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TOXICOPATHOLOGICAL STUDIES ON INDUCED CHLORPYRIFOS TOXICITY IN WISTAR RATS (*Rattus norvegicus*)

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ABSTARCT

In the present study twenty four young albino Wistar rats were divided uniformly into four equal groups viz. A, B, C and D. Group A served as control, while, Group B, C and D rats were orally administered with Chlorpyrifos @ 4, 8 and 16 mg/kg b.wt. respectively for 28 days. The clinical symptoms were characterized by decreased physical activity, dullness, depression, piloerection, shivering, sweating, salivation and reduced body weight. There was significant ($P < 0.05$) reduction in the body weight at 28th day post treatment in treatment groups. In chlorpyrifos treated groups , histopathological changes observed in liver were characterized by sinusoidal congestion, bile duct proliferation, focal hepatic necrosis and portal vein congestion. In kidney, severe intertubular hemorrhages, tubular degeneration and shrinkage in size of glomeruli with wide Bowman's capsular space was observed in rats receiving the high dose of chlorpyrifos. Brain showed cytoplasmic vacuolation, neuronal degeneration and partial blockage of blood capillary with perivascular

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oedema and swollen endothelial cells of capillary. In ovary, hemorrhages, epithelial damage, degenerating ova along with Graffian follicle and corpus luteum was observed. Graffian follicle showed clustered nuclei of granulosa cells , While fallopian tube showed varying degree of damage to mucosal folds .In spleen ,depletion of lymphoid cells in germinal center. Lung showed emphysema, thick interalveolar septa, congestion of alveolar capillaries and hemorrhages. It is concluded that chlorpyrifos in very high dose cause adverse effects in rats.

Key words: Chlorpyrifos, wistar rats, toxicopathology

INTRODUCTION

Environmental pollution from pesticides is an important issue that attracts wide spread public concern. Among them, some organophosphate and organochlorine pesticides are routinely used in agriculture (Forget, 1991). Organophosphates (OP's) are the esters of pentavalent phosphorous acid, exhibiting wide range of toxicity in mammals (Sultatos, 1994). Chlorpyrifos (O-O-diethyl-O- {3, 5, 6 trichloro-2-pyridyl}-phosphorothioate) is one of the most heavily used organophosphate pesticides in domestic and agricultural applications throughout the world (Asperlin, 1994). Chlorpyrifos causes deleterious effects through acetylcholinesterase inhibition at synapse of central and peripheral nervous system (Gordon et al.,1997) and produces nausea, vomiting, salivation, diarrhoea, tremor and convulsion like symptoms (Kamrin,1997). The acute oral LD50 of chlorpyrifos is 118-245 mg kg^{-1} for rats, 1000 mg kg^{-1} for rabbit and 504 mg kg^{-1} for guinea pigs (USEPA, 2000). In agricultural applications, chlorpyrifos is registered to control a broad range of insect pests across many crops including cotton, sugarcane, vegetables, pome and stone fruit, turf and ornamental crops. In the home and commercial sites it is registered for the control of pests such as cockroaches, termites and fleas. It is also registered for use in dog and cat

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flea collars and shampoos and in flea sprays for dogs. It is also widely used in early spray programs in brassica because it achieves a broad spectrum of activity against cutworm, aphid, cabbage moth and cabbage white butterfly with a single application. Chlorpyrifos is moderately fat-soluble, and was readily absorbed by oral and inhalation routes but dermal absorption was relatively low (about 2% in humans). This experiment was carried out to evaluate the toxicopathologic effects of the chlorpyrifos in wistar rats..

MATERIALS AND METHODS

The study was conducted on 24 young albino Wistar rats were divided uniformly into four equal groups viz. A, B, C and D . Group A served as control, while Group B, C, and D rats were orally administered with chlorpyrofis @ 4 mg/kg b.wt (low dose), 8 mg/kg b.wt (mid dose) and 16 mg/kg b.wt (high dose) respectively for 28 days. The Institutional Animals Ethical Committee (IAEC) approved the experimental protocol which met the National guidelines as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Chlorpyrifos (20%) obtained from the market was used for inducing the toxicity. All the other chemicals used in the study were of standard analytical grade. All the animals used in this study were placed in cages in an air conditioned room maintained at a temperature of 25ºC ± 30ºC , 12 hour light and dark and humidity (45-75 %) was maintained throughout the course of study. Rice husk was used as bedding material. Throughout the experiment, the animals were provided standard pellet diet (M/S Pranav Agro Industries Ltd. Baroda, India.) and water *ad libitum.* Essential cleanliness conditions were also maintained. All animals were observed daily for any abnormal physical or behavioral changes.The body weight of each rat was recorded one day before initiation of treatment (Day 0) and at weekly intervals throughout the period of study. At the day 29^{th} of study animals were sacrificed by decapitation method and gross lesions were noted and tissues obtained for histological evaluation included liver, lung, heart, kidney, brain, stomach, spleen, intestine and

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ovary subsequently preserved in 10 per cent neutral buffered formalin for at least 24-48 hours. Further these tissues were processed by routine method of dehydration in graded alcohol, clearing in xylene and embedding in paraffin. Sections of 5-6 µ thicknesses were prepared and processed by routine Hematoxylene and Eosin method to study the general histopathological alterations (Luna, 1968)

RESULTS AND DISCUSSION

No mortality was observed in any rats through the study period. The clinical symptoms were characterized by decreased physical activity, dullness, depression, piloerection, shivering, sweating, salivation and reduced body weight in Group C and D rats. There was significant ($P < 0.05$) reduction in the body weight at 28th day post treatment in Group B, C and D rats when compared with Group A control rats. Almost similar clinical signs were also observed in the chlorpyrifos treated rats were also earlier reported by Ambali *et al*., (2007) in mice treated with CPF (21.3 mg/kg b. wt.) at 7th and 9th weeks of dosing. Similarly, Akhtar *et al*., (2009) also observed piloerection, reduced body weight and diarrhea in rats @ 9 mg/kg b. wt for 90 days.

There was no significant reduction in body weight of female rats up to 14th day. The significant ($P < 0.05$) decrease in body weight was observed in rats of Group B and Group D on 21st day and 28th day onwards as compared with the control group. The most significant ($P < 0.05$) dose dependant reduction in the body weight at 28th day post treatment was observed in Group D followed by Group C and Group B rats when compared with Group A control rats.(Table - I). The significant decrease in body weight gain in rats belonging to high dose group was also reported earlier by Akhtar *et al*., (2009) on administration of CPF orally to male Wistar rats @ 3, 6 and 9 mg/kg b. wt. for 90 days. Almost similar findings were reported by Ambali *et al*., (2010^{b}) on administration of CPF (42.5 mg/kg b.wt.) alone and along with vitamin C (100 mg/kg b.wt.) in Wistar rats. Significant decrease ($p \leq 0.01$) in body weight was also

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observed by Mansour and Mossaa (2010) on administration of CPF orally @ 6.75 mg/kg b. wt. in male and female rats for 28 consecutive days. Anorexia and general weakness of the animals may be the reason for weight loss in animals exposed to high dose of chlorpyrifos.

Table 1. Effect of Chlorpyrifos on weekly body weight in rats of different experimental groups (Values are Mean ± S.E., n=6 rats)

Day	Group-A Control	Group-B Low dose	Group-C Mid dose	Group-D High dose
0 day	220.83±6.508^{a}	218.33±5.270^{a}	221.50±5.942^{a}	219.16±6.635^{a}
7th day	227.83±5.856^{a}	224.16±4.190^{a}	224.50±5.590^{a}	221.50±5.469^{a}
14th day	237.00±6.454^{a}	226.00±4.912^{a}	224.66±5.577^{a}	217.00±5.354^{a}
21st day	242.66±6.227^{a}	**223.00±4.725^{b}**	**218.33±5.806^{ab}**	**209.00±5.573^{b}**
28th day	246.83±6.326^{a}	**219.83±4.519^{b}**	**211.16±5.844^{c}**	**198.66±6.162^{c}**

Mean with similar superscripts in row do not differ significantly (P < 0.05).

Histopathological changes were observed in liver, kidney, brain, ovary, fallopian tube, lung and spleen. The lesions were marked and pronounced in Group D rats, whereas, Group C rats showed mild to moderate pathological changes. Liver showed sinusoidal congestion, bile duct proliferation, focal hepatic necrosis and portal vein congestion in Group C and D(Fig- 1) . In kidney, severe intertubular hemorrhages, tubular degeneration and shrinkage in size of glomeruli with wide Bowman's capsular space was observed in Group D rats receiving the high dose of CPF(Fig- 2,3). Brain showed cytoplasmic vacuolation, neuronal degeneration and partial blockage of blood capillary with perivascular oedema and swollen endothelial cells of capillary(Fig- 4). In ovary, hemorrhages, epithelial damage, degenerating ova along with Graffian follicle and corpus luteum was observed (Fig- 5). Graffian follicle showed clustered nuclei of granulosa cells in Group C and D rats. While fallopian tube showed varying degree of damage to mucosal folds in Group C and D rats. In spleen of Group D rats found depletion of lymphoid

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cells in germinal center. Lung showed emphysema, thick interalveolar septa, congestion of alveolar capillaries and hemorrhages in Group C and D rats. Almost similar pathological changes were observed by many workers after administration of chlorpyrifos. Glomerular shrinkage and widened urinary space of Bowman's capsule reported by Surana *et al.,* (2008) in Quinalphos (QP) toxicity at the dose of 14 mg/kg body weight in male wistar rats in 15 days study. El-Hossary *et al*., (2009[b]) also observed shrunken glomeruli surrounded by wide Bowman's space in chlorpyrifos toxicity study in rats. Partially occluded blood capillary with slight perivascular edema, neuronal degeneration were reported by El-Hossary *et al*., (2009[a]). Extravasations of RBCs with perivascular edema, degeneration and vacuolation was reported by El-Hossary *et al*., (2009[c]) in chlorpyrifos toxicity as a single oral dose of 63 mg/kg in rats. Madhavi and Kumar (2010) also found vacuolated spaces in Matured graffian follicle with clustered nuclei of granulosa cells and degeneration in ova in 21 days study of chlorpyrifos in mice. Madhavi and Saraswathi (2011) also reported many vacuolated spaces, degenerated ova in different stages, ruptured epithelium and degenerated corpus luteum with matured graffian follicle at periphery in ovary.

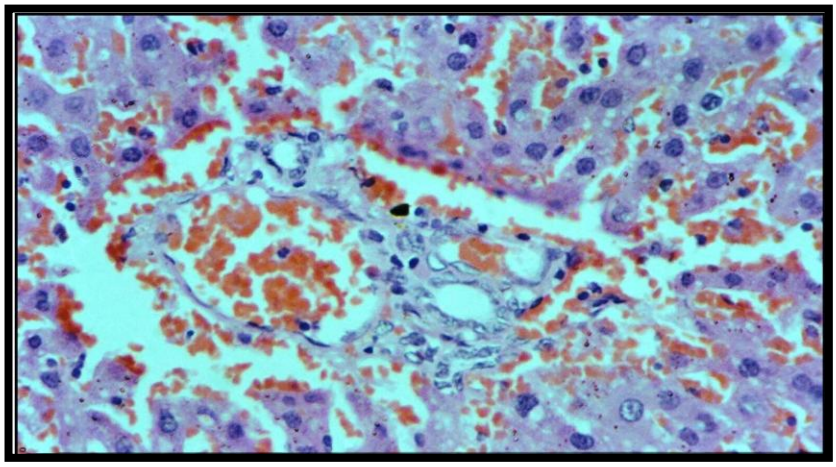

Fig.1-Group-D-Photomicrograph of liver showing portal vein congestion and sinusoidal Congestion (H.E.x 400)

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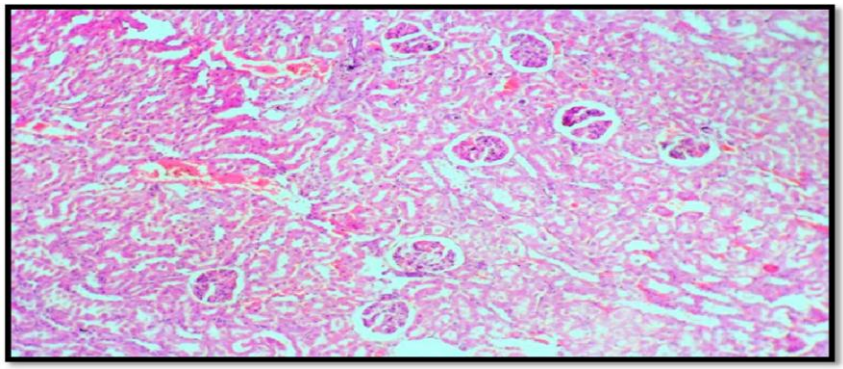

Fig. 2-Group -D-Photomicrograph of kidney showing shrunken Glomeruli with wide bowman's capsule and hemorrhages (H.E.x 100)

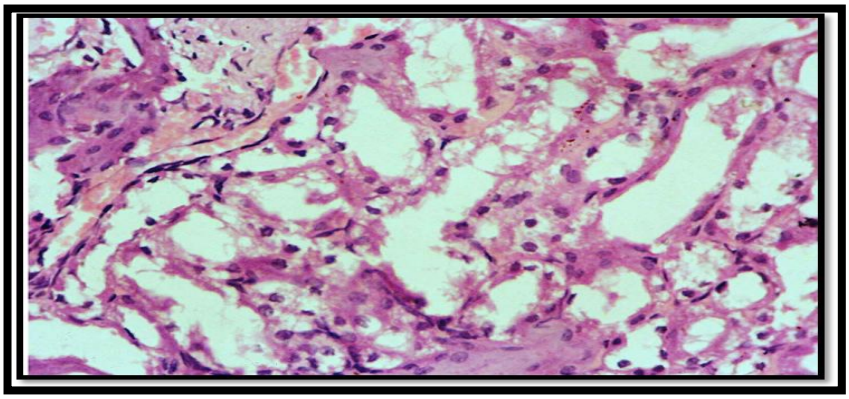

Fig.3-Group-D-Photomicrograph of kidney showing severe tubular degeneration with hyaline casts and vacuolation (H.E.x 400)

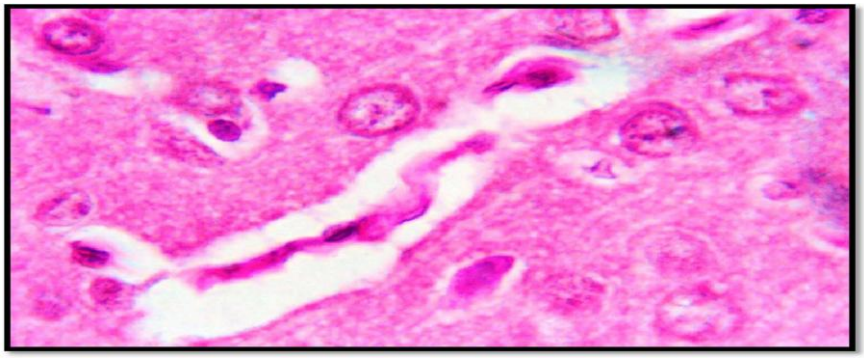

Fig.4-Group-D-Photomicrograph of brain showing neuronal degeneration, cytoplasmic vacuolation and necrosis (H.E.x 400)

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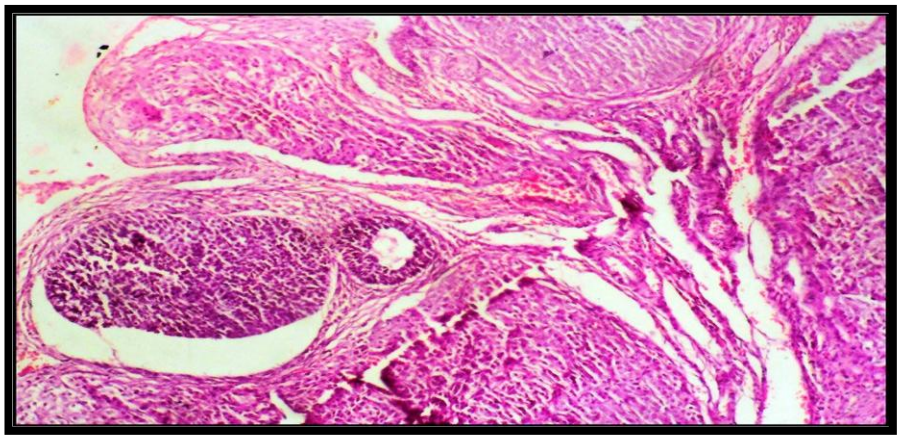

Fig.5-Group-D-Photomicrograph of ovary showing increased vaculolation , degeneration and hemorrhages (H.E.x 100)

CONCLUSION

Oral administration of chlorpyrifos at the dose of 16 mg/kg for a period of 28 days induces toxicity symptoms like decreased physical activity, dullness, depression, piloerection, shivering, sweating and salivation. There was dose dependent significant reduction in body weight in group b, c and d as compared to group a control rats. Pathomorphological examination of different tissues revealed moderate to severe degenerative, vascular and necrotic changes especially in liver, kidney, brain and ovary of group d rats.

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REFERENCES

Akhtar, N., Srivastava, M. K. and Raizada , R. B. (2009) Assessment of chlorpyrifos toxicity on certain organs in rat, Rattus norvegicus. *Journal of Environmental Biology*, **30** (6): 1047-1053.

Ambali, S., Akanbi, D., Igbokwe., N., Shittu., M. Kawu, M.and Ayo, J. (2007) Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of Vitamin C. *The Journal of Toxicological Sciences*. **32**(2): 111-120.

Ambali, S. F., Onukak, C., Idris, S. B., Yaqub, L. S., Shittu, M., Aliyu H. and Kawu M. U. (2010[b]) Vitamin C attenuates short-term hematological and biochemical alterations induced by acute chlorpyrifos exposure in Wistar rats. *Journal of Medicine and Medical Sciences*, **1**(10): 465-477.

Asperlin, A. (1994). Pesticide industry sales and usage-1992 and 1993 market estimates. U.S. Environmental Agency report no. 733-K-94-001.

El-Hossary, G. G., Mansour S. M. and Mohamed A. S. (2009[a]) Neurotoxic Effects of Chlorpyrifos and the Possible Protective Role of Antioxidant Supplements: an Experimental Study. *Journal of Applied Sciences Research*. **5**(9): 1218-1222.

El-Hossary, G. G., El-Gohary, A A., Ahmed, N. S., Mohamed A. S. and Mansour S. M. (2009[b]) Amelioration of Chlorpyrifos Induced Retinal and Renal Toxicity by Vitamin D3. *Australian Journal of Basic and Applied Sciences*, **3**(3): 2304-2314.

El-Hossary, G. G., Mansour, S. M. and Mohamed, A. S. (2009[c]) Neurotoxic Effects of Chlorpyrifos and the Possible Protective Role of Antioxidant Supplements: an Experimental Study. *Journal of Applied Sciences Research*. 4(7).

Forget, G. (1991). Pesticide and the third world. *J. Toxicol. Environ. Hlth*., 32, 11-31

Gordon, C.J., A.G. Tholeatheus and Y. Ying.(1997). Hypothermia and delayed fever in male rats exposed to chlorpyrifos. *Toxicol.,* 118, 149-155.

Kamrin, M.A. (1997). Organophosphates In Pesticide profile: Toxicity, environmental impact and fate, *CRC Lewis Press, Michigan.* pp. 135-240

Madhavi K. and Kumar R. (2010) *In vivo* toxicological evaluation of organophosphate pesticide on female albino mice: therapeutic effects of curcumin. *International Journal of Pharmaceutical Sciences and Research*, **1** (9).

Madhavi K. and Saraswathi V. S. (2011) In vivo toxicological evaluation of chlorpyrifos pesticide on female albino mice: therapeutic effects of curcuma longa. *International Journal of Pharmaceutical Sciences and Research*, **2**(2): 439-447.

Mansour, S. A. and Mossa A. H. (2010) Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. *Pesticide Biochemistry and Physiology*. **96**: 14-23

Luna, A. G. (1968). Manual of histological staining methods of the armed forced institute of pathology, 3rd Edn. McGraw Hill book Co, London, Pp.124-125.

Sultatos, L.G. (1994). Mammalian toxicology of organophosphorous pesticides. *J. Toxicol. Environ. Hlth.,* 43, 271-289

Surana, B., Mehta, J., Seshadri, S. (2008).Toxicological Effects of Quinalphos and its Subsequent Reversal by Using Root Extract of *Withania somnifera* and Leaf Pulp of *Aloe barbadensis. Journal of the Indian Society of Toxicology*: **4**(2).