separated serum of different groups of control, and treated mice according to Okhwa *et al*., 1979 [12].

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

RESULTS AND DISCUSSION:

Acute Toxicity: When the CPF was administered at the dose levels of 35, 45, 55, 65, and 75 mg/kg body weight the percentage mortality as observed to as 0, 10, 25, 50, 60. Thus LD_{50} was established to be 65mg/kg body weight.

Effects of CPF and bioremediation on the Lipid Peroxidation Level: Mice were treated with CPF at 10 and 20 mg/kg body weight concentrations for 14 and 21 days. After the toxicity was induced, bioremediation was done using curcumin at 100 mg/kg body weight for group I for 7 days and crude turmeric at 200 mg/kg body weight for group II. After completion of every dose separated serum was evaluated for the lipid levels. As per the results control showed normal MDA level (1.6 nmol/ml), when compared to control, in CPF treated mice with 10 mg/kg body weight it showed 37.4 nmol/ml after 14 days treatment and 35.0 nmol/ml after 21 days treatment, where as when with CPF at 20 mg/kg body weight showed prominent increase in MDA from 38.74 nmol/ml after 14 days treatment to 67.50 nmol/ml after 21 days treatment. After treating with curcumin it showed only 35.26 nmol/ml where as when treated with crude it showed 46.112 nmol/ml. (**Table 3 and Fig . 1**).

Effect of CPF and bioremediation on the FSH levels of mice: FSH levels were measured using Direct ELISA method and it was reported as follows. When it was compared with control (0.05) there was a gradual decrease in FSH level to 0.036u/ml (14 days), 0.031u/ml (21 days) in group I and to 0.029u/ml (14 days), 0.020u/ml (21 days) in group II. Curcumin treated showed prominent high levels 0.033u/ml but where as crude treated showed only 0.03 /ml (**Table 4 & Fig. 2**).

TABLE 3: MDA LEVELS IN CONTROL, TREATED AND REMEDIAL MICE

Sample Type Level	Absorbance (λ_{max} = 532 nm)	MDA (nmol/ml)
Control	0.013±0.002	1.6±0.47
CPF@10mg/kg body weight for 14 days	0.150±0.023**	37.4±2.42**
CPF@10mg/kg body weight for 21 days	0.155±0.084**	35.0±1.87**
CPF@20mg/kg body weight for 14 days	0.135±0.074**	38.7±1.56**
CPF @ 20mg/kg body weight for 21 days	0.140±0.035**	67.5±0.75**
CPF@10mg/kg body weight (21 days)+ curcumin@100mg/kg body weight (7 days)	0.130±0.080**	35.3±0.63**
CPF@20mg/kg body weight (21 days) + crude turmeric@200mg/kg body weight (7 days)	0.170±0.043**	46.1±0.38**

Values are expressed as Mean ± S.D; n=12. Statistical significance: (**p<0.01) One way ANOVA followed by Dunnett test

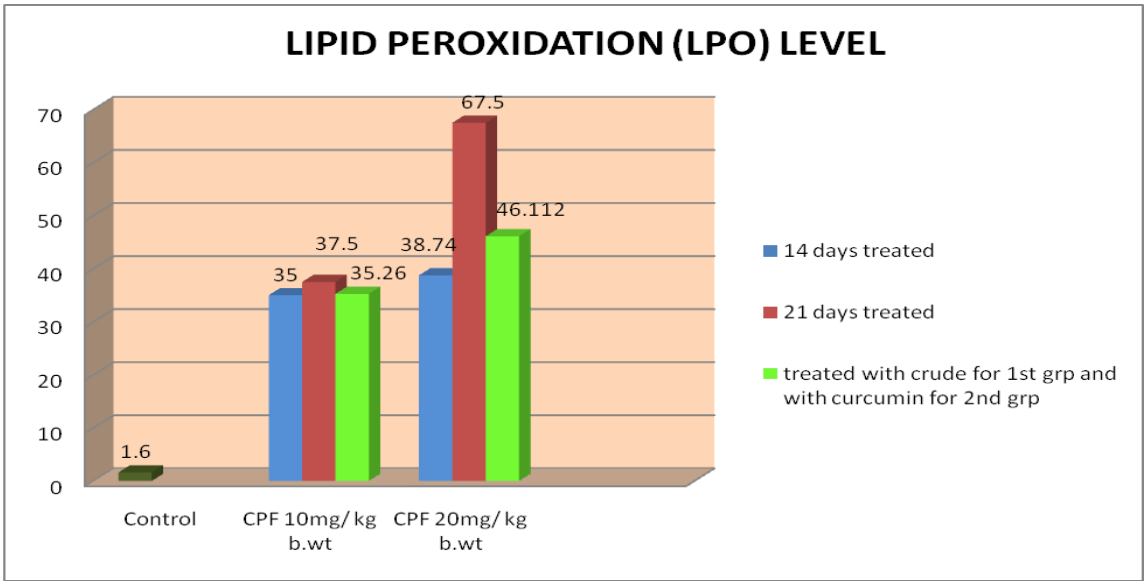

FIG. 1: LIPID PEROXIDATION PROFILE IN CONTROL AND DIFFERENT GROUPS OF TREATED MICE

TABLE 4: EFFECT OF CPF AND BIOREMEDIATION ON THE FSH LEVELS

Sample	Concentration (u/ml)
Control	0.05±0.002
CPF @ 10mg/kg body weight for 14 days	0.036±0.001**
CPF @ 10mg/kg body weight for 21 days	0.031±0.007**
CPF @ 20mg/kg body weight for 14 days	0.029±0.005**
CPF @ 20mg/kg body weight for 2 days	0.020±0.004**
CPF @10mg/kg body weight (21 days) + curcumin @100mg/kg body weight (7 days)	0.033±0.006**
CPF @20mg/kg body weight (21 days) + crude turmeric @200mg/kg body weight (7 days)	0.030±0.009**

Values are expressed as Mean ± S.D; n=12. Statistical significance: (**p<0.01) One way ANOVA followed by Dunnett test

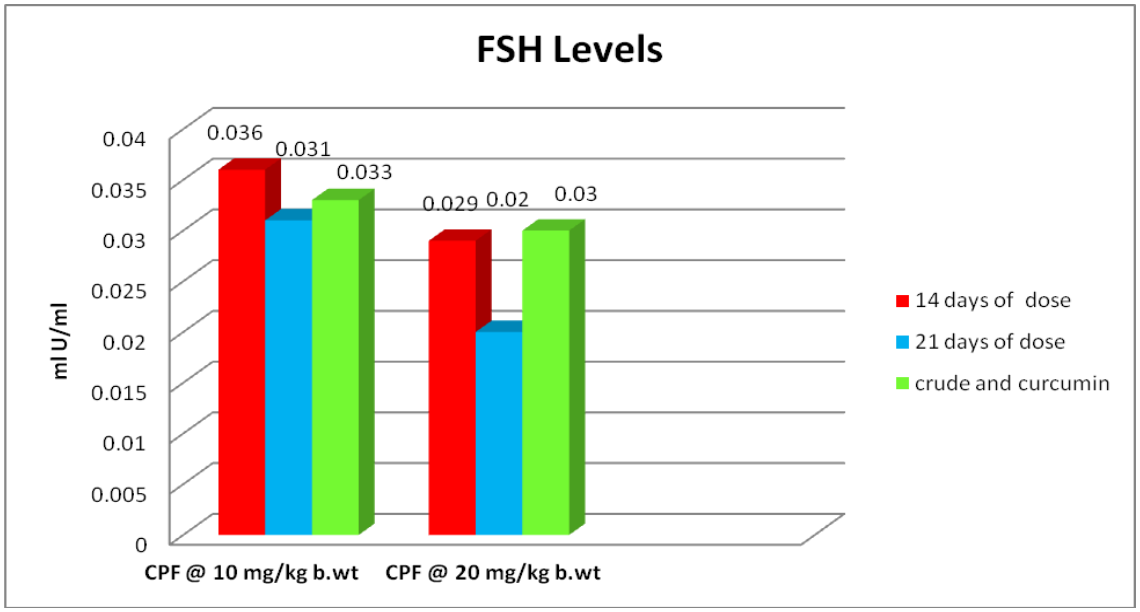

FIG. 2: COMPARATIVE FSH LEVELS IN THE DIFFERENT GROUP OF CPF TREATED AND HERBAL TREATED MICE

Histopathological observations: Chlorpyrifos pesticide showed deleterious effects on the histology of ovary and uterus of female mice.

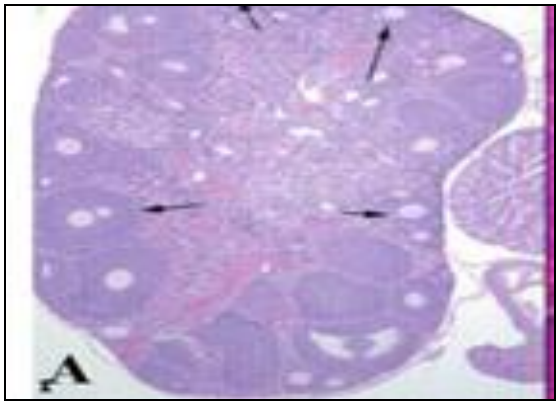

PLATE I, FIG. A: IN CONTROL OVARY WITH CONTINUES GERMINAL EPITHELIUN WITH WELL DEFINED DIFFERENT STAGES OF GRAFFIAN FOLLICLE, MATURE GRAFFIAN FOLLICLE

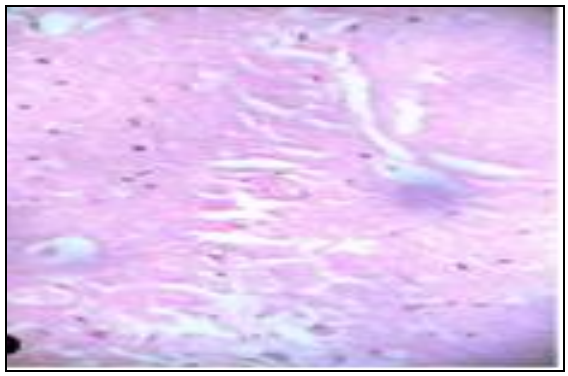

PLATE I, FIG. B: CONTROL OVARY WITH WELL DEFINED OVARIAN CORTEX AND MEDULLA REGION

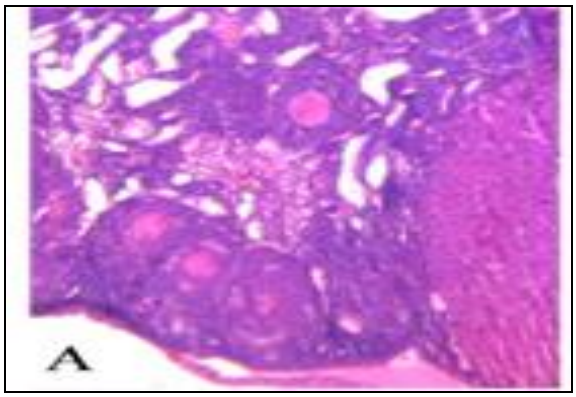

PLATE II, FIG. A: 14 DAYS CPF TREATED OVARY AT 10mg/kg b.wt SHOWING DEGENERATED OVA IN DIFFERENT STAGES AND RUPTURE EPITHELIUM IS SEEN

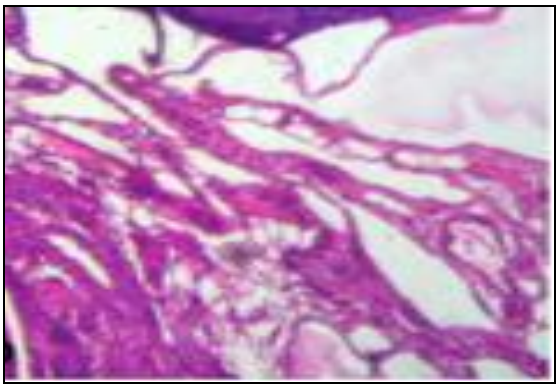

PLATE II, FIG. B: 14 DAYS CPF TREATED OVARY AT 20mg/kg b.wt SHOWING VERY ENLARGED CELLS OF OVARIAN MEDULLA WITH MANY VACUOLATED SPACES

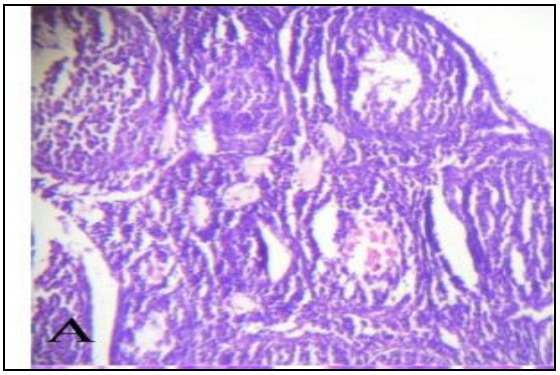

PLATE III, FIG. A: 21 DAYS CPF TREATED OVARY AT 10mg/kg b.wt SHOWING DEGENERATED OVA WITH MANY VACUOLATED SPACES

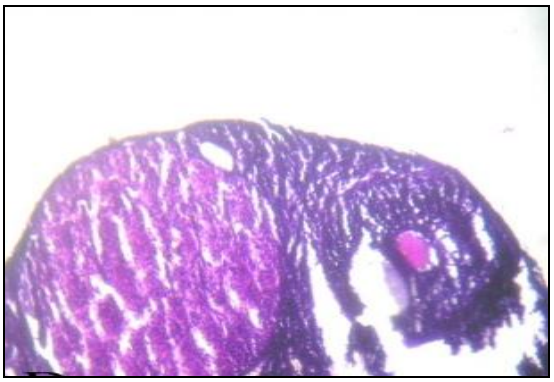

PLATE III, FIG. B : 21 DAYS CPF TREATED OVARY AT 20mg/kg b.wt SHOWING RUPTURE EPITHELIUM AND DEGENERATED CARPUS LUTEUM WITH MATURED GRAFFIAN FOLLICLE AT PERIPHERY

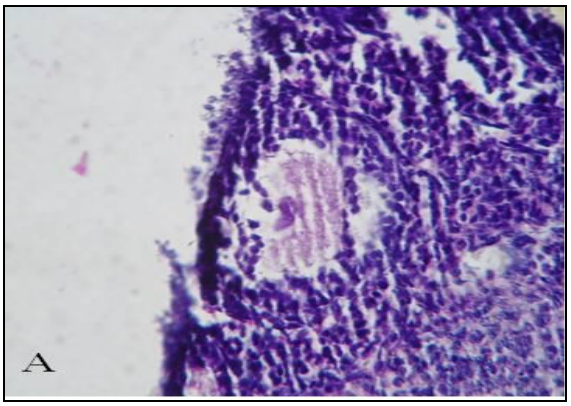

PLATE IV, FIG. A: 10mg/kg b.wt CPF TRETAED MICE FOLLOWED BY CURCUMIN TREATED FOR 7 DAYS SHOWED THE REGENERATING OVA

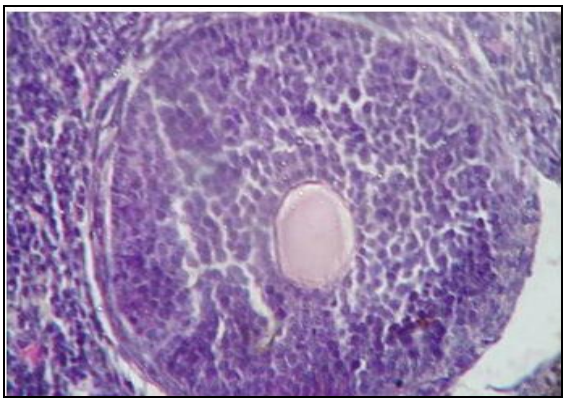

PLATE IV, FIG. B: 20mg/kg b.wt CPF TREATED MICE FOLLOWED BY CRUDE TURMERIC TREATED FOR 7 DAYS SHOWED ALMOST COMPLETE

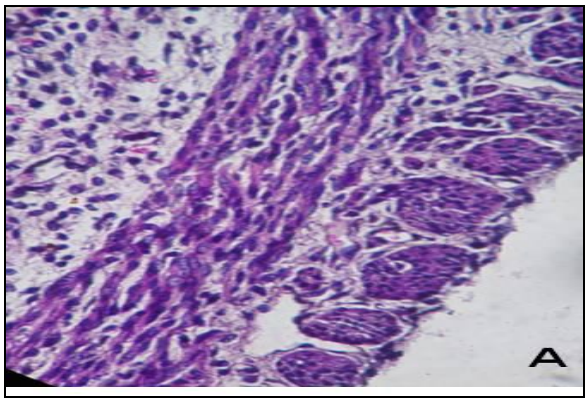

PLATE V, FIG. A: SHOWING UTERUS OF NORMAL MICE WITH WELL DEFINED CIRCULAR AND LONGITUDINAL

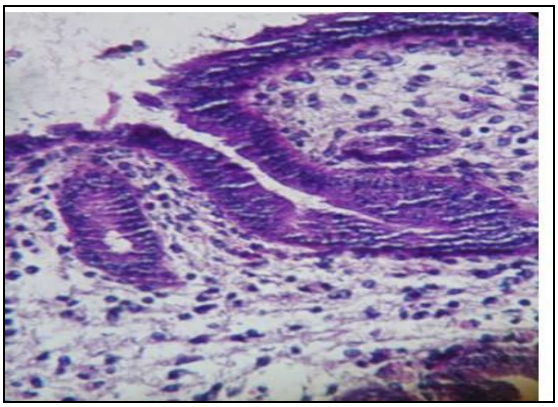

PLATE V, FIG. B: SHOWING UTERUS OF NORMAL MICE WITH WELL STRUCTURED ENDOMETRIAL CELLS

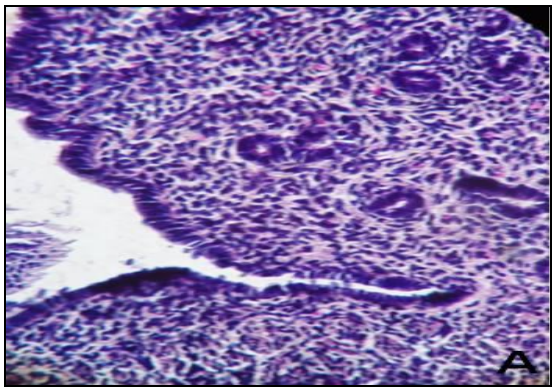

PLATE VI, FIG. A: SHOWING UTERUS OF CPF AT 10mg/kg b.wt FOR 14 DAYS MICE WITH DEGENERATION IN CIRCULAR AND LONGITUDINAL MUSCLES ON PHERIPHERY

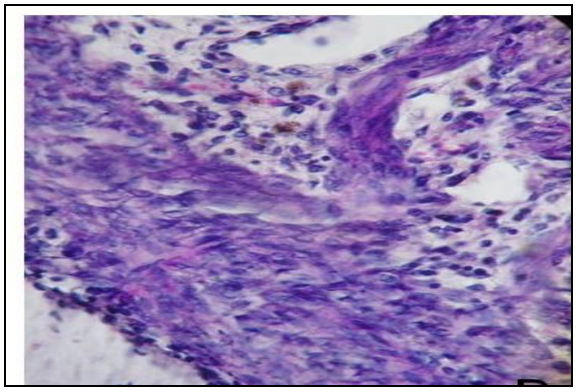

PLATE VI, FIG. B: SHOW UTERUS OF CPF AT 20mg/kg b.wt FOR 14 DAYS WITH VACUOLATED STRUCTURED OF ENDOMETRIAL CELLS AND GLANDS IN UTERUS WHILE LONGITUDINAL

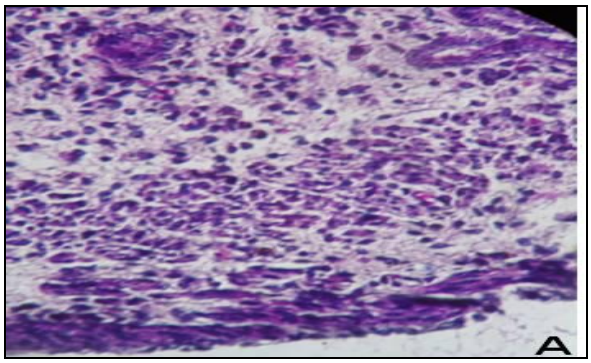

PLATE VII, FIG. A: SHOWING UTERUS OF CPF AT 10mg/kg b.wt FOR 21 DAYS MICE WITH DEGENERATION IN CIRCULAR MUSCLES. LONGITUDINAL MUSCLES WERE ALSO RUDIMENTARY ON PERIPHERY WITH MANY VACUOLATED SPACES

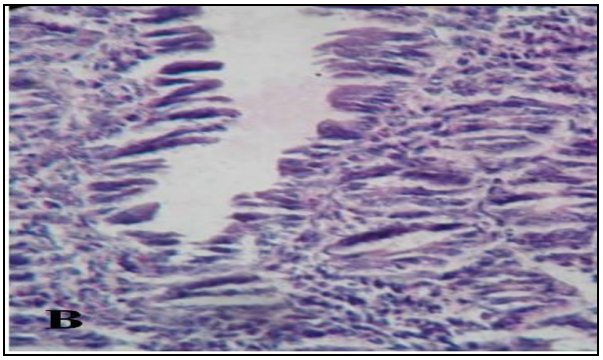

PLATE VII, FIG B: SHOWING UTERUS OF CPF AT 20mg/kg b.wt FOR 21 DAYS MICE WITH DEGENERATION IN CIRCULAR MUSCLES IS OBSERVED. LONGITUDINAL MUSCLES WERE ALSO RUDIMENTARY ON PERIPHERY. WITH MANY VACUOLATED SPACES AND ENDOMETRIAL GLANDS WERE ALSO RUDIMENTARY IN STRUCTURE

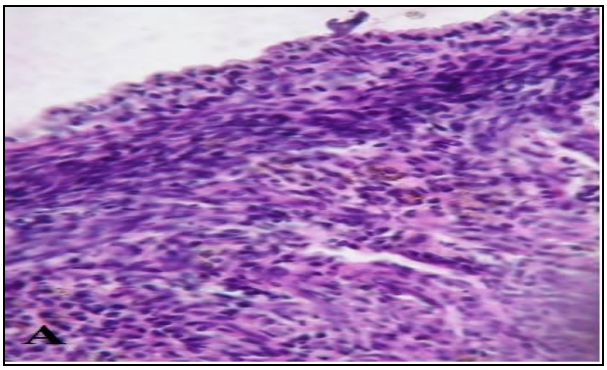

PLATE VIII, FIG. A: UTERUS OF CPF AT 10mg/kg b.wt FOR 21 DAYS FOLLOWED BY CRUDE TURMERIC FOR 7 DAYS MICE SHOW RESTORATION IN LITTLE EXTENT ON CIRCULAR AND LONGITUDINAL MUSCLES AND ENDOMETRIAL CELLS AND GLANDS

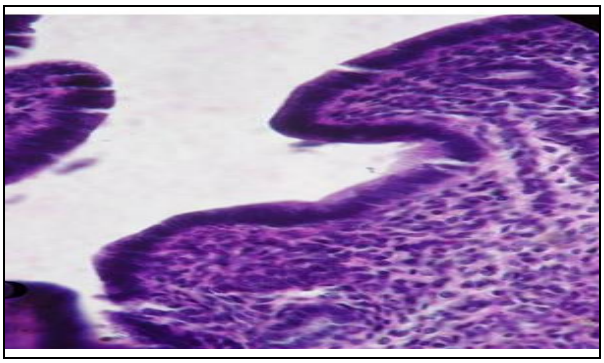

PLATE VIII, FIG. B: UTERUS OF CPF AT 20mg/kg b.wt FOR 21 DAYS FOLLOWED BY CURCUMIN FOR 7 DAYS MICE SHOW RESTORATION TO GREATER EXTENT ON CIRCULAR AND LONGITUDINAL MUSCLES AND ENDOMETRIAL CELLS AND GLANDS WERE ALSO

ACKNOWLEDGEMENT: It is author's privilege and gratefulness to thank Mahavir Cancer Sansthan and Research Center for allowing to undertake the work and also for laboratory and infrastructure facilities. Also conveys special thanks and regards to the Director, Dr. J. K. Singh, H.O.D., Prof A. Nath, Dr. Arun Kumar, Dr. Mohammad Ali for their guidance throughout. Also thankful to one and all, who helped directly and indirectly and whose name is not mentioned here.

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