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RESEARCH ARTICLE

Chlorpyrifos-induced hematological, biochemical and histoarchitectural alterations in albino mice

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Abbreviations:

CPF: chlorpyrifos, AChE: acetylcholinesterase ALT: Alanine aminotransaminase, AST: Aspartate aminotransaminase, LDH: Lactate dehydrogenase, TLC: Total leucocyte count, Hb: Haemoglobin, PCV: Packed cell volume, L/N: lymphocytes/neutrophils, g: gram, b.w.: body weight

Abstract

Effect of 2 and 8 weeks exposure to sub-lethal doses of chlorpyrifos on haematological, biochemical and histopathological parameters in male swiss albino mice was evaluated. The animals were randomly divided into two groups I and II, of 25 animals each, given the pesticide treatment for 2 weeks and 8 weeks respectively. Both the groups were further divided into 5 sub-groups (A, B, C, D and E). The sub-group A served as the control, and B, C, and D and E were treated with $LD_{50}/80^{th}$, $LD_{50}/40^{th}$, $LD_{50}/20^{th}$ and LD_{50} of chlorpyrifos, respectively. A significant dose dependent decrease in AChE, hemoglobin level, packed cell volume, L/N ratio and total leucocyte count and significant increase ALT, AST and LDH levels of exposed subjects were observed. As even the dose of $LD_{50}/80^{th}$ showed marked affect on the treated animals, this dose was further evaluated for its histopathological effects on liver, kidney, spleen and lymph nodes. Two weeks exposure to this dose led to mild diffused granular degeneration in liver and necrosis of renal tubular epithelium with mononuclear cell infiltration in kidney. Eight weeks exposure was marked with bile-duct proliferation, necrosis of hepatocytes and vacuolar degeneration in liver tissue; however regenerative attempts with normal tubular epithelium were seen in kidney. Marked hyperplasia of lymph nodes was observed in mice on 8 weeks exposure to this dose of chlorpyrifos. Thus, even the lowest tested dose posed marked alterations in the exposed animals.

1. Introduction

Organophosphate insecticides are one of the most widely used chemicals in agriculture and public health (Ambali et al., 2010). Chlorpyrifos, an organophosphate insecticide, induces neurotoxicity and tissue damage with observable signs of poisoning. The primary mechanism of toxicity is associated with its ability and especially that of its metabolite, CPF-oxon to inhibit acetylcholinesterase (AChE), an enzyme that normally terminates neurotransmission at cholinergic synapses (Eaton et al., 2008). Prolonged exposure to chlorpyrifos has been shown to cause anaemia (Ambali et al., 2009) and severe damage to the vital organs. In the present study, we investigated the chlorpyrifos-evoked haematological, biochemical and related histopathological alteration in albino mice upon 2 weeks and 8 weeks exposure have been correlated with the different dilutions of LD_{50} of chlorpyrifos administered.

2. Material and methods:

2.1 Procurement and oral administration of experimental animals

Two months old male swiss albino mice weighing 35.6g (average weight) were obtained from and housed in Small Animal Colony (SAC), Livestock Production and Management, Guru Angad Dev Veterinary and Animals Sciences University (GADVASU), Ludhiana. The animals were acclimatized for 10 days prior to the start of the experiment. The mice were provided with standard diet containing pelleted food and water *ab libitum*. The

experimental protocol met the National guidelines on the proper care and use of animal in the laboratory research. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC). The animals were randomly divided into two groups (I and II) of 25 animals each. Both the groups were further divided into 5 sub-groups (A, B, C, D and E) of 5 animals each. Group I and group II were orally administered with the pesticide for 2 weeks and 8 weeks, respectively. The sub-group A was the control; groups B, C, D and E were treated with $LD_{50}/80^{th}$, $LD_{50}/40^{th}$, $LD_{50}/20^{th}$ and LD_{50} of chlorpyrifos, respectively. LD_{50} of chlorpyrifos of 60 mg/kg bw was confirmed taken into consideration for calculation of various sub-lethal doses (Gosselin, 1984). Chlorpyrifos (Dursban 20% EC) was obtained from entomology farm, Punjab Agricultural University, Ludhiana.

2.2 Haemto-biochemical evaluation

At the end of the experiment after 2 and 8 weeks, blood from retro-orbital venous plexus of the animals was collected in vials containing heparin (10 IU/ml) and centrifuged at 1500 rpm for 15 min for plasma separation for Acetylcholinesterase (Knedel and Klin. 1967), Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST), and Lactate dehydrogenase (LDH) determination (Anon., 1976). The animals were sacrificed; the weight of liver and kidney of all the treated animals was recorded. White blood count was done as per procedure given by Jain (1986). Hemoglobin (Hb) content of blood was estimated by Sahli's haemoglobinometer [8] and Packed cell volume (PCV) by microhaematocrit method (Coles, 1986).

2.3 Histopathological studies

For histopathology, liver, kidney, spleen and lymphnode tissues were collected at necropsy in 10% neutral buffered formalin. Thin sections (5 µ) after routine hematoxylin and eosin staining were examined for histopathological changes (Luna, 1968).

2.4 Statistical analysis

Data is expressed as Mean ± SD and subjected to one-way analysis of variance (Singh, 1991).

3. Result and discussion

3.1 Effect of daily oral administration of Chlorpyrifos on biochemical parameters

Results of the effect of chlorpyrifos exposure on plasma enzyme profile of albino mice are shown in Table 1. The daily oral administration of chlorpyrifos led to a significant dose dependent inhibition of plasma acetylcholinesterase both upon 2 weeks and 8 weeks exposure. The synaptic concentration of acetylcholine builds up leading to hyper-excitation of the central nervous system occurs (Slotkin et al., 2008). Significant elevation was found in the level of ALT, AST and LDH, both in time and does dependent manner, more being in the group treated with higher does and exposed chronically (for 8 weeks). Results indicated that the administration of chlorpyrifos had necrotic effect on vital organs like kidney and liver, thus causing the leakage of enzymes into blood. ALT is the marker of liver function, increase in the activity of which indicates hepatocellular degeneration and AST, being organ specific, marks the degenerative changes occurring in chlorpyrifos exposed animals. A similar increase in AST and ALT was observed in goats (Kaur et al., 2000) and rats (Krishna et al., 2009; Wang et al., 2009) exposed to chlorpyrifos. Studies with other insecticides also showed similar trends previously in chicks exposed to fenvalerate (Singh et al., 2001), poultry exposed to deltamethrin (Jayasree et al., 2003), and dwarf bucks exposed to cypermethrin (Khan et al., 2009). Increase in LDH level indicates tissue damage. Tissue necrosis attributed to increased levels of lactate dehydrogenase in blood sera has been reported due to various chemicals (Asztalos and Nemcsók, 1985). Significant increase in LDH activity was reported in gill and brain of tilapia exposed to organophosphates (Rao, 2006) and in broiler chicks exposed to deltamethrin (Jayasree et al., 2003).

3.2 Effect of daily oral administration of Chlorpyrifos on haematological parameters

Results of the effect of chlorpyrifos on lymphocyte-neutrophil ratio, total leucocyte count, hemoglobin level, and packed cell count of albino mice are shown in table 2. The two weeks treatment of chlorpyrifos led to a dose dependent decrease in the lymphocyte-neutrophil ratio in all the treated groups, indicating a relative decrease in lymphocyte count and increase in neutrophil count whereas the decrease in this ration was significant ($P<0.05$) only in the group treated with $LD_{50}/20^{th}$ of CPF upon eight weeks exposure. Repeated CPF exposure has been seen to cause lymphopenic leucopenia. The lymphopenia observed in the CPF-exposed mice may be due to either the decreased production and/or increased rate of removal due to rapid destruction. The pesticides are toxic to the cells of immune system through the induction of necrosis and apoptosis (Ambali et al., 2010), which may be an outcome

of the oxidative stress provoked by chlorpyrifos. However, lymphoproliferative changes were seen in the animals exposure to sub lethal dose of the pesticide for eight weeks which may be attributed to the relative increase in the lymphocytes (Figure 3c). A significant decrease in hemoglobin level was observed in all the groups given CPF administration for both 2 and 8 weeks. Similar significant decrease was observed in the level of PCV and TLC in all the chlorpyrifos treated animals. The lower values of Hb and PCV observed in CPF treated animals indicate that the repeated exposure to CPF causes anaemia. The reason for anaemia in the CPF treated groups is not known. It may however be related to disruption of erythropoiesis or an increase in RBC destruction (Vural et al., 1986). A decrease in serum iron concentration which eventually results in reduced hemoglobin production has also been reported (Goel et al., 2006).

Table 1: Effect of daily oral administration of chlorpyrifos on acetylcholinesterase (AChE), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and lactate dehydrogenase (LDH) in albino mice

Groups	AChE (IU/L)		AST (IU/L)		ALT (IU/L)		LDH (IU/L)	
	2 weeks	**8 weeks**	**2 weeks**	**8 weeks**	**2 weeks**	**8 weeks**	**2 weeks**	**8 weeks**
Group A (control)	431.98 ±8.82	379.34 ±11.84	86.0 ± 1.529	90.67 ± 2.188	26.67 ± 1.203	24.67 ± 1.455	370.88 ±19.98	421.58 ±9.709
Group B ($LD_{50}/80^{th}$)	279* ±17.67	258* ±9.77	100.67* ± 3.26	121.67* ± 2.335	31.67* ± 0.667	33.67* ± 0.44	462.18 ±34.43	487.6 ±11.67
Group C ($LD_{50}/40^{th}$)	224.52* ±13.85	164.8* ±15.73	110.67* ± 3.65	135.67* ± 2.335	33.67* ± 0.356	43.33* ± 1.02	553.2 ±30.80	575.84 ±17.48
Group D ($LD_{50}/20^{th}$)	150.8* ±11.009	82.4* ±12.75	118.67* ± 4.58	145.67* ± 0.333	36.37* ± 0.334	47.33* ± 0.94	605.8 ±13.25	698.48 ±11.67
Group E (LD_{50})	69.6* ±16.71	-	164.0* ± 2.98	-	55.0* ± 2.646	-	842.2 ±32.04	

- The values are represented as Mean ± SE
- The values having superscript '*' refer to the values that differ significantly from the values of control (Group A) ($p \leq 0.05$)

Table 2: Effect of daily oral administration of chlorpyrifos on total leucocyte count (TLC), haemoglobin count (Hb), packed cell volume (PCV) and lymphocyte-neutrophil ratio (L/N ratio) in albino mice

Groups	TLC (cells/mm^3)		Hb (g%)		PCV (%)		L/ N ratio	
	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks
Group A (control)	3540 ±147.64	4028.4 ±133.11	13.4±0.25	12.78 ±0.23	40.62 ±1.54	37.04 ±5.13	3.47* ±0.54	2.59±0.39
Group B ($LD_{50}/80^{th}$)	2866.6* ±178.26	2976* ±243.26	10.34* ±0.77	12.52 ±0.19	32.08* ±1.68	36.98 ±1.04	1.45* ±0.27	2.03 ±0.145
Group C ($LD_{50}/40^{th}$)	2422 * ±164.07	2934.8* ±232.33	9.84* ±0.88	11.76* ±0.73	29.62* ±2.32	35.7 ±2.43	1.03* ±0.12	2.26 ±0.41
Group D ($LD_{50}/20^{th}$)	1514.2* ±121.65	2643.8* ±314.84	8.72* ±0.46	11.22* ±0.30	28.66* ±0.98	33.42 ±1.31	0.67* ±0.13	1.801* ±0.307
Group E (LD_{50})	828 * ±138.81	-	5.16* ±0.709	-	15.98* ±4.043	-	0.54* ±0.07	-

- The values are represented as Mean ± SE
- The values having superscript '*' refer to the values that differ significantly from the values of control (Group A) ($p \leq 0.05$)

Figure 1a: Liver of animal treated with $LD_{50}/80^{th}$ of chlorpyrifos for 2 weeks showing mild diffused granular degeneration (arrows) at 40X

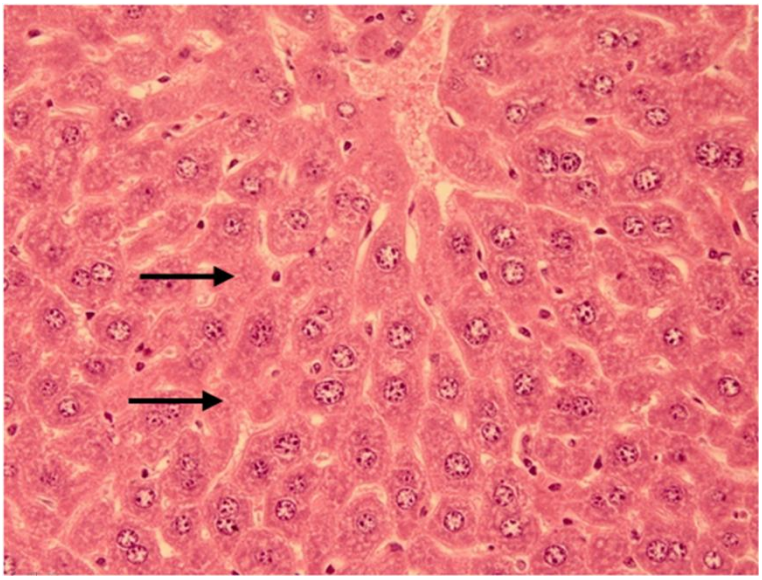


Figure 1b: Liver of animal treated with $LD_{50}/80^{th}$ of chlorpyrifos for 8 weeks showing necrosis (white arrow) and vacuolar degeneration (yellow arrow) at 20 X

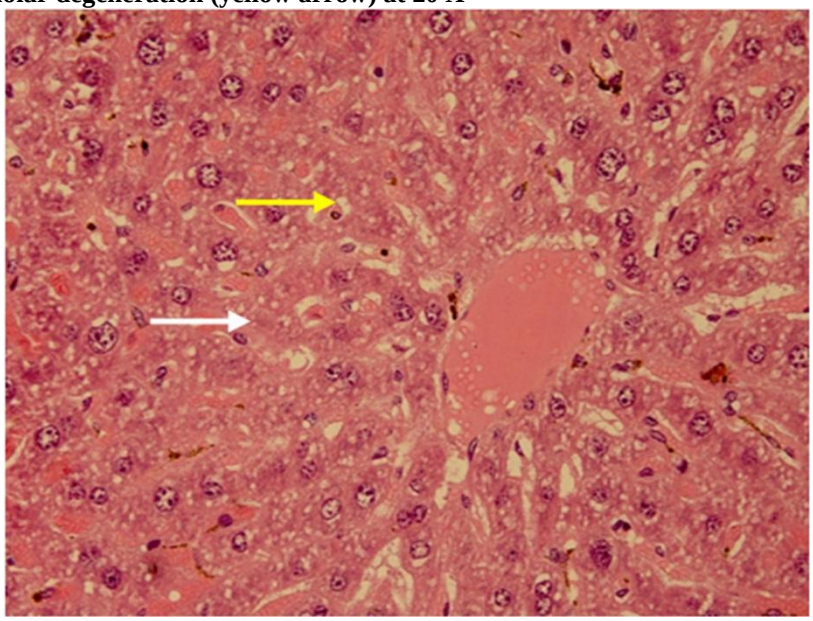


Figure 1c: Liver of animal treated with $LD_{50}/80^{th}$ of chlorpyrifos for 8 weeks showing bile duct proliferation (white arrow) and necrosis (yellow arrow) of hepatocytes at 40 X

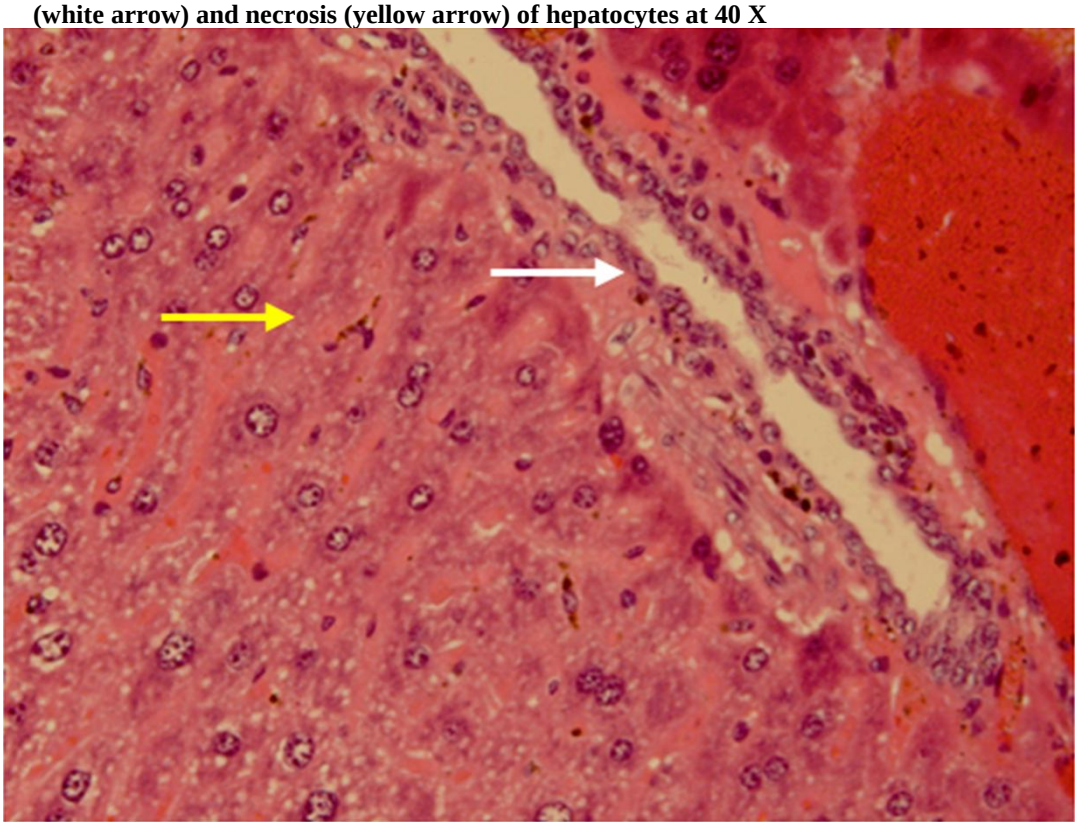


Figure 2a: Kidney of animal treated with $LD_{50}/80^{th}$ of chlorpyrifos for 2 weeks showing degeneration and necrosis of tubular epithelium (blue arrow) with mononuclear cell infiltration (black arrow) at 20 X

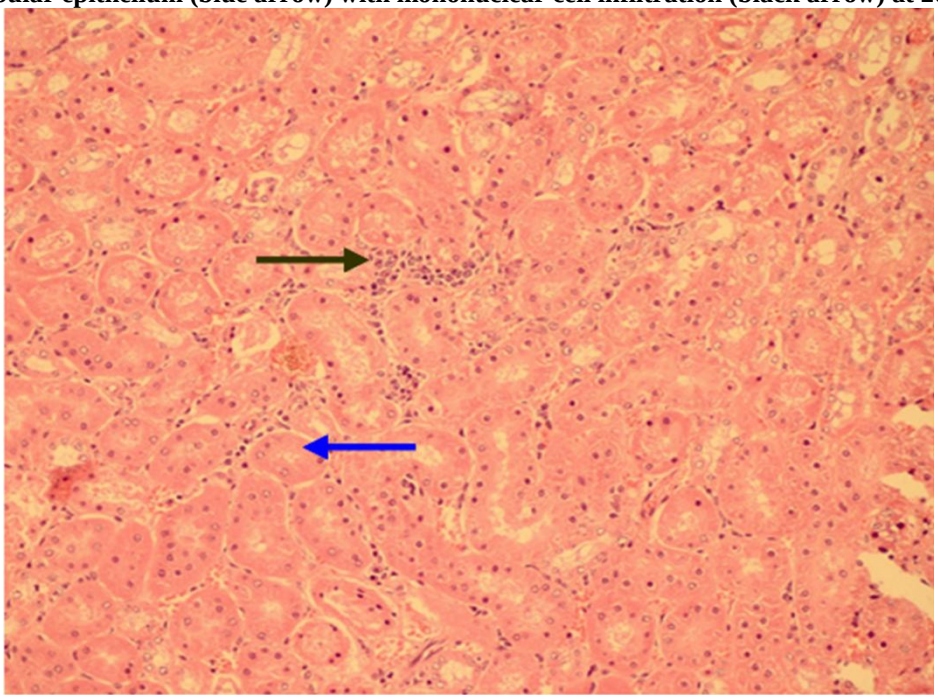


Figure 2b: Kidney of animal treated with $LD_{50}/80^{th}$ of chlorpyrifos for 2 weeks showing degeneration and necrosis of tubular epithelium (arrow) at 40 X

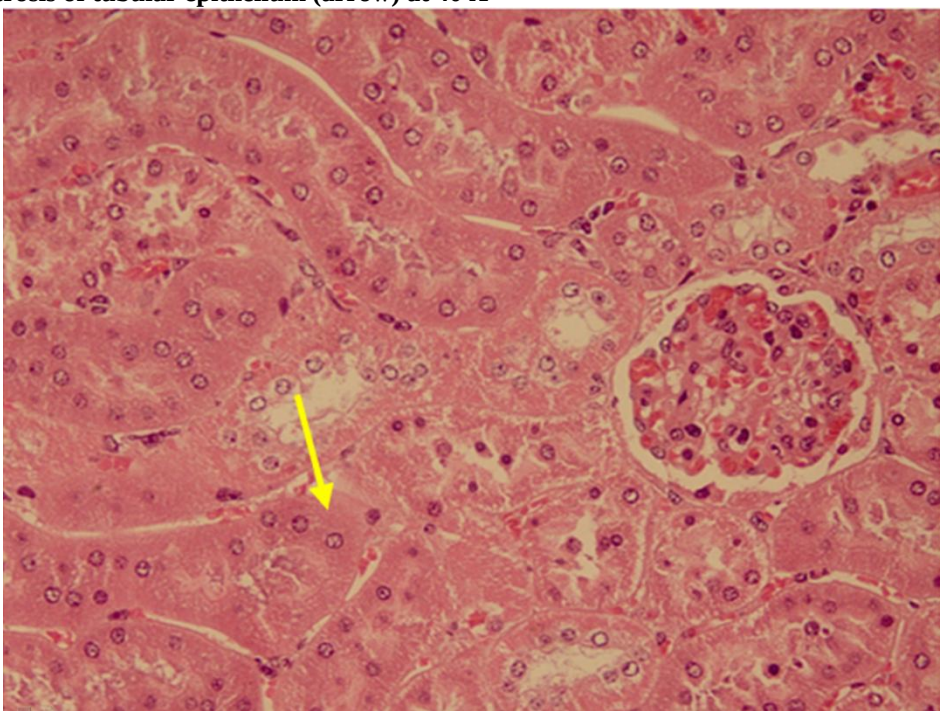


Figure 2c: Kidney of animal treated with $LD_{50}/80^{th}$ of chlorpyrifos for 8 weeks showing normal tubular epithelium with regenerative attempts (arrows) at 20 X

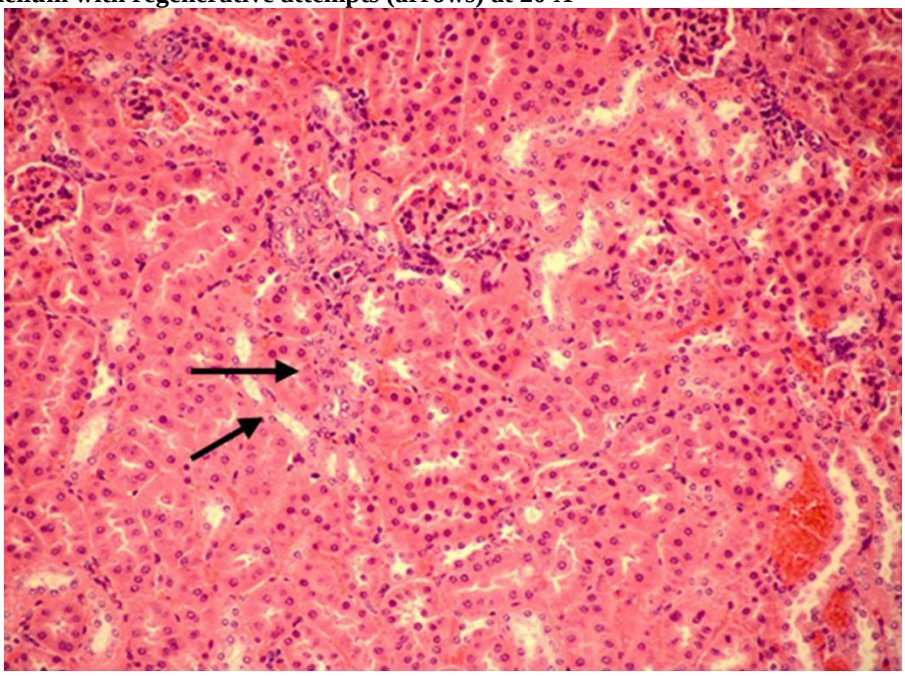


Figure 3a: Lymph node of animal treated with $LD_{50}/80^{th}$ of chlorpyrifos for 8 weeks showing hyperplasia of lymphoid tissue (arrow)

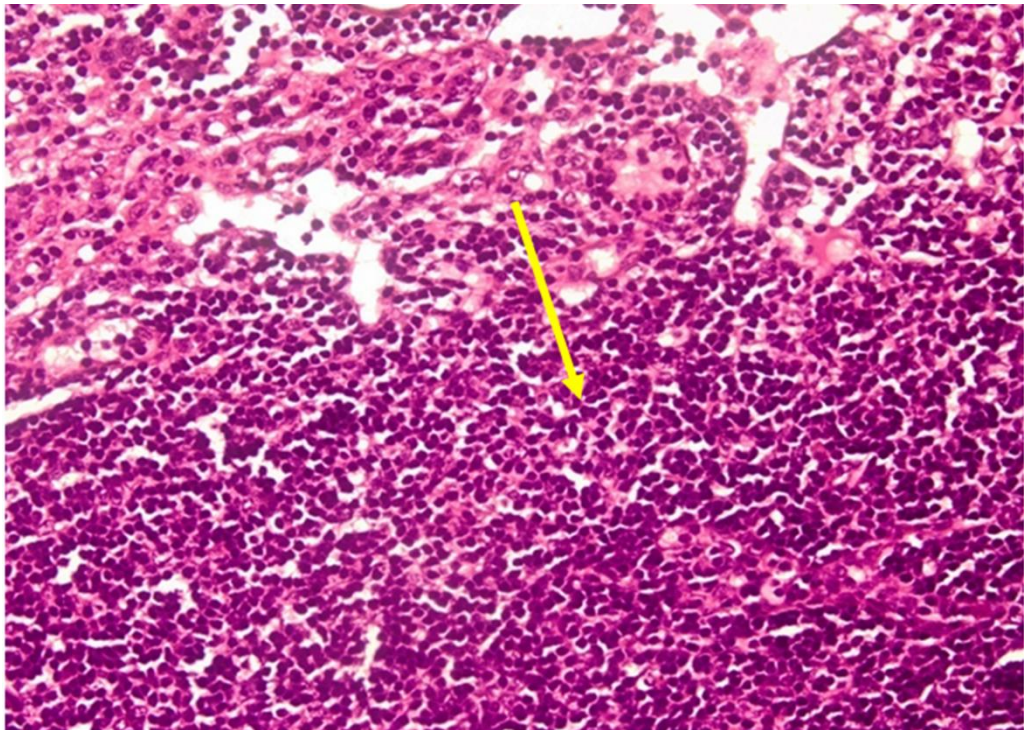
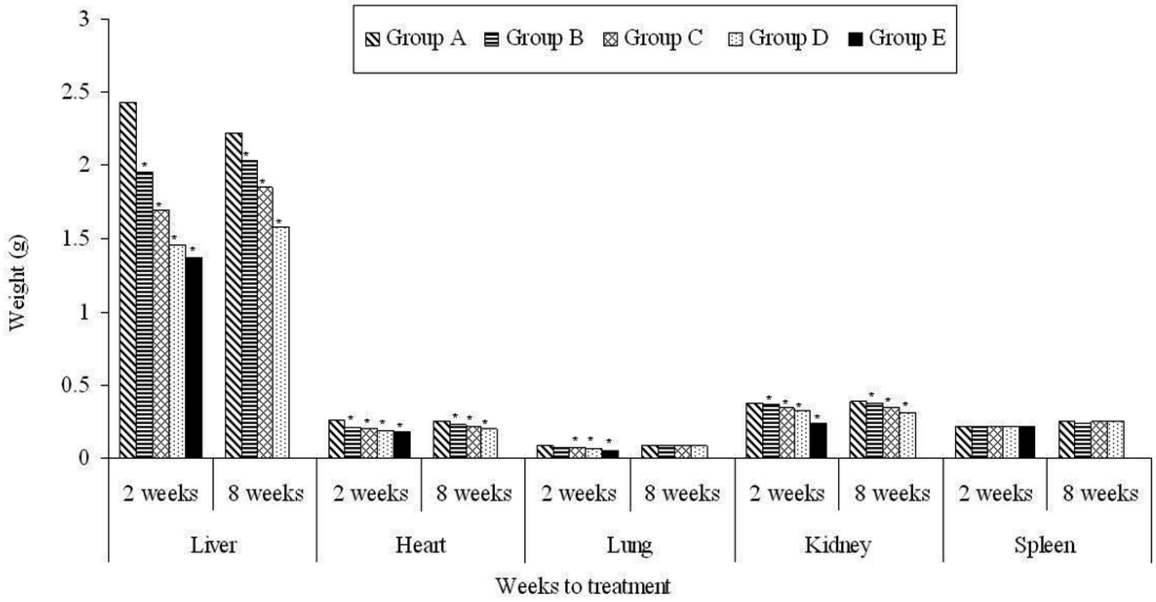


Figure 4: Effect of daily oral administration of chlorpyrifos on the weight of vital organs of albino mice



3.3 Effect of daily oral administration of Chlorpyrifos on histopathological parameters

From the findings of biochemical and haematological analysis, it was drawn that even the dose as low as $LD_{50}/80^{th}$ of CPF has toxicological effect thus increasing the level of ALT, AST and LDH in plasma. Histopathological changes in kidney and liver tissues of the mice treated with this dose were in accordance. Liver of animals treated for 2 weeks showed mild diffused granular degeneration (Figure 1a) and that of animals treated for 8 weeks showed necrosis and vacuolar degeneration, bile duct proliferation and necrosis of hepatocytes (Figure 1b and 1c). These changes indicated that the degeneration of liver increased with the time of exposure. This can also be corroborated with time dependent increase in the liver function enzymes. Histopathological changes observed in liver indicated time-related degeneration of liver upon exposure to $LD_{50}/80^{th}$ of chlorpyrifos for two and eight weeks. This fact was also depicted by the increasing AST and ALT level, which are the markers of liver function. It is reported that chlorpyrifos laid oxidative stress and tissue damage in the liver, kidney, brain and foetus in pregnant rats (Goel et al., 2006). Kidney tissue of the animals treated for 2 weeks with $LD_{50}/80^{th}$ of chlorpyrifos showed degeneration and necrosis of renal tubular epithelium with mononuclear cell infiltration and congestion of glomeruli (Figure 2a and 2b) and that of animals treated for 8 weeks showed regenerative attempts with normal tubular epithelium (Figure 2c). The regenerative attempts shown by the animals indicated an adaptive response of the exposed animals to the insecticide toxicity. Necrotic changes in kidney tissue; chronic glomerulonephritis, glomerulosclerosis, odenoma of glomerulus, in rats exposed to endosulfan have been reported (Zama et al., 2007). Increase in LDH level showed that tissue damage had taken place. Difference in increase in LDH activity after two and eight weeks of treatment was insignificant, thereby indicating time-independent tissue degeneration with some repair taking place alongside. Insecticide treated fishes showed degenerative changes in liver tissues **(**Choudhary et al., 2003**)**. Biliary hyperplasia was observed at certain regions of the hepatic tissue. This might be indicating the regenerating hepatic cells to withstand the toxic stress condition. Degenerative changes in liver and kidney in fishes exposed to deltamethrin (Radhaiah and Jayantha, 1992) and in rats exposed to chlorpyrifos and carbaryl alone or in combination (Krishna et al., 2009) have been observed. Alterations of hepatic pathology after 8 weeks of treatment in intoxicated rats were also reported (Yildirim et al., 2006). The histopathology of lymph-nodes showed hyperplasia of lymphoid tissue (Figure 3a) after eight weeks of chlorpyrifos administration. However, the histopathology of spleen showed not significant alteration in animals exposed for two or eight weeks.

3.4 Effect of daily oral administration of Chlorpyrifos on weight of vital organs

The effect of various sub-lethal doses of CPF on the weight of vital organs is depicted in Figure 4. A dose dependent decrease in weight of liver of the animals exposed to CPF for two weeks was correlated with the

histopathological changes seen in the liver autopsy. Decrease in weight of heart of the exposed animals indicated that CPF induced cardiopathy. In post mortem examination also, a dose-dependent myocardial haemorrhages were observed.

A significant dose dependent decrease in weight of lung was seen in two weeks treated animals; however the decrease in eight weeks treatment was insignificant. It indicates that the degeneration of lung tissues may be more in two weeks treated animals. Decrease in the lung weight may be due to alveolar degeneration; shrinkage in lungs was seen in dose dependent manner (as seen in post mortem examination)

Significant decrease was seen in the weight of kidney as also attributed to the histopathological degenerations. Spleen showed a relative increase in the weight in eight weeks treated animals as compared to two weeks treated animals. This may indicate that chlorpyrifos has immunosuppressive effect at two weeks treatment. Relative increase in spleen weight must have taken place in response to this suppression. Cumulatively, the exposed animals showed an adaptive response by increase in weight of vital organs in 8 weeks exposure which functions to detoxify the xenobiotic.

It has been reported that the body weight and organ/body weight ratios remained unaffected in male Fisher-344 rats exposed to chlorpyrifos (Blakley et al., 1999). However, a dose dependent decrease in weight of thymus and spleen in rats exposed to chlorpyrifos has been observed (Ehrich et al., 2004). Increase in relative liver weight in rats exposed to chlorpyrifos-methyl (Kang, 2004) and malathion and spinosad (Mansour, 2008) has been reported.

In conclusion, the study has shown that both 8 weeks and 2 weeks administration of sub lethal dose of chlorpyrifos as low as $LD_{50}/80^{th}$ causes an alteration in histopathological, biochemical, haematological parameters indicating degenerative changes leading to damages to the vital organs along with relative decrease in weights of almost all vital organs.

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