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Histological and Biochemical Alterations in Liver of Rattus Rattus Trapped From Vegetable Fields

KEYWORDS: Pesticides, biochemical, histological, liver.

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ABSTRACT *Pesticide toxicity has gained considerable attention in the recent years leading to a postulation that they are a major cause of environmental deterioration. A number of pesticides are sprayed on vegetable crops and field rats are continuously being exposed to the mixed concentration of pesticides. The present study was designed to assess the histological and biochemical alterations in liver of the female rats due to pesticide exposure trapped from the vegetable fields. Enzyme assays have showed a significant increase (P≤0.05) in alanine transferases (ALT), asparate transferases (AST), acid phosphatase (ACP), alkaline phosphatase (AKP), total lipids, phospholipids and cholesterol in liver homogenate which may be due to pesticidal stress. Total proteins in liver showed a non significant (P≥0.05) increase. Histological alterations (vacuolization, nuclear fragmentation, infilteration, pyknosis and sinusoidal dilations) have been observed and indicated that pesticides have caused a serious damage to the organ.*

INTRODUCTION

Environmental toxicants can induce adverse effects in normal cell functioning of humans as well as other organisms. Excessive use of pesticides in fields has been recognized as a major reason of environmental deterioration. Environmental and occupational exposure to these pesticides also cause severe effect on neural regulation, absorption, metabolism and elimination due to impaired functions of liver and other vital organs (Abd El Raouf and Girgis, 2011). Pesticides preliminarily acts on central nervous system either as nerve poisons or as acetyl cholinesterase inhibitors. Since liver is associated with metabolism and elimination of toxicants from the body, its biochemical parameters are considered as key points to elucidate toxicity of the chemicals. Toxic compounds interfere with carbohydrate, protein and lipid metabolism, that is concerned with normal physiology of the animal. Pesticide induced biochemical changes are reflected by the change in histoarchitecture of the tissue. Significant histopathologic changes such as fibrosis, necrosis, cellular hypertrophy and hyperplasia, pyknosis and vacuolization has been found in the brain, thymus, spleen, liver, kidney in the rats treated with betahexacyclohexane (BHC), monocrotophos, mancozeb, propineb, maneb (Ksheerasagar et al 2011). There are few reports on work done on effects of chlorpyrifos, imidacloprid, monocrotophos, carbaryl, dihydrogen monoxide used in vegetable fields on the physiology of rats as a model animal.

MATERIAL AND METHODS

Rats (Rattus rattus) were trapped from the vegetable fields of Faridkot, once a month, using multi rat traps and brought to the laboratory. The rats trapped from the fields of Punjab Agricultural University (PAU) served as control.

The collected rats were humanely anaesthetized and sacrificed. Liver was removed and washed thrice with 0.1 M Phosphate Buffer Saline (PBS) and weighed. 1gm of tissue was homogenized in two ml of PBS and centrifuged at 5000 rpm. Supernatant collected from liver was stored at -4°C until the analysis of biochemical parameters.

BIOCHEMICAL ESTIMATION

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined by colorimetric method of Reitman and Frankel as described by Bergmeyer (1974). Activities of Acid phosphatase and alkaline phosphatase were estimated as per the method of Bessey et al (1946). Total proteins were determined by the method of Lowry et al (1951). Estimation of total lipids, cholesterol and phospholipids was done in the liver by the method of Folch et al (1957), Chiamori and Henry (1959) and Ames (1966) respectively.

HISTOLOGICAL STUDIES

Liver was cleared from adhering tissues and fixed in Alcoholic Bouin's fixative for 24 hours and after following standard procedure, embedded in paraffin wax (58-60°C) and 5µm thick serial sections were obtained. The sections were stained in haematoxylin, counterstained with eosin and mounted in DPX. These sections were examined for sinusoidal dilations, mononuclear cell infiltration and pyknosis.

STATISTICAL ANALYSIS

Statistical significance of biochemical parameters was obtained by students t- test at 5% level (P<0.05) of significance.

RESULTS

BIOCHEMICAL OBSERVATION

Rats collected from the fields of Faridkot showed a significant (P≤0.05) increase in the levels of ALT, AST, ACP and AKP in liver as compared to control rats of PAU (Table 1). But there was a non significant (P≥0.05) increase in the total proteins of liver of field rats as compared to control. Total lipid extracted from the liver of the treated rats showed a significant (P≤0.05) increase in total lipid content. Phospholipids and cholesterol activity estimated from the lipids extracted also increased significantly (P≤0.05) as compared to those extracted from the control rats of PAU.

HISTOLOGICAL OBSERVATION

The liver of control groups revealed normal hepatocytes arranged in cords which are separated from each other by sinusoids radiating from the central veins towards the periphery of liver lobules. No infiltration of leucocytes was observed in the sinusoids. No signs of nuclear fragmentation, vacuolization were observed (Fig 1 A-D).

Liver of rats collected from Faridkot showed a slight loosening in arrangement of hepatic cords around central vein. The loss of radial arrangement of hepatocytes was also seen. The dilation of central vein (CV) occurred and showed infiltration of large mass of leucocytes inflammatory cells in CV in the female rats. Histopathological examination of the liver from

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treated group animals revealed various cellular and lobular abnormalities, including intralobular vein (ILV) membrane dilation, presence of hepatocytes in ILV, vacuolization, nuclear fragmentation, nuclear vacuolization, hepatocyte membrane damage, pyknosis (Fig 1 E-H).

DISCUSSION

Great part of the pesticides is used in agriculture and gets accumulated in the body of humans and other animals. This gradual increase in the concentration of pesticides in animal body can cause many diseases like gastric cancer, cytogenetic damage, kidney infections and others (Bhushan et al 2013). Our present study revealed elevated levels of alanine and amino transferases in the liver of the female rats collected from the fields of Faridkot. Aminotransferases are sensitive inductors of hepatocellular damage under oxidative stress caused by xenobiotics, which histologically presented as cytoplasmic vacuolization in this study which is in agreement with Bhushan et al (2013). The elevation in the liver enzyme activities may be due to liver dysfunction with a consequent reduction in enzyme biosynthesis. Elevated ALT and AST in the present study point towards active utilization of amino acids in energy yielding metabolic processes such as gluconeogenesis. It also reflects the genetic abnormality in production in order to overcome pesticide induced oxidative stress (Bhushan et al 2013; Abd-Elhady 2013).

Acid phosphatase (ACP) and Alkaline phosphatase (AKP) are lysosomal enzymes, which catalyse the splitting of phosphoric acid from certain phosphoric esters and commonly found in most tissues of the body. These enzymes are generally located on absorptive or secretory surface of cells as membrane bound enzymes. ACP hydrolysis the ester linkage of phosphate esters at acidic pH (between 5 to 6) and helps in autolysis of the degenerated cells. AKP splits phosphorus esters at alkaline pH (10) and mediates membrane transport and is intimately associated in protein synthesis, and glycogen metabolism (Ksheerasagar et al 2011). Our present study showed increased levels of alkaline and acid phosphatases in liver of the treated rats. It has been suggested that an increase in AKP levels occur due to damage of the liver cells (Vohra and Khera, 2013). In a similar manner, increase in the specific activity of ACP in the rat tissues, only confirmed the increase in the dephosphorylation potential within the rat cells due to the pesticides. The lack of phosphorylated compounds within the animal cells probably led to reduction in stored phosphate, the phosphate depletion affecting the calcium:phosphorous ratio within each cell, eventually resulting in membrane damage and lack of energy compounds (Muthuviveganandavel et al 2011).

A significant increase in the total protein content was observed by Vohra and Khera (2013) in liver of the rats treated with imidacloprid, but the results obtained from the rats collected from the fields of Faridkot revealed a non significant increase in the total protein content in liver, indicating no severe damage to the liver and also no interference with the protein metabolism.

Present study showed significant increase in total lipids, cholesterol and phospholipid contents in liver of rats collected from the fields of Faridkot which is in agreement with Bhushan et al (2013). Increase in total lipid levels in liver may be due to increased lipogenesis which reflects abnormal carbohydrate metabolism. It led to excessive conversion of pyruvate to free fatty acid. Increased cholesterol is likely to have substantially contributed to the total lipid levels in treated rats (Saxena and Doneriya 2004) and may have played a role in the significant increase in phospholipid content (Pereira et al 2006).

Similarly various histopathological changes in the liver of the rats collected from the fields of Faridkot in the form of dilations, pyknosis, vacuolization, nuclear fragmentation are in agreement with the observations of Bhushan et al (2013).

CONCLUSION

Increased activity of various enzymes in the liver of the female rats collected from the fields of Faridkot indicates that the amount of pesticides being sprayed in the fields is causing a great damage to the tissue. Our findings are supported by the liver histology showing damage in the tissue.

Table 1: Liver Biochemical Parameters of Rats trapped from PAU (control) and Faridkot

Parameters	FARIDKOT	CONTROL
Protein (mg/g tissue weight)	6.70 ± 0.35	4.80 ± 0.61
ALT (µ mole/g liver)	43.90±2.20	29.50±1.30
AST (µ mole/g liver)	83.23±7.10	60.04±5.50
ACP (µmoles/min/mg of protein)	10.10±0.57	6.37±0.14
AKP (µmoles/min/mg of protein)	86.04±2.80	67.05±0.74
Total lipids (mg/g wt of tissue)	0.02±0.00	0.01±0.00
Cholesterol (mg/g tissue weight)	5.94±0.071	4.85±0.06
Phospholiids (mg/g tissue weight)	10.74±0.27	6.32±0.26

Values represent the mean ± SE
Significantly different from control at P<0.05

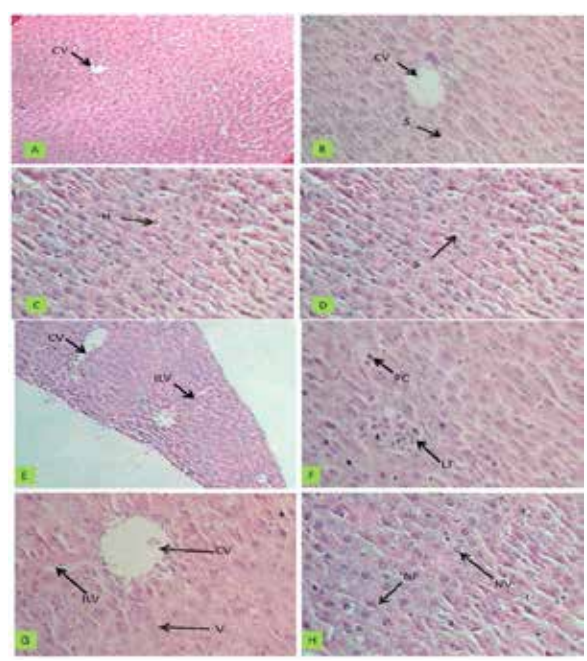

Fig 1. A-D (Rattus rattus control) E-H (Rattus rattus treated). CV- central vein, S- sinusoids, H- hepatocytes, ILV- intralobular vein, PC- pyknotic cells, LI- leucocyte infilteration, V- vacuolization, NF- nulear fragmentation, NV- nuclear vacuolization (HE stained sections; 100X and 400X).

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