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RESEARCH ARTICLE

CHLORPYRIFOS INDUCED HISTOLOGICAL AND BIOCHEMICAL CHANGES IN THE LIVER AND KIDNEY TISSUES OF FEMALE WISTAR RATS

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Abstract

Chlorpyrifos (CPF) is a widely used organophosphorous insecticide in agricultural and domestic platforms. In our previous studies we observed a remarkable toxicity of CPF in the reproductive organs of female Wistar rats. To study effect of CPF on liver and kidney two groups of Wistar albino rats were dosed orally for 8 weeks with chlorpyrifos in vegetable oil (0.1mg/kg bw and 2.5mg/kg bw) and control group was given only vegetable oil. A significant ($P<0.05$) increase in total plasma proteins in rats treated with higher dose (2.5 mg/kg bw) was observed. A non-significant increase in liver total proteins was observed in treated rats as compared to control but there was a significant ($P<0.01$) decrease in the liver glycogen valuein higher dose (2.5 mg/kg bw) group. No significant change in blood urea value was found in the treated rats as compared to control. A significant ($P<0.01$) increase in liver alanine aminotransferase and lactate dehydrogenase enzyme activity was observed in both the treated groups as compared to control.Degenerative histological changes like leucocyte infiltration, proliferation in Kupffer cells, dilated blood sinusoids and cytoplasmic vacuolization in liver and kidney were observed in the treated rats. The results show that the sub-chronic exposure of CPF affects liver and kidney adversely.

INTRODUCTION

Pesticides constitute a diverse class of chemicals extensively used for prevention of harmful effects caused by pests. Among the large number of different pesticides those that are of particular concern are organophosphate (OPs). Due to their potential for short and long term hazardous effects, continuous biomonitoring of the levels of pesticides and their metabolites in humans is an essential step towards the evaluation of risk assessment and the prediction of adverse health effects in populations with either occupational or background environmental exposure to pesticides (Kavallakis and Tsatsakis, 2012, Tsatsakis et al., 2012).

Pesticide applications to the home can result in both dermal and respiratory exposure. Injudicious use of these compounds often leads to the presence of pesticide residues in harvested produce in amounts exceeding prescribed maximum residue limits (MRL), which may pose serious health risks(Szpir, 2006; FSSAI, 2009). There is a sequential rise in the production and consumption of pesticides in India during last three decades. The consumption pattern of pesticides in India differs from rest of the world, 76 per cent of total pesticide consumption in India as against 44 per cent worldwide. Of the total pesticide usage 45 per cent goes to cotton crop followed by paddy and wheat, and 10–12 per centof total pesticides are used for fruits and vegetables (Kumari et al., 2003; Bhattacharyya et al., 2009).

Chlorpyrifos (CPF) is a widely used, broad spectrum and moderately toxic OP insecticide used for agricultural and domestic platforms (EPA, 2008). Although EPA has imposed restrictions on the use of CPF on agricultural crops

(EPA, 2000), it is still highly used in the agricultural crops (Donaldson et al., 2002).Chlorpyrifos has been found to disturb biochemical and physiological functions of red blood cells (RBC) through lipid peroxidation (Ambali, 2011a, Nishi and Hundal, 2013). A non-reparable DNA strand breakage in mouse lymphocytes after chlorpyrifos exposure has been observed (Cui et al., 2011). Chlorpyrifos has been found to be related to reproductive and developmental defects (Adigun et al, 2010; Shalaby et al., 2013). In our previous study we observed an increased reproductive damage in female Wistar rats at doses equivalent and higher to NOEL level (Nishi and Hundal, 2013). The present study was designed to determine the toxicity of CPF on liver and kidney.

1. Material and methods

1.1 Experimental animals

Healthy adult female Wistar albino rats of 12 weeks of age and weighing between 140-160 g were procured from Department of Livestock Production and Management, Guru AngadDev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Animals were maintained at the Animal House in the Department of Zoology, Punjab Agricultural University, Ludhiana in polypropylene cages under controlled conditions (23±2ºC temperature; 40±5% relative humidity). Water and standard pelleted feed were provided *ad libitum*. Rats were acclimatized to the laboratory environment for 15 days prior to the start of experiments. TheInstitutional Animal Ethics Committee (IAEC) approved this experimental protocol (VPS/2008/874-885).

1.2 Chemicals and experimental designs

Commercial grade Chlorpyrifos (20% EC, Eldrin, Tc) was purchased from Crystal Phosphate Limited, Nathupur, Sonepat, Haryana, India. Different dilutions for the doses of the insecticide to be administered were made with vegetable oil. The doses (0.1mg/kg bw and 2.5mg/kg bw) selected were based on our previous studies in which a toxic effect of CPF was observed on the reproductive organs of female Wistar rats (Nishi and Hundal, 2013). The study was conducted for 8 weeks to determine sub-chronic toxicity of CPF on liver and kidney of female Wistar rats.

The rats were divided into three groups of four animals each. Two groups were given chlorpyrifos at a dose level of 0.1mg/kg bw (T1) and 2.5 mg/kg bw (T2) for eight weeks on daily basis by oral intubation. Same amount of vegetable oil i.e. 1.25ml/kg was given to the control group (C) orally through intubation. The animals were sacrificed on the completion of the experiment. Liver and kidney of the rats were collected, weighed and processed for analysis.

2.3 Biochemical assays

Total proteins were estimated in liver and plasma by method of Lowry et al., (1951). Glycogen content in the liver tissues was estimated by the method of Morales et al., (1973).Aminotransferases in liver were estimated by the method of Reitman and Frankel as described by Bergmeyer (1974).Activity of Lactate dehydrogenase in liver was estimated by the method of King (1965). Urea content in plasma was estimated by the method of Levine (1961).

2.4 Histological analysis

For histological analysis the liver and kidney were quickly removed from the experimental animals, washed in 0.9 per cent (w/v) cold normal saline, pat dried and weighed on an electrical balance. After completing fixation in Bouin's fixative for 24 hours, the tissue was dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax (58-60ºC). The 5µm thick sections were cut serially with the help of microtome and after usual de-waxing followed by rehydration in descending series of ethanol to water, the sections were stained with haematoxylin, counter stained with eosin, dehydrated in ascending ethanol series, cleared in xylene and mounted in DPX. Stained tissues were processed for histological analyses (100X/400X) under light microscope (Olympus CH20i attached with Magnüs Micro Image Projection System, New Delhi, India).

2.5 Statistical analysis

Values obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by paired t-test, using GraphPad Prism version 6 from GraphPad Software, San Diego, CA, USA (www.graphpad.com). All the parameters were compared at 5% level of significance.

3. Results

No significant changes in the feed intake, body weight and organ (Liver and kidney) were observed in treated and control rats.

3.1 Biochemical

Total plasma proteins were increased in both the treated groups in a dose dependent manner (Table 1). But the increase was statistically significant in T2 group. No significant difference in blood urea level was observed in both the treated and control groups. There was a non-significant increase in liver total proteins in treated rats as

compared to control (Table 2). No significant change in blood urea value was found in the treated rats as compared to control. A significant decrease in the liver glycogen value was observed in T2 group (Table 2). Also, a non-significant decrease in glycogen was observed in T1 group. A remarkably significant increase in the Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) activities was observed in both the treated groups (Table 2). There was no significant change in the Aspartate aminotransferase (AST) enzyme activity.

3.2 Histological analysis of liver

Liver tissue microscopic analysis showed presence of pyknotic nuclei (necrosis) which indicates CPF induced injury. Leucocyte infiltration, proliferation in Kupffer cells, dilated blood sinusoids and cytoplasmic vacuolization were observed in the treated rats as compared to control (Figure I, A-F).

3.3 Histological analysis of kidney

In the present study the changes noticed in CPF treated rat kidney were mainly the shrinkage of glomerulus at initial stage of treatment, degeneration of glomerulus and renal tubules, vacuolization of renal tubules, deposition of eosin-positive substances in the glomeruli and renal tubules, and infiltration of leucocytes in rats treated with CPF as compared to control (Figure 2, A-C). Kidney sections of control rats showed normal histoarchitecture of the glomeruli and renal tubules.

Table 1 Biochemical parameters in plasma

Treatment	Total protein (g/dL)	Blood Urea Value (mg/dL)
T1	8.584±0.370	16.193±0.350
T2	8.931±0.393*	16.058±0.144
Control	7.126±0.506	17.311±0.393

All the values are Mean ± SE values of 4 animals in each groups.
Values are significant at * P<0.05, ** P<0.01

Table 2 Biochemical parameters in liver

Treatment	Total protein (mg/g of liver)	Glycogen (mg/g of liver)	AST (µmol/g of liver)	ALT (µmol/g of liver)	LDH (µmol/min/g of liver)
T1	8.095±0.338	10.909±0.824	15.365±1.510	17.614±0.844***	119.23±12.463**
T2	8.168±0.34	8.329±0.545**	16.030±0.536	22.917±1.079***	110.58±12.099**
Control	7.282±0.204	12.000±0.273	12.106±1.033	10.871±0.317	44.231±5.088

All the values are Mean ± SE values of 4 animals in each groups.
Values are significant at * P<0.05, ** P<0.01, *** P<0.001

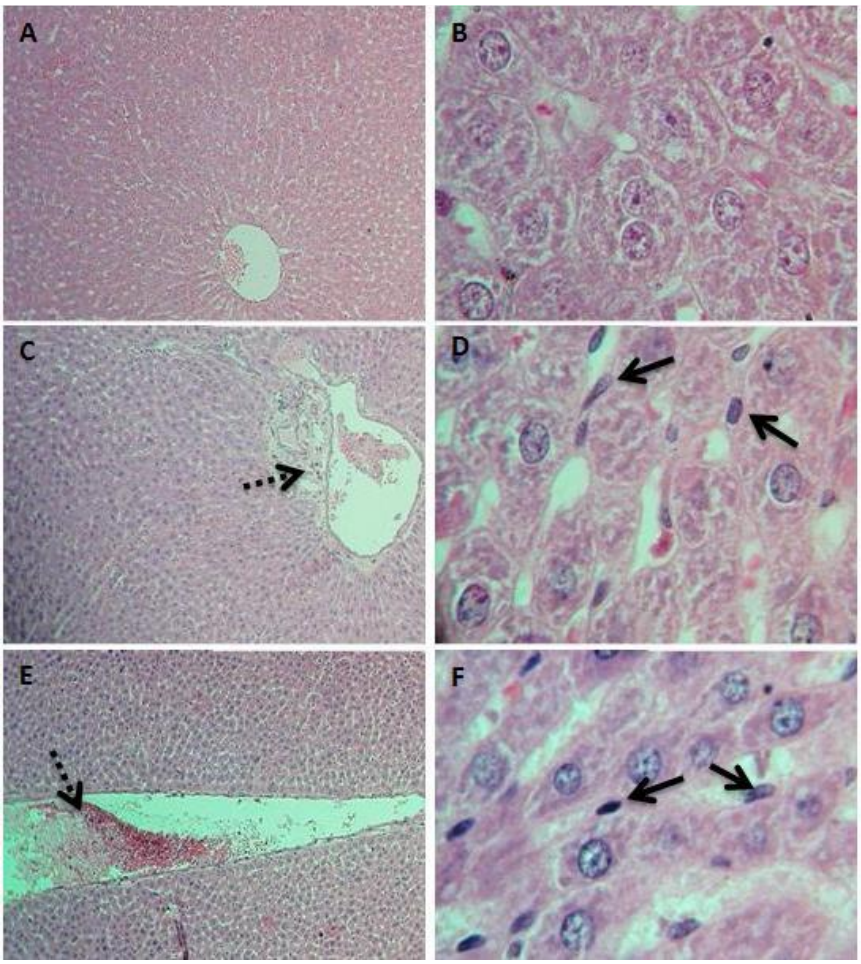

Figure 1. Chlorpyrifos induced changes in rat liver histology. The dotted arrows show increased leucocytic infiltration and solid arrows show increased Kupffer cell activity in lower (C,D; 0.1mg/kg bw), and higher (E,F; 2.5mg/kg bw) dose groups as compared to control (A,B).

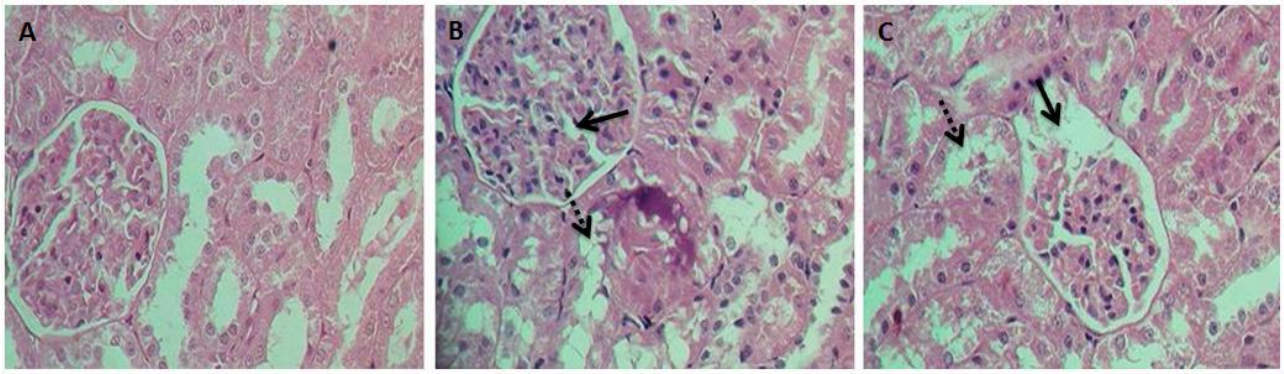

Figure 2. Chlorpyrifos induced changes in rat kidney histology. The dotted arrows show increased vacuolization and solid arrows show increased glomeruli damage in lower (B; 0.1mg/kg bw), and higher (C; 2.5mg/kg bw) dose groups as compared to control (A).

4. Discussion

4.1 Biochemical analysis

Decreased glycogen content in liver of treated rats might be due to increased utilization of glycogen by the tissue to mitigate the stress caused by CPF (Rao, 1999). A decrease in the glycogen level in rat and mice liver due to pesticide exposure has been observed in previous studies (Fayez and Kilgore, 1992; Parimala and Kaliwal, 2005; Ksheersagar and Kaliwal, 2006; Bhushan et al., 2013). Liver plays a pivotal role in regulating the concentration of glucose in blood. It exhibits net uptake of sugar when the concentration of glucose in portal blood is high and provides a release of glucose when the blood sugar is low. Hypoxic conditions in tissues have been reported to cause an elevation in glycogen phosphorylase activity to compensate the increased energy demand (Dua et al., 2010). The increased ALT might be indicative of stress on liver. The increased ALT results in increased transaminase reactions resulting in the formation of alanine and glutamate amino acids. This increase in the amino acid synthesis might have resulted in increase in the total proteins following CPF exposure. CPF exposure has been found to increase LDH secretion *in vivo* in rat liver and *in vitro* in PC-12 neuroactive cell lines (Bagchi et al., 1995). LDH constitutes a major checkpoint of anaerobic glycolysis, by catalyzing the reduction of pyruvate into lactate. Thus, an increase in LDH indicates increased glycolytic activity as NAD^+ produced in the conversion of pyruvate to lactate is used in glycolysis (Parra-Bonilla *et al* 2010) thereby, providing an evidence for a depleted liver glycogen content in the present study. This enzyme has recently received a great deal of attention since it may constitute a valid therapeutic target for diseases so different as malaria and cancer (Granchi et al., 2010). Increased liver LDH activity might have resulted in increased synthesis of lactic acid and is indicative of oxidative stress and hypoxia (Jovanovic et al., 2010).

4.2 Histological analysis of liver

These results are in agreement with the previous studies (Acker et al., 2012; Uzun and Kalender, 2013; Ma et al., 2013). Oral administration of high-dose groups (12 mg/kg) CPF led to a significant increase in levels of reactive oxygen species, DNA–protein crosslinks, 8-hydroxy-2-deoxyguanosine and malondialdehyde, decreases in acetylcholinesterase activity and glutathione level, as well as causing hepatic and renal histopathological change (Ma et al., 2013). Congestion of central vein, dilation of sinusoids, diffuse kupffer cells proliferation, mononuclear cell infiltration, pyknosis, eosinophilic cytoplasm after 4 weeks of chlorpyrifos exposure in rat liver tissues was observed (Uzun and Kalender, 2013). Oxidative damage in the rat liver was observed (Acker et al., 2012). Kupffer cells play a vital role in protecting the organ damage by removing particles and colloids from the blood stream (Bradfield, 1974). Kupffer cell proliferation indicates an increased defensive process against CPF toxicity in the present study. The susceptibility of the liver to toxic compounds is due to the central role it plays in the biotransformation and disposition of xenobiotics (Murray et al., 1999). Leucocyte infiltration and blood sinusoid dilation indicate inflammation in the liver of treated rats. These observations indicated marker changes in the overall histoarchitecture of liver in response to CPF, which could be due to its toxic effects primarily by the generation of reactive oxygen species causing damage to the various membrane components of the cell.

In a study on chicken Kammon et al., (2010) also found degenerative changes in the chicken liver after CPF exposure. They observed degeneration, coagulative necrosis and hemorrhages in the chicken liver. Histopathological changes including necrosis of hepatocytes, infiltration of lymphocyte cells, hyperemia, and proliferation of fibroblasts were observed in liver tissue of the dermally CPF exposed rabbits (Solati et al., 2012).

4.3 Histological analysis of kidney

Kidney function such as renal blood flow, concentrating substances, and biotransformation of the parent compounds makes this tissue sensitive to a variety of toxins. Our results were in agreement with the results of Heikal et al., (2012) showing a degenerating kidney due to a continuous 28 day treatment of CPF in male rats. Hemorrhages, vacuolar degeneration of tubular epithelial cells as well as focal coagulative necrosis in the CPF treated chicken kidney has also been observed (Kammom et al., 2010). However, the kidneys, the major detoxification organs for many xenobiotics, are frequently susceptible to the nephrotoxic effects. Although hisopathologic examination showed lesions in kidney tissues produced by CPF, the blood urea value remain unchanged in treated rats as compared to control.

4.4 Conclusion

The use of CPF in domestic and agricultural platforms provides a great help in minimizing the damages caused by insects, but on the other hand its long term exposure is harmful for animal as well as human population. The present investigation provides an insight of CPF induced toxicity on liver and kidney, the vital organs for maintaining the homeostasis. Increased damage to these vital organs even at low doses of CPF could be compared withthe adverse effect of CPF on human population.

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